THE CONTROL OF LARVAL COLOUR IN PHLOGOPHORA METICULOSA L. (LEPIDOPTERA : NOCTUIDAE) AND SOME OF ITS CONSEQUENCES.

by

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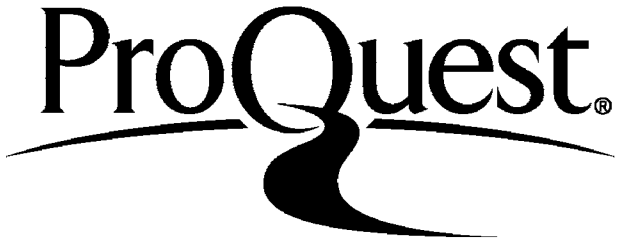

ABSTRACT OF THESIS

Michael E.N. Majerus

The Control of Larval Colour in Phlogophora Meticulosa L. (Lepidoptera : Noctuidae) and some of its consequences.

The larvae of many Lepidoptera of the family Noctuidae have both green and brown forms. This trait had never been studied in depth. (Chapter 1)

Larvae of Phlogophora meticulosa exhibit considerable colour variation. A method was developed to score larval colour. (Chapter 2)

Eight colour types were defined, these being named "early green", "3rd instar green", and the main colour types "green, olive, brown, plain yellow, yellow-green and yellow-brown". Larval colour in the 1st, 2nd and sometimes 3rd instars is determined by foodplant colour. The seven colour types found in the the late instars, (3rd instar green is replaced by the green main colour type in subsequent instars), are controlled by five unlinked major genes which have a total of eleven alleles. The system involves a complex arrangement of dominance and epistatic effects. (Chapter 3)

The change from foodplant to genetic control of larval colour is correlated with a change from a positive to a negative phototactic response. (Chapter 4)

Study of allelic and phenotypic frequencies in wild populations has indicated that the genetic polymorphism is balanced from year to year, although the frequencies of some alleles show seasonal variation. (Chapter 5)

Tests to determine the nature of the selective forces, which maintain the polymorphism, have indicated that choice of mate is random. Heterozygote advantage is implicated in the maintenance of at least two of the genes. (Chapter 6)

Selection due to viral infection and parasitism both affect the maintenance of at least one gene, and beetle and small mammal predation may also do so. (Chapter 7)

Experiments on the effects of bird predation have uncovered a complicated system of bird-larval morph relationships.(Chapter 8)

Arguments are put forward to explain the switch from food-plant to genetic control of colour, and to explain the lack of linkage between the five major genes controlling larval colour. The relative importance of the selective agencies scrutinised is considered. It is suggested that bird predation is extremely important in the maintenance of the genetic system. The degree to which larval morphs are exposed to differential predation by birds is determined by the cryptic qualities of the larvae, and, because birds select apostatically to some extent, their abundance. Further lines of research are suggested. (Chapter 9)

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PREFACE

To my mother, Mrs. Muriel Majerus, and to the memory of my father, Mr. Fernand Majerus.

> "I lingered round them, under that benign sky: watched the moths fluttering among the heath and harebells; listened to the soft wind breathing through the grass, and wondered how anyone could ever imagine unquiet slumbers for the sleepers in that quiet earth".
>
> (E. Brontë - 1847)

My aim in this work has been to study a phenomenon which is widespread in Lepidoptera, that is to say larval colour variation involving both green and brown forms. This phenomenon has been investigated in Phlogophora meticulosa L. The work was broadly split into two parts, the first being to determine the ways in which larval colour variation, in P. meticulosa, is controlled, and the second being to look into the maintenance of these control mechanisms.

The thesis is organised into nine chapters. The first is introductory and provides a background to work on larval variation. A description of the moth P. meticulosa, the basic techniques used in rearing and the method of colour scoring larvae are given in the second chapter. The experimental work is described in the following six chapters. The final chapter focuses on the main conclusions and points out possible lines for future research.

Acknowledgements

First and foremost I thank my supervisor Dr. John Pontin for his invaluable encouragement, criticism and discussion, throughout all stages of the work.

I am also grateful to many other friends and colleagues

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A great many people gave me help at various times, and I thank them all. They include those people who allowed me to work on their land, and everyone, particularly my mother, who fed and looked after larvae for me when I was unable to. I am especially grateful to Mrs. Jenny Hawkins who typed the thesis for me, and to all those who have read, criticised and corrected parts of the work, particularly Miss Vicki McLean, without whose advice on English and style the thesis would probably have been largely incomprehensible.

I also wish to thank a motley crew who have harassed and helped me throughout, particularly with moral support. They ensured that I preserved a grain of sanity and kept things in perspective when prospects seemed bleak. Without them I have no doubt that this thesis would never have been completed. They include Peter and Eileen, Mike, Iain and Boo, Keith, Nigel, Richard and Mike, Gail, Antos and Dave, Genesis and the Moodies,

and last but most importantly, Vicki.

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ABBREVIATIONS AND OTHER NOTES

Gr = Green (larva)

OL = Olive (larva)

Br = Brown (larva)

PY = Plain Yellow (larva)

YG = Yellow-green (larva)

YB = Yellow-brown (larva)

(The numbers 3, 4 or 5, followed by any of the above colour abbreviations, indicate the instars in which the colour occurred).

EG = Early green (larva)

3IG = Third instar green (larva)

GP = Green-pink (larva)

In some tables abbreviations are used for alleles:

(Gene A)	+ =	a^+
	- =	a
(Gene B)	' =	b'
	o =	b^o
(Gene C)	+ =	c^+
	- =	c
(Gene D)	+ =	d^+
	- =	d
(Gene E)	+ =	e^+
	' =	e'
	o =	e^o

An x associated with an allele indicates that the nature of the allele was unknown.

Parentheses around a pair of alleles indicates that the parent in which these alleles occur was unknown.

Due to the epistatic and dominance effects between genes D and E, the exact genotype of larvae, with respect to these

genes, was rarely known. Thus, the following abbreviations have been used in association with these genes.

(D) ++ or (E) ++ means that either D and/or E were homozygous dominant.

(D) + or (E) + means that the dominant allele of at least one of these genes was present.

Gene A and its recessive allelomorph, a, when used in isolation in the text are sometimes underlined to avoid confusion with the indefinite article.

The symbol $\underline{X}^2$ represents chi-squared, and the subscript number (as in X^2_3) equals the number of degrees of freedom for a particular test.

When referring to chi-squared tests; Significant means that a probability value is less than 0.05, Highly significant means that a probability is less than 0.01.

Throughout, times given are based on Greenwich Mean time.

CHAPTER 1 GENERAL INTRODUCTION

A great deal of research has been carried out on variation in imaginal characters of Lepidoptera; however, since Poulton's experiments on the colours of lepidopterous larvae in the late nineteenth century, the subject of larval variation has received little close scrutiny.

Butterflies and moths in the adult state are eminently suitable for research in the field of ecological genetics for three main reasons. Firstly, the wings of Lepidoptera provide a site in which variation can be recognised and measured with comparative ease due to the nature of the wing structure and the wide variety of colours and patterns which occur. Secondly, breeding experiments designed to produce data for genetic research may give fruitful results in a short time as the females lay a considerable number of eggs, and the life cycle of Lepidoptera is rarely longer than a year. Indeed, in many species it is naturally shorter, or the length of the life cycle may be lessened by breeding under special conditions. Finally, the techniques used in breeding large quantities of Lepidoptera are comparatively simple for many species.

The lack of work on the control of variation in lepidopterous larvae is, therefore, rather surprising for although colour variations in larvae may not be as conspicuous to the eye as those of the imagines, wide variations do occur in many species, and a great number have been described in the literature, particularly that of the nineteenth century. Obviously, with regard to lifespan and breeding techniques, larvae are as suitable as the adult insects for the study of variation. Indeed, they have a slight advantage

in that by the time the larval stage ends, mortality will necessarily be less than by the time the adult stage is reached; so data on larval variation are likely to be less affected by differential mortality than data on imaginal variation. One added interesting feature which is not applicable to the adult insects is that larvae have a number of instars - usually from four to seven - and larval colour often varies from instar to instar.

The work that has been carried out on larval variation may be split into three main categories; pigment studies, environmental variation, and genetic variation.

Much of the work on pigments has involved analysis of the pigments present in larvae, such as the work of Junge (1941), who discovered that the bright green integument of Sphinx ligustri L., is caused by a mixture of a yellow carotenoid (probably lutein) and a blue-green chromoprotein (probably mesobiliverdum). Early work of this type was usually connected with investigations into insect-plant relationships rather than the control of variations in colour and pattern. More recently, a thorough study of the carotenoids of the Large White Butterfly (Pieris brassicae L.) and its foodplant, the cabbage (Brassica oleracea var. capitata L.) (Feltwell and Valadon, 1972, 1974; Feltwell, 1973), has indicated that P. brassicae does not synthesise new carotenoids from those taken in with their food. Further studies showed that a number of other species of Lepidoptera, representing ten families, also do not form new carotenoids - the families being, Pieridae, Danaidae, Sphingidae, Saturnidae, Arctiidae, Geometridae, Noctuidae, Lymantriidae, Notodontidae, and Zygaenidae (Feltwell, 1973;

Mummery et al., 1975). This feature generally appears to be the rule in Lepidoptera, although exceptions have been noted such as Papilio xuthus L., which is able to oxidise beta-carotene and lutein to keto-carotenoids (Harashima et al., 1972). Thus it is probable that foodplant carotenoids always affect larval colour to some extent, even though the extent may be slight if other 'masking' pigments are present.

It is notable that not only does larval colour vary from instar to instar in many species, but the instars in which colour changes occur may vary within a species. Bückmann (1974) proposes that colour changes in insects which are part of the normal development should be termed ontogenetic colour changes. He goes on to say that:-

> "In all cases investigated so far these ontogenetic colour changes are caused by the hormonal system of moulting and metamorphosis, ecdyson and juvenile hormone - even if the colour change does not coincide with a moult .
>
> "Other colour changes caused by environmental conditions may be caused in three ways:-
>
> 1) Direct action of environmental conditions as temperature or food on the pigment metabolism in the integument.
>
> 2) Modification of the pigmentation by endocrine control of the pigment metabolism by the corpora allata.
>
> 3) Modification of the development of the pigmentation by special neurosecrets of the ventral ganglia. Such occurs evidently in most cases, where sensory reception controls the development by way of the central nervous system and the hormones".

Some aspects of the way in which certain environmental

factors affect larval colour have been investigated, such as the effect of overcrowding larvae. In Spodoptera exempta Walker and S. exigua Hübner, larval colour varies from green to grey when reared in isolation; however, when kept under crowded conditions larval colour becomes considerably darker (Faure, 1943). These darker larvae are also much more active than the paler ones. This is possibly a result of mutual disturbance, and the more intense pigmentation may be due to a generally enhanced rate of metabolism (Fox and Vevers, 1960). Similar tendencies are reported in Spodoptera abyssinia Guen. (Matthée, 1945, 1947); Lacanobia oleracea L., Orthosia cruda D. and S., Orthosia gothica L., Orthosia incerta Hufn., Autographa gamma L., and Saturnurnia pavonia L., (Long, 1953). The increase in pigmentation for A. gamma is very marked, ranging as it does from pale green through a whole range of colours to dark brown. (The change in colour has even been noted in the wild state when larval density has become unusually high). Long (1953) does mention that not all species respond in this way, Lathoe populi L., Mimas tiliae L., Orthosia stabilis D. and S., and Pieris brassicae showing negligible response, and in those species which do respond to overcrowding the degree of darkening may vary for individual larvae.

Poulton (1885, 1886, 1887, 1892, 1893) extensively examined the possible influence of the surroundings upon larval coloration. He used mainly arbivorous larvae of the Geometridae for his experiments and found that they are very sensitive to the background colour of their immediate surroundings. Opisthograptis luteolata L., and Biston betularia L., produced the most striking

results. In general, larvae of both species bred amongst green leaves were of the green form, whilst those reared amongst brown twigs became brown. Larvae of other species which have a series of brown forms but no green ones, were also found to be susceptible, the shade of the ground colour depending on the darkness of the twigs which the larvae rested upon. The Geometrids, Crocallis elinguaria L., Ennomos quercinaria Hufn., Selenia lunularia Hubn., and Xanthorhoe montanata D. and S. were found to be sensitive as were several species of Catocala (Noctuidae). Poulton also produced lichen-marked larvae of Odontopera bidentata Clerck, and Gastropacha quercifolia L., and others by keeping them in cages with bits of white paper mixed with their food. Cockayne (1928) notes that these experiments are borne out by observations in the field on Allophyes oxyacanthae L., which often have a mottled pattern in the New Forest. There they feed on blackthorn covered with lichens, and the proportion of lichen-marked larvae is seemingly dependent on the profusion of lichen. On the other hand, Cockayne notes that he never met this form at all in the London area, nor around Sheffield or in Lincolnshire where lichens are absent or scarce. Cockayne states that similar situations were observed in O. luteolata and O. bidentata in a wood at Gight in Scotland. In the low part of the wood where most of the Mountain Ash (Sorbus aucuparia L.) trees are dying and are covered with lichen, most O. luteolata larvae are green or mottled with very few of the brown type, whilst in the higher part of the wood where the trees are healthier, more leafy and much less lichen-covered, green larvae were the preponderate form with many more brown larvae and

very few of the mottled type. In O. bidentata lichen-marked larvae were common in the lower part of the wood and brown ones rare, but higher up all were brown.

Poulton found that the larvae he experimented with were most sensitive to their surroundings in the third, and to a lesser extent, second instars. The effect was accumulative, in that the longer a larva was exposed to a special environment, the greater was the effect produced by it on colour variation.

The difference in colour in these species is generally thought to be due to the distribution and quantity of various pigments in the epidermis of the larvae.

A number of other Geometrids have both brown and green forms of larvae which seem to differ only in pigmentation and Cockayne (1928) speculates that these would probably prove to be sensitive in the same way.

Most of the work that has been carried out on the genetics of larval characters has dealt with monogenic characters and in very few species has extensive work been undertaken. The exceptions include Gerould's work on Colias philodice Latr. (Gerould, 1921, 1922, 1926). He describes two colour types, blue-green and olive-green, which are inherited independently of one another as autosomal recessives to the typical grass-green form, the blue-green form being epistatic to olive-green. Gerould ascribed the blue-green coloration to the absence of a yellow carotene normally derived from the food and present in the larval haemolymph. This seems to indicate that the carotene is actually being broken down by the action of the blue-green allele. This allele also provides one of the few examples where an allele

has been shown to be expressed phenotypically in the larva, pupa and imago, the pupa being blue-green instead of the normal grass-green, whilst the adult has a distinctly blue hue rather than the normal light green. Gerould also suggests that the olive-green is due to an orange pigment in the larval epidermis. Olive-green forms have also been noted in Pieris napi L., (Bowden, 1958, 1959) and Colias croceus Geoff. (Herman and Lorkovic, 1961). In both these species, the olive-green form is inherited as an autosomal recessive. Sevastopulo (1959), commenting on the similarity of the olive-green forms of two of these species, mentions that he has observed aberrant yellow-green larvae in Colotis eucharis Fab. He gives no breeding data to show that the form is inherited but, nevertheless, speculates that the form could be a genetic homologue of the previous cases. The fact that the blue-green larval form of C. philodice is inherited independently to the olive-green form shows that more than one locus is involved in the control of larval coloration, and this fact alone should induce caution in the too ready acclaim of homology.

Gorodenski (1970) worked on Chlosyne lacinia Geyer, which has three larval forms, nigra the black form, bicolor which is black with a mid-dorsal orange band extending the length of the body, and rufa, the orange form. The genetic mechanism controlling these three forms is a non-linked, two-locus epistatic system. The bicolor and rufa determining alleles are dominant over their recessive homologues, with the dominant rufa allele epistatic over the bicolor locus. Neck (1971) has shown that genetic modifiers and environmental influences act upon this basic three-phenotype

polymorphic system with the result that a genetically discontinuous mechanism of variation is modified into a system of almost continuous variation.

The other major exception is Bombyx mori L. More genes have been identified in this species than in any other of the order. However, these constitute rather a special situation as the moth is unknown in the wild state, and much of the experimental breeding which has been carried out on it relates to the commercial production of silk. By far the majority of known mutations modify the various larval characteristics, the reason for this being that this stage has been most fully examined. In all, over seventy alleles which affect larval morphology have been recognised. Larval body colour may be changed from the typical grey to chocolate brown, lemon, albino, dilute black, sooty or reddish brown. Three reviews of the work on B. mori are those of Kikkawa (1953), Tanaka (1953) and Yokoyama (1959). These are complemented by the monograph by Tazima (1964).

One of the most widespread types of larval polymorphism is the occurrence of green compared with brown or blackish colouring, of which some Geometrid examples have already been mentioned. Ford noted this occurrence in his book on Moths (1955), where he wrote:-

> "Such variation has been reported in a great many species widely scattered among the Lepidoptera. It is by no means fully understood and has indeed never been subjected to the rigorous experiments which would be needed to interpret it".

Species with both green and brown larval forms are most common amongst the Noctuidae; often both forms are approximately

equal in abundance, for example Ceramica pisi L., but in some cases, such as Xylena vetusta Hubn., the brown form is scarce, whilst in others, for instance Noctua pronuba L., the green is the scarce form (Cockayne, 1928). The blood and fat tissues in the majority of these species are green and the differences between forms are merely pigmentary. Experiments on the sensitivity of these Noctuid larvae to environmental factors are few and generally inconclusive. Neither Professor Poulton nor his co-worker, Miss Gould, found any indication that they were influenced by their surroundings. Most of the larvae of this group hide amongst low herbage during the day-time and so the need for acquiring colours like those of their environment would appear to be much less than in the case of arbivorous Geometrid larvae. Indeed, Cockayne (1928) indicates that he suspects that even in Geometrid larvae there is an hereditary difference between green and brown forms, but that it can, to a large extent, be overcome by environment, whilst in the majority of Noctuids the difference is solely hereditary.

Federley (1916) investigated the green and brown forms of Deilephila elpenor L. larvae, in which the change from green to brown is similar to that of Noctuid larvae, with respect to the suddenness and completeness of the changes which occur and the variability in the stage at which they occur. Federley, starting with a brood in which forty-two larvae were brown and ten were green, believed that green would prove to be recessive to brown; however, subsequent crosses of brown x brown, green x green and the reciprocal crosses of brown x green produced no green larvae. Robinson (1971) states that at present the only

explanations worth considering are, (a) that the possibly recessive green form has an extremely poor manifestation in certain circumstances or (b) that the difference between the forms is caused by an environmental threshold effect. The situation, however, is complicated by the discovery of a bilateral mosaic larva, half green and half brown (Oertel, 1910), which implies that at least some aspect of the variation has a genetic basis.

Ford (1940b) comments on the occurrence of either green or brown forms with an apparent lack of intermediates in a number of species of Cyclophora (Geometridae). After examining five of the British species, he found that broods of entirely green larvae may be obtained from either brown x brown or green x green crosses as well as the reciprocal matings of brown x green. Ford takes the view that the colour change is determined environmentally, probably by a temperature differential acting over a brief but critical phase of larval growth. Since a group of larvae will usually grow at different speeds, this theory could explain both the occurrence of mixed broods and the comparative absence of intermediates. However, as no substantive evidence has been obtained it must still be considered as speculative.

Another speculative explanation of the green-brown variation involves the action of visual stimuli on larval ocelli, and this certainly seems to be true with regard to pupal variation in some Lepidoptera species (Poulton, 1887; Kayser-Wegmann, 1975; Rothschild, Valadon and Mummery, 1977).

There are many reports that green and brown larvae habitually rest upon backgrounds which they match. The full grown

larva of the Pine Hawkmoth (Hyloicus pinastri L.) may be either mottled brown all over, or it may have a mottled brown band along the dorsal surface and be mottled green on the sides. The brown form is better concealed at least to the human eye, when sitting exposed on the branches of the foodplant, whereas the brown and green one is more difficult to detect when it is resting along a twig with needles upon it. Sheppard (1961a) notes that there is some evidence that the two types of larvae tend to take up a position appropriate to the particular form. Similarly, the green and brown forms of various Noctuid larvae, for instance the Angleshades moth (Phlogophora meticulosa L.), are reported to have a tendency to rest respectively on the green or brown parts of the foodplant (Ford, 1955). If these observations are verified, then an interesting question arises. Do the larvae of genetically polymorphic species actively rest in situations where they match their surroundings, or do the surroundings act upon larval colour and possibly also induce in larvae a behavioural pattern, which leads to larvae subsequently resting upon backgrounds which they match?

Extensive and rigorously controlled experiments will be needed if the situation is to be clarified, or if any of these suggestions and theories are to be verified.

The aim of this study has been to investigate in depth the causes and consequences of larval colour variation in a species of Lepidoptera, which has both green and brown forms.

CHAPTER 2 MATERIALS AND METHODS

Introduction

Throughout this study, the materials and methods used have been kept as simple as possible with the proviso that this simplicity did not reduce the effectiveness of the experiments. The reasons for this are threefold. Firstly, as many people breed Lepidoptera as a hobby, it is to be hoped that some of these enthusiasts may wish to repeat some of the experiments in this study either with Phlogophora meticulosa or with other species. The use in this study of equipment normally available to the amateur would enable direct comparisons to be made between data from this and ensuing studies.

Secondly, simple equipment enabled a large number of larvae to be reared and used for statistical analysis[1].

The third reason for simplicity, and possibly the deciding factor in the selection of equipment, was purely financial. During the first two years of this study, I was self-financed, and this effectively limited the choice of equipment used in the work to that which I already owned, or which was available at the Zoology and Botany Departments of Royal Holloway College.

The research covered a number of different subjects which may be listed as follows:-

i) Selecting a species which showed larval colour variation and which was easily reared, of convenient size, and abundant in the wild.

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[1] In fact, the results of some of the experiments showed that it was indeed fortunate that this view had been taken, because some of the larval colour types which occur in P. meticulosa are very rare, and may have been overlooked if a smaller number of larvae had been studied.

ii) Analysis of the type of variation.
iii) The effect of certain environmental factors on larval variation.
iv) The genetic control of variation.
v) Larval behaviour.
vi) Gene frequency analysis.
vii) Reproductive behaviour with respect to choice of mate.
viii) Heterozygous advantage.
ix) The effect of predators and parasites on larval variation.

Because of the wide range of experiments used to investigate these subjects, I feel it is impractical to outline here the materials and methods used for all the experiments. Rather, I will confine myself, in this chapter, to describing the moth used, and to outlining the basic methods employed when rearing or colour scoring larvae, leaving the description of the materials and methods used for specific tests and experiments, to appropriate points in the other chapters.

A The Moth.

The ecology and larval morphology of over eighty species of Lepidoptera were considered before Phlogophora meticulosa L. (the Angleshades Moth) was selected for this study. It was chosen due to:-

i) The larval variation it exhibits.
ii) The ease with which it may be obtained and bred in large quantities.
iii) The lack of true diapause at any stage in its development, which meant that a relatively large number of consecutive broods could be reared in a fairly short time.

iv) The interesting problems that seasonal changes in the environment may pose in relation to the insect's life cycle, which is often irregular.

Phlogophora meticulosa is a member of the sub-family Amphipyrinae of the family Noctuidae. It is a medium-sized moth with a wingspan of about 50 mm, its forewing ground colour is pinky-brown with a triangular central band of olive and the outer margin unevenly dentated. The hindwing is of a pale ochre-brown colour. When at rest, the wings are held along the body and are broadly wrinkled. In this position, the moth is very like a crumpled, decaying leaf and is undoubtedly cryptic. The imago varies little, although the olive colour may be replaced with red, and in such specimens, the ground colour is more rosy. The hindwing ground colour may also occasionally be suffused with dark grey scales.

The larvae is eruciform, up to 40 mm long when in the 5th instar[1], and the colour varies from pale yellow through green and olive to dark brown. It is minutely dotted with white and a central line runs along the back. This may be interrupted and has dark v-shaped marks along each side of it, which vary in intensity, appearing in some cases to be totally absent. The lines along the sides of the larvae connecting the dark ringed spiracles are usually

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[1] P.meticulosa normally has five larval instars, but occasionally six instars were recorded, a situation which is possibly analagous to that of A. gamma, which may have five, six or seven larval instars (Long, 1953). The reason for the occurrence of an additional instar is not known, but it does not appear to be correlated with any of the environmental factors used in the later tests, (see chapter 3B), nor with a simple genetic mechanism. In all cases where a 6th larval instar was recorded, the colour of the larvae was the same in the 5th and 6th instars.

whitish, but they may be tinged with the ground colour. Larval instars may be distinguished by measuring the width of the head capsule, or by measuring the overall body length. (See chapter 2C).

The pupa is smooth in texture and chestnut brown in colour. It is surrounded by a thin silken web, either amongst the leaf debris at ground level, or just below the surface in light soil.

The moth comes readily to mercury-vapour light-traps and females taken in this way are virtually always fertile.

P. meticulosa does not have a rigid annual cycle and it may be found in all stages of development, during all seasons of the year. Generally, there seem to be two generations a year, although in abnormally warm years, (e.g. 1976), there may be three broods, and under optimum conditions in captivity, eight broods a year may be obtained. The adults occur most commonly during April and May, and again in September and October in normal years, but may be found in any month and egg-laying females have even been taken in January and February. For this reason, the occurrence of a large number of moths on the wing will be referred to as a main emergence, rather than a brood or generation as is common practice.

Watson & Whalley (1975) record that there are two generations each year. One flies from August to late autumn. These moths overwinter and reappear in May to lay eggs. The caterpillers resulting from these eggs overwinter, then pupate and produce adults in May and June, so that during May there are two generations on the wing.

My own observations in southern England oppose this view. Those females I have taken in September and October lay eggs and die within a couple of months of emerging. Adults taken during January and February may be quite fresh, indicating they have emerged recently, and lay eggs as soon as the minimum night temperature rises above about 6°C. Larvae resulting from eggs laid in April, May or June always seem to feed up, pupate and produce adults by October at the latest. In fact, if the arrangement of brooding suggested by Watson & Whalley were to be true, one wonders which broods would give rise to September-emerging adults.

The larvae feed mainly between dusk and dawn, spending the daylight hours amongst the herbage and debris close to the ground. They show no true winter diapause and will continue to be active throughout the year, when the temperature is above 4°C. The larvae are highly polyphagous, feeding mainly at night on the foliage of a very wide range of low growing plants. In captivity, they may also eat the leaves of many deciduous trees as well as the flowers, seed-pods and stems of many plants, although they will not commonly do so in the wild. Personal observations have indicated that the favoured natural foodplants seem to be sorrels and docks (Rumex spp.) and plantains (Plantago spp.), and the results of the first behaviour experiments accord with this view. (See chapter 4).

B Basic breeding methods.

The methods used for breeding P. meticulosa varied, depending on the particular type of experiment for which the

resulting larvae were to be used. I will, thus, confine myself here to explaining the methods used for rearing large numbers of larvae for genetic analysis and predation experiments. Other methods used, such as those in tests to determine the effect of various environmental factors, will be dealt with later in the introduction of the relevant section.

The original stocks used in this study were obtained from females captured in a standard 125 watt Robinson mercury-vapour light-trap. All these females were taken either in the grounds of the Zoology Department, Royal Holloway College, Englefield Green, Surrey, or at my home on Picket Hill, near Ringwood in Hampshire.

The females were placed in circular perspex boxes. These had a diameter of 105 mm and each was aerated by a muslin covered hole with a diameter of 30 mm in the lid. The females readily laid eggs in these boxes, either singly or in batches, attached to the sides, top or bottom, over the first few nights after capture. Normally the majority of eggs were laid during the first three nights, and no female continued laying after eight nights, even though some remained alive for up to two months if fed on a sugar solution. The only exception was when females were kept at temperatures below 6°C. In these conditions, the females would not lay at all until the temperature increased. Nine days after the first eggs were laid, a few leaves of common sorrel (R. acetosa L.) or sheep's sorrel (R. acetosella L.) were put in the box.

With broods used in the colour analysis and genetic experiments, as soon as larvae hatched they were transferred, using

a fine paint brush, to similar boxes containing sorrel. 25 larvae were put into each box. When larvae reached the 4th instar, they were transferred to oblong boxes 310 mm long, 155 mm wide and 85 mm high, with white sides and a perspex top. In the 5th instar a thin layer of soil and leaf litter was placed on the bottom of these boxes for larvae to pupate amongst. The larvae were fed on sorrel throughout, the foodplant being renewed every 12 to 48 hours depending on the temperature and humidity.

When significant colour changes occurred, so that a box contained larvae of two distinct colour-types, these were then separated so that reverse colour changes would be noticed if they occurred.

The boxes were kept in an insectary during the summer months and in a heated green-house during the winter so that the temperature rarely fell below 5°C.

Great care was taken to make sure that boxes were marked with precise information as to the origin and history of the larvae they contained, and that any discrepancies that occurred, due possibly to escape or to additional larvae being introduced with the foodplant, were recorded so that the results obtained were as reliable as possible.

When rearing larvae purely for predation experiments, various boxes, often of large dimensions, were used and up to 200 larvae were reared together. Otherwise, these larvae were reared in the same way and under the same conditions as those for genetic analysis. Again, larvae of different colour-types were always segregated and all observed larval colour changes were recorded.

After the larvae had pupated, the pupae were removed from their silken webs and those which were required for further breeding purposes were placed in small wood and muslin emergence cages. These were specially designed with partitions, so that all the pupae and resulting adults would remain separate. Those which were not needed were placed in large emergence cages and the resulting imagines were marked with cellulose paint and subsequently released, at least ten miles away from any sampling areas. Marking the released adults was necessary to enable a check to be kept on whether the gene pools of populations in sampling areas were being contaminated, at least in the short term, by released insects. At least five times the number of pupae likely to be needed, were kept segregated to guard against pupal mortality and to make sure that sufficient numbers of both males and females were acquired.

After emergence, the adult moths were put into cylindrical hanging cages of muslin, supported by a wire frame. They were put in the cages as soon after emergence as two suitable moths became available. The cages were hung outside when the minimum night temperature was expected to be above $8^{o}C$ and pairing usually took place on the first night. When the night temperature was below $8^{o}C$, mating rarely took place if the cages were hung outside. Unfortunately, the cages could not be simply hung inside in higher temperatures, as the moths will only be stimulated properly if an air current is present. This is because the pheromones from the female must be spread to attract the male, and then the pheromones from the male abdominal scent glands and brushes, which are

particularly well developed in P. meticulosa, must be distributed so that they stimulate the female to respond to him (Ford, 1955). Therefore, various arrangements using electric fires and fans were tried to induce matings in cold weather. The most successful method was to hang the cages at one end of a shed, and then place a strong electric fire at the other end, four metres away, with a thick wet towel hanging half a metre in front of it and an air fan beside the fire pointing towards the towel and cages. This kept the temperature of the shed at around 14°C and kept the air fairly humid. With this arrangement, a mating success rate of about 70% was achieved. After mating had taken place, females were transferred to the small circular perspex boxes for egglaying.

Care was taken at all stages to watch for disease in the stock, particularly the viral and bacterial diseases which attack larvae, and following Robinson's suggestion (1971), a second group of stocks were kept in isolation from the main stock to guard against the possibility of losing all of a particular brood or strain through a disease epidemic. The second set of stock were kept under conditions as similar as possible to those of the main stock. Tests to compare the effects of conditions on ten broods, which were split so that half of each brood was kept at each location, produced no significant differential variation in reaction to their environment.

(for 1st instar, $X^2_5 = 3.107$, $0.7 > p > 0.5$;
for 2nd instar, $X^2_5 = 5.261$, $0.5 > p > 0.3$;
for 3rd instar, $X^2_8 = 9.334$, $0.5 > p > 0.3$;
for 4th instar, $X^2_9 = 8.016$, $0.7 > p > 0.5$;
for 5th instar, $X^2_{10} = 9.811$, $0.5 > p > 0.3$.

The method used to colour score these larvae was the same as that used for the environmental temperature experiments, see chapter 3B).

Obviously, throughout the study, an accurate method of larval instar determination was needed. For this reason, a number of larvae were reared and the width of their head capsules and total body-length were measured periodically. The results of the mean head width and the mean body-length of larvae in each instar are given in Table 2Bi, together with the ratios of mean body-length between successive instars. The mean ratio of the width of the head capsules between successive instars is 1.615 which is slightly higher than the value predicted by Dyar's law (i.e. a ratio of 1.4)[1]. Similar calculations for body-length give a ratio of 1.628, which is considerably higher than the expected ratio, (i.e. 1.26), for Prizibram's rule. Throughout all experiments larval instar determination was based upon measurements of the width of the head capsule, body-length only being considered in doubtful cases.

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[1] In lepidopterous larvae the head width is, in fact, usually extremely variable, being more properly related to the weight of larvae at the end of the preceding instar (Long, 1953). This, with other observations, has led a number of authors, such as Beck (1950) to conclude that Dyar's progression factors have no fundamental basis.

Table 2Bi.

Mean head widths and body lengths with progression factors (i.e. ratio between successive instars).

Instar	Number of larvae measured	Width of head capsule (in mm)	Progression factor	Mean body length (in mm)	Body length limits (in mm)	Progression factor
First	94	0.48 ± 0.016		4.63 ± 0.711	2.70 - 5.41	
			1.54			1.58
Second	89	0.74 ± 0.017		7.30 ± 1.310	6.01 - 8.80	
			1.68			1.77
Third	87	1.24 ± 0.022		12.91 ± 2.211	11.22 - 15.20	
			1.55			1.63
Fourth	85	1.92 ± 0.043		21.07 ± 3.159	18.64 - 25.93	
			1.69			1.53
Fifth	81	3.24 ± 0.103		32.21 ± 4.213	28.34 - 39.97	
		mean progression	1.615		mean progression	1.628

C Colour Analysis.

The ground colour of P. meticulosa larvae varies from pale yellow, through ochre, green and olive to dark brown. Because of this, a constant and reliable method of scoring colour had to be developed, firstly, to discover whether the variation between individual larvae was continuous or discontinuous; if it were continuous, then it would be more likely that the variation is controlled either by a series of polygenes, or by environmental factors; if discontinuous, then control by one or a few major genes, producing a true genetic polymorphism in Ford's sense of the phrase (Ford,1940a), or by environmental factors, under the influence of a threshold effect, is indicated as more probable. Secondly, once the true nature of the variation had been determined, a constant method of scoring would be needed to investigate the frequency of the various forms in the wild, and for selection experiments to be carried out to give results which could be correlated with larval colour.

After careful consideration of the various techniques available, it was decided that simple visual scoring of larvae against a colour chart under constant conditions would be the easiest and most effective method to employ.

The techniques used by biologists to characterise colours of solid biological objects are very unsatisfactory, particularly when working in the field. There are various drawbacks and inaccuracies in all of these methods and I feel it would be worthwhile to outline some of those inherent in direct methods of colour matching, which have particular relevance to the system

used in this study.

i) As described in chapter 2A, the larvae of P. meticulosa are far from uniform in colour, having a pale dorsal and two pale lateral stripes, one each side of the body, being covered in minute dots, and having variable dark markings along the back and sides. To make an accurate visual comparison of such a composite object with a homogeneous surface, such as paint on paper, is strictly impossible. However, by selecting one small specific unpatterned area on the larvae for use, a comparison can be made, although the result is at best a crude approximation.

ii) The incidence of light falling on an object, and the direction from which the object is viewed, may cause apparent variation in the colour.

iii) To compare two coloured surfaces satisfactorily, it is necessary that these should lie in juxtaposition, without the presence of another coloured surface between them, and this is often difficult or impossible with any set of colour standards, such as the Munsell or Ridgeway colour charts, which are bound in book form. Furthermore, the appearance of any coloured surface, which is less than about 100 mm square will be liable to interference from the surrounding surfaces when viewed directly.

iv) Even with the extensive range of colours contained in the larger colour standard books, it is frequently impossible to exactly match a particular sample. This is because the human eye can detect far smaller differences in shade and hue than are portrayed in any of these books.

Sumner (1927) outlines the difficulties of direct visual colour-matching with reference to mammal pelages. He notes that some sort of quantitative method, whereby colour variations can be expressed in figures, is necessary for statistical studies and observes that this is impossible to accomplish through any system of matching standards.

Using an adaptation of Munsell's colour index system, I believe I have been able to produce a system which bypasses this problem so that colour can be expressed in figures, and different samples can be tested for homogeneity.

The method employed was to make up an extremely extensive colour chart, containing some 641 different colours. The chart was made up by mixing specific amounts of various green, yellow, brown, black and white paints. Each colour on the chart consisted of a 100 mm square patch of paint. 71 hues, which to avoid confusion will be referred to as the Mj hues, were produced by making up quantities of 15 paints, which were to me, exactly equivalent in colour to a specific value and chroma index of the 15 Munsell chart hues[1], between 10R and 5G inclusive, which are referred to as Munsell base colours. Four equally divided intermediates between each of the adjacent Munsell base colours were then produced. Thus, hue Mj1 is equivalent to Munsell's 10R4/8; hue Mj2 is a mixture of 4 parts 10R4/8 and 1 part 2.5YR4/8; hue Mj 3 is a mixture of 3 parts 10R4/8 and 2 parts 2.5YR4/8, until hue 6 is pure 2.5YR4/8 and so on through each of the other 13

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[1] In the Munsell colour system the hue of a colour indicates its relation to red, yellow, green, blue and purple; the value, its relative lightness or darkness; and the chroma its strength.

Munsell hues used. Nine "shades" were made of each of the 71 Mj hues by adding fixed amounts of black or white paints to each of these hues. Shade 0 is the undiluted Mj hue. Shade -1 is a mixture of 4 parts hue and 1 part black, similarly, shade -2, -3 and -4 involve mixtures of 4 parts hue and 2, 3 and 4 parts black paint, respectively. The shades +1, +2, +3 and +4 were made up in the same fashion with white in place of black, shade +4 being the palest. The final two colours in the chart were simply black and white. The hue, value and chroma index numbers of the 15 Munsell "base colours" used are given in Table 2Ci, together with the equivalent Majerus index numbers.

When referring to colours from the chart, these are written with the hue first followed by an oblique line and then the shade number. The hue type may be represented by either the Munsell notation or the Majerus number, depending on which is considered more appropriate. Thus, the darkest shade of the first hue may be written as either 10R/-4, if the Munsell notation is used for hue, or Mj 1/-4, if the Majerus hue number is used.

The choice of Munsell value and chroma indices for the colours on which the Majerus chart was based, was made with a view to ensuring that the colour variation exhibited by P. meticulosa larvae was covered as completely as possible, and no attempt was made to ensure that the differences between colours were consistent. If consistent differences had been required, then taking the same value and chroma indices for each of the 15 base colours may have produced more similar differences, however, picking Munsell colours in this way would have meant that the colour of many larvae would not have been represented on the chart. On the other hand, because

Table 2Ci.

The Value and Chroma indices of the 15 Munsell hues upon which the Majerus colour chart was based.

Munsell hue number	Value index	Chroma index	Equivalent Majerus colour
10R	4	8	Mj 1/0
2.5YR	4	8	Mj 6/0
5YR	4	6	Mj 11/0
7.5YR	5	8	Mj 16/0
10YR	6	10	Mj 21/0
2.5Y	8	12	Mj 26/0
5Y	8	12	Mj 31/0
7.5Y	8	10	Mj 36/0
10Y	8	12	Mj 41/0
2.5GY	7	8	Mj 46/0
5GY	7	10	Mj 51/0
7.5GY	7	10	Mj 56/0
10GY	6	10	Mj 61/0
2.5G	6	8	Mj 66/0
5G	5	8	Mj 71/0

the value and chroma indices of the base colours varied, the figures should not be supposed to give an accurate quantitative indication of one colour in relation to another, except when comparing shades of the same hue, or colours based on the same two Munsell base colours.

To reduce errors inherent in direct visual matching, all scoring was carried out under similar controlled conditions. The larva to be scored was gently rolled on a tissue to remove any moisture, which might cause light to be reflected or refracted abnormally. The larva was then placed on a small piece of perspex varying from 5 mm x 10 mm to 10 mm x 50 mm, depending on the size of the larva to be viewed. The perspex was attached to a wire which was held in a clamp, and was manoeuvred so as to be 1 mm above the colour chart. Two 100 watt lamps were situated, one on either side of the larva, shining down on it at an angle of 45 degrees to the vertical and one metre distant from the subject. One of the chart colours was then selected and the chart moved so that the subject was directly over the centre of the colour square. Other colours were then compared until a "match" was achieved. When matching, the larva was initially viewed as a whole to give a general impression, but for final scoring the dorsal surface on the left of the dorsal stripe of the 7th segment was used.

Even with the large number of colours in the chart, it was frequently impossible to exactly "match" a particular specimen, as its colour fell between two of the chart colours and in these cases, a closest approximation was given. When occasionally this could not be done, because the larval colour appeared to fall exactly

between two of the chart hues, or shades, it was assigned to the lower numbered hue or shade. When dealing with 1st, 2nd and 3rd instar larvae, a binocular microscope was used for viewing due to the difficulty of scoring such small larvae with the naked eye. This method could possibly cause anomalies in the scoring of 1st, 2nd and 3rd instar larvae, compared with the 4th and 5th instars, if light reflected from the larvae and the colour standards was differently affected by the microscope lenses. However, comparison of the scores given to the 5th instar larvae using the microscope and naked-eye methods showed that the two methods produced identical scores. As far as possible, larvae were scored for colour at least 24 hours after their previous ecdysis to ensure that the full pigmentation applicable to a particular instar had been attained.

Possibly, some sort of adaptation of an Ives tint photometer, similar to that used for mammal pelages by Sumner (1927), could be used effectively to score larval colour in the future. However, this type of method was rejected for this particular study as it was considered preferable to disturb larvae as little as possible, and obviously no method which might in any way damage the larvae could be considered. Also, it was decided that it would be profitable to use a simple method to ensure that a large number of larvae could be scored, rather than using a method which would severely limit the number of larvae which could be scored in a few hours.

CHAPTER 3 THE COLOUR VARIATION OF P. METICULOSA LARVAE AND ITS CONTROL.

The investigation into larval colour variation and its control was carried out in three sections. The first of these was designed to describe variation. The second tested whether various environmental factors affected larval colour, whilst the third investigated the possibility of larval colour being wholly or partly genetically controlled.

A. The variation in larval colour.

Methods

To investigate the type of variation which occurs in larvae of P. meticulosa, seventeen broods - referred to as the Colour Analysis Broods (CAB) 1 to 17 inclusive -, from light trapped females, taken in September, 1975 in Ringwood, were studied. 3,517 first instar larvae resulted from these broods, a number which had dropped to 3,245 by the 5th instar due to larval mortality. 1,000 of these larvae taken at random from the stock during each of the first four instars and 3,000 5th instar larvae were accurately scored for colour using the Majerus colour chart (see chapter 2C).[1]

In these and other tests, the method used to obtain a random sample of larvae from a larger sample involved taking a computed set of random numbers. Each set was appropriate to the size of the sample needed and the number of larvae being selected from. For instance, for a sample of 1,000 larvae from a total of 3,517, a set of 1,000 whole numbers were selected with the proviso

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[1] The rest of the larvae from the 17 broods were scored less accurately for use in allelic frequency estimates (see chapter 5).

that all were greater than zero and less than 3,518, and that no number was repeated. The 3,517 larvae were then counted and each time the number reached in the count was the same as one of the random numbers, the relevant larva was put on one side for use in the appropriate test.

Results and analysis of results.

The results from these five samples are shown in Tables 3Ai - v inclusive.

Looking at the results from the 3,000 5th instar larvae which were scored, it was obvious that the distribution was discontinuous, with larvae falling into six definite groups. These were named the main colour types Green (Gr), Olive (OL), Brown (Br), Plain Yellow (PY), Yellow Green (YG) and Yellow Brown (YB). On the basis of the results in Table 3Av, definite limits were assigned to each of these main colour types by taking the limits as two Mj hues numerically higher or lower than the maximum or minimum Mj hue number which was represented by larvae in each shade of a group. This treatment gave the following limits:-

Green (Gr) varying between shades -3 and +3 inclusive:

shade -3 varying from hue Mj 56 to Mj 61,
shade -2 varying from hue Mj 54 to Mj 64,
shade -1 varying from hue Mj 53 to Mj 66,
shade 0 varying from hue Mj 52 to Mj 68,
shade +1 varying from hue Mj 49 to Mj 67,
shade +2 varying from hue Mj 55 to Mj 70,
shade +3 varying from hue Mj 62 to Mj 66.

Table 3Ai.

Tests to determine the nature of larval colour variation.

First Instar Sample Colour Scores.

Munsell hue No.	Majerus (Mj) hue No.	Majerus Shade Number -4	-3	-2	-1	0	1	2
2.5GY	46							
	47			1				
	48		2		1	1		
	49			1	2			
	50		1	2		1		
5GY	51			1	1	3	2	
	52		1	2	2	7	3	
	53			3	4	6	2	
	54		1	8	14	17	3	
	55	1	2	15	24	28	4	1
7.5GY	56	3	5	23	38	33	8	1
	57	4	7	30	63	49	9	
	58		14	41	87	36	13	2
	59	2	9	51	55	26	4	
	60	2	9	24	29	18	3	
10GY	61	2	5	13	11	13	1	
	62	1	4	10	7	9	3	
	63		6	4	3	8	2	
	64		2	1	2	5		
	65		1	3	1	6	1	
2.5G	66		1	1	1		1	
	67			2		2		
	68			1	1	1		
	69							
	70							
5G	71							

Table 3Aii.

Tests to determine the nature of larval colour variation.

Second Instar Sample Colour Scores.

Munsell hue No.	Majerus (Mj) hue No.	Majerus Shade Number -4	-3	-2	-1	0	1	2
2.5GY	46			1		1		
	47							
	48			1	1	1		
	49		1		2	1	1	
	50			1	1	1	2	
5GY	51		2	1	2	1	3	
	52		1	2	5	4	7	
	53			7	10	10	6	
	54		2	3	14	13	6	1
	55		4	13	27	17	10	2
7.5GY	56		2	30	46	21	7	1
	57	1	2	41	59	39	5	
	58		1	43	83	47	3	
	59	1		31	55	32	1	
	60		2	25	44	26	2	
10GY	61			19	18	17	2	
	62		1	17	11	7		
	63	1	1	14	9	4	1	
	64		3	8	5	2		
	65		1	2	3			
2.5G	66			3	2	1	1	
	67		1	1	2	1		
	68				1		1	
	69			1		1		
	70							
5G	71							

Table 3Aiii.

Tests to determine the nature of larval colour variation.

Third Instar Sample Colour Scores.

Munsell hue No.	Majerus (Mj) hue No.	Majerus Shade Number -4	-3	-2	-1	0	1	2	3
2.5YR	6								
	7								
	8								
	9								
	10								1
5YR	11			1					
	12				1				
	13								
	14			1		1			
	15			1	3				
7.5YR	16		1	2	1				
	17		1	3	2				
	18	1			1				
	19			1					
	20			1					
10YR	21				1				
	22								
	23								
	24								
	25								
2.5Y	26								
	27								
	28								
	29								
	30								
5Y	31								
	32							1	
	33								
	34						1	2	
	35							1	
7.5Y	36								
	37								
	38								
	39								
	40		1	2					
10Y	41		1		1				
	42		2	3					
	43			7	2				
	44		4	10	2				
	45		7	14	3				1
2.5GY	46		9	9	4				
	47		3	5	2				1
	48			3					
	49		3	1					
	50								

Table 3Aiii. (Continued)

Tests to determine the nature of larval colour variation.

Third Instar Sample Colour Scores.

Munsell hue No.	Majerus (Mj) hue No.	Majerus Shade Number -4	-3	-2	-1	0	1	2	3
5GY	51				1	1			
	52								
	53			1	2	1	1		
	54		1	1	2	2			
	55			3	10	4	2	1	
7.5GY	56			14	16	7	2		
	57	1	2	26	33	10	2		
	58	4	2	64	96	37	6	1	
	59	2	7	61	71	51	10	3	
	60		13	32	47	30	7	2	
10GY	61	2	4	22	32	15	11		
	62		2	14	9	10	4		
	63			6	6	8	3	1	
	64			2	4	3	1		
	65		1	1	2	1	2	1	
2.5G	66					2	1		
	67		1	1	2		1		
	68				1	1	1		
	69								
	70								
5G	71								

Table 3Aiv.

Tests to determine the nature of larval colour variation.

Fourth Instar Sample Colour Scores.

Munsell hue No.	Majerus (Mj) hue No.	Majerus Shade Number -4	-3	-2	-1	0	1	2	3
2.5YR	6								
	7		1						
	8			1					
	9	1	2		1	1			1
	10	1	1	1					
5YR	11		1	2		2			1
	12			1	3				
	13	1	1	4	1				
	14	1	1	3					
	15		4	6					
7.5YR	16	1	1	6	1				
	17		4	7					
	18		6	9					
	19		3	2					
	20	1		1					
10YR	21		2	3					
	22		1	1					
	23	1	1						
	24		1						
	25		1						
2.5Y	26		1	1					
	27	1							
	28								
	29		2						
	30	1							
5Y	31								
	32							1	
	33						2	2	1
	34	1						3	
	35						1	2	1
7.5Y	36						1		
	37						1	1	
	38								
	39								
	40		1	1					
10Y	41			3	2				
	42		1	7	5				
	43	1	8	19	8				
	44		21	28	11				
	45	2	30	35	16	1			
2.5GY	46	5	43	51	12				1
	47	4	21	38	3				
	48	2	16	21	1				1
	49	1	7	6				1	1
	50		2	1					1

Table 3Aiv. (Continued)

Tests to determine the nature of larval colour variation.

Fourth Instar Sample Colour Scores.

Munsell hue No.	Majerus (Mj) hue No.	Majerus Shade Number -4	-3	-2	-1	0	1	2	3
5GY	51		1						
	52						1		
	53					1	1		
	54					3	3		
	55				1	7	4	1	
7.5GY	56				4	14	5	1	
	57			1	11	24	14	2	
	58			5	24	42	10	2	
	59		1	3	47	27	8	3	
	60			7	30	21	4		
10GY	61		1	8	17	22	3	1	
	62		1	4	10	4	4		
	63		2	3	4	4	2		
	64		2	4	2	3	1		
	65		1	2	1	1	1		
2.5G	66			1	1				
	67								
	68								
	69								
	70								
5G	71								

Table 3Av.

Tests to determine the nature of larval colour variation.

Fifth Instar Sample Colour Scores.

Munsell hue No.	Majerus (Mj) hue No.	Majerus Shade Number -4	-3	-2	-1	0	1	2	3	4
10R	1									
	2									
	3									
	4				1					
	5		1	1						
2.5YR	6		1	1	2					
	7	2	7	5	14	2			1	
	8	2	9	15	19	3		2	2	
	9	3	8	17	10	5		3	3	
	10	4	13	20	16	4			3	
5YR	11	4	22	29	12	6		1	1	
	12	6	30	39	16	7		1		
	13	13	37	43	15	6			1	
	14	12	39	49	18	2				
	15	16	62	58	19	4				
7.5YR	16	17	84	82	24	2				
	17	21	80	75	22	1				
	18	15	86	61	17					
	19	8	61	50	11					
	20	7	42	28	4					
10YR	21	9	37	19	3					
	22	5	34	17	2					
	23	6	14	6						
	24	8	11	3						
	25	4	2	2						
2.5Y	26	6	4							
	27	9	5							
	28	10	4					1		
	29	8	3							
	30	6	1				1	1	2	
5Y	31	4	2				1	2		
	32	2	1				2	4	1	1
	33	4				1	2	3	1	
	34	2	1			1	3	3		
	35	1					1	2	1	
7.5Y	36	1					2	1		
	37									
	38									
	39				1					
	40			2						
10Y	41		4	7	3					
	42		13	19	8					
	43		25	25	10				1	
	44	1	33	39	14	1				
	45	6	42	51	17			1	2	

Table 3Av. (Continued)

Tests to determine the nature of larval colour variation.

Fifth Instar Sample Colour Scores.

Munsell hue No.	Majerus (Mj) hue No.	Majerus Shade Number -4	-3	-2	-1	0	1	2	3	4
2.5GY	46	4	36	46	11			2	2	
	47	2	37	22	3			1	2	
	48	2	19	17					1	
	49		6	3					1	
	50		2	1						
5GY	51						2			
	52						4			
	53						9			
	54					2	7			
	55				3	4	11			
7.5GY	56			2	14	12	13			
	57			9	27	21	19	1		
	58		1	13	44	21	19	4		
	59		2	13	43	33	15	2		
	60			4	27	26	12	2		
10GY	61			2	11	28	10	6		
	62			1	4	23	7	2		
	63				4	14	7	4		
	64				1	10	3	3	1	
	65					6	1	1		
2.5G	66					4				
	67									
	68							1		
	69									
	70									
5G	71									

Olive (OL) varying between shades -4 and 0 inclusive:

shade -4 varying from hue Mj 42 to Mj 50,
shade -3 varying from hue Mj 39 to Mj 52,
shade -2 varying from hue Mj 38 to Mj 52,
shade -1 varying from hue Mj 37 to Mj 49,
shade 0 varying from hue Mj 42 to Mj 46.

Brown (Br) varying between shades -4 and 0 inclusive:

shade -4 varying from hue Mj 5 to Mj 38,
shade -3 varying from hue Mj 3 to Mj 36,
shade -2 varying from hue Mj 3 to Mj 27,
shade -1 varying from hue Mj 2 to Mj 24,
shade 0 varying from hue Mj 5 to Mj 19.

Plain Yellow (PY) varying between shades 0 and +4 inclusive:

shade 0 varying from hue Mj 31 to Mj 36,
shade +1 varying from hue Mj 28 to Mj 38,
shade +2 varying from hue Mj 26 to Mj 38,
shade +3 varying from hue Mj 28 to Mj 37,
shade +4 varying from hue Mj 30 to Mj 34.

Yellow Green (YG) varying between shades +2 and +3 inclusive:

shade +2 varying from hue Mj 43 to Mj 49,
shade +3 varying from hue Mj 41 to Mj 51.

Yellow Brown (YB) varying between shades +2 and +3 inclusive:

shade +2 varying from hue Mj 6 to Mj 14,
shade +3 varying from hue Mj 5 to Mj 15.

The results from 4th instar larvae (Table 3Aiv) again show a discontinuous distribution with six groups, and all larvae, apart from a few of the green colour, fall within the defined limits of the six main colour types. Indeed, the distributions for 4th and 5th instar larval colour are very similar when compared qualatively. The main differences are the slightly closer limits that would be assigned to the main colour types if limits were defined from 4th instar results in the same way as for 5th instar larvae. This difference is probably due, at least in part, to

the smaller size of the 4th instar sample. However, if the results are compared quantitatively quite obvious differences are evident. The proportion of the four main colour types Gr, OL, PY and YG are all higher in the 4th instar sample, whilst those of the other two main colour types, Br and YB, are much higher in the 5th instar sample.

To cater for the few 4th instar larval scores which fell close to, but outside the 5th instar Gr colour type, the limits of this group were extended to cover colours Mj 56/-3 to Mj 67/-3 inclusive, and Mj 54/-2 to Mj 68/-2 inclusive.

When the 3rd instar results (Table 3Aiii) were studied, again six groups were evident; however, not all larvae fell within the limits of the six main colour types defined from the 5th instar results. Five of the groups fell within the confines of the OL, Br, PY, YG and YB main colour type limits, but,whilst the limits of the sixth group approximated to the Gr main colour type limits, a few larvae had scores which fell close to, but outside these limits. Therefore, limits were set for 3rd instar green larvae in the same way as the limits for the main colour types were defined, so that the 3rd instar green colour type (3IG) was defined as:-

varying between shades -4 and +2 inclusive:

shade -4 varying from hue Mj 55 to Mj 63,
shade -3 varying from hue Mj 52 to Mj 69,
shade -2 varying from hue Mj 53[1] to Mj 69,
shade -1 varying from hue Mj 50[1] to Mj 70,
shade 0 varying from hue Mj 49 to Mj 70,
shade +1 varying from hue Mj 51 to Mj 70,
shade +2 varying from hue Mj 53 to Mj 67.

The frequency of larvae in the six colour groups was also quite different from that of 4th instar larvae, more larvae being assigned to the 3IG colour type than the Gr main colour type, and less to the five other main colour types than in the 4th instar.

The distribution of colour scores for 1st and 2nd instar larvae (Tables 3Ai and 3Aii respectively) is radically different from those of the later instars. The distribution is continuous, with only one group. This group has a similar distribution to the Gr main colour type, but with slightly wider limits. Indeed, whilst the distribution is similar in shape to that of the Gr and 3IG colour types, and might be considered to approximate a "three-dimensional normal distribution", some of the peripheral scores fall within the OL main colour type limits.

Limits were set for this distribution, which was called the early green colour type (EG), using 1st instar scores as the

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[1] The lower hue values for shades -2 and -1 should strictly speaking be Mj 51 and Mj 49 respectively, but these two values fall just inside the OL main colour type limits. Therefore, the 3IG limits were adjusted so as not to overlap with those of the OL limits whilst still containing all the larvae scored in the 3IG group. As it happened, no 3rd, 4th or 5th instar larvae were scored either Mj 52/-2, Mj 51/-2 or Mj 49/-1, at any time.

distribution was slightly wider in the 1st instar. These limits vary between shades -4 and +2 inclusive:

shade -4 varying from hue Mj 53 to Mj 64,
shade -3 varying from hue Mj 46 to Mj 68,
shade -2 varying from hue Mj 45 to Mj 70,
shade -1 varying from hue Mj 46 to Mj 70,
shade 0 varying from hue Mj 46 to Mj 70,
shade +1 varying from hue Mj 49 to Mj 68,
shade +2 varying from hue Mj 53 to Mj 60.

All 2nd instar scores fall within these limits.

Therefore, it appears that the system follows a pattern in which 1st and 2nd instar larvae are of a green colour which varies considerably in both shade and hue. A radical change takes place between instars two and three when five more completely distinct colour types arise, and of the remaining larvae, some fall close to, but outside, the early green colour type. Further changes in the frequency of larvae assigned to the various colour types coincide with the 3rd and 4th larval ecdyses, and the variability of the green colour type is reduced at the 3rd larval ecdysis.

This system indicates that the 1st and 2nd instar larval colour variation is continuous and so will probably be controlled either environmentally, or by a polygenic hereditary system. On the other hand, 3rd, 4th and 5th instar larval colour variation is discontinuous and is more likely to be controlled by environmental factors under the influence of a threshold switch effect or by a small number of major genes.

B. The effect of environment on larval colour variation.

It was decided that the investigation into larval colour

control should be tackled in two stages; the first being an investigation into the effect of various environmental factors, and the second involving a series of breeding experiments to test for genetic control.

The effect of four main groups of environmental factors were studied. These were temperature, larval density, light and foodplant. Except for some of the samples in the foodplant experiments, larvae for all these tests came from a stock of about 9,000 larvae from 183 broods which were the result of random matings between imagines from the CAB1 - CAB17 broods. 50 eggs were taken at random from each of the broods and mixed together before being re-split at random into samples of 25 eggs, (in the overcrowding experiments the number of larvae in samples varied). Each series of experiments involved special rearing conditions and so the methods used in conjunction with the four groups of environmental factors will be dealt with individually. (Plates 1 - 4 show some of the larval colour types).

(a) The effect of temperature.

Materials and methods.

For the temperature experiments, larval samples were reared in Fisons 50 G2 Mk III Growth Cabinets, each of which was illuminated by four interior 18 inch warm white 15 watt Cryselco bulbs for 12 hours a day from 6 a.m. to 6 p.m. and unlit for the rest of the time.

15 samples of 25 eggs were taken from the stock. These were named the "environmental temperature samples 1 - 10" (ET1 - 10) and the "environmental temperature reserve samples 1 - 5" (ETR1 - 5). Three of these samples (ET1, ET2 and ETR1)

Plate 1

(a) 1st instar larvae, (approximately 18 hours old) x 4. (Foodplant:Ribwort Plantain (Plantago lanceolata))

(b) 1st instar larvae, (left, 5 days old; right 2 days old). x 7. (Foodplant : Common Sorrel (Rumex acetosa))

Plate 2

(a) 2nd instar larvae. x 10
(Foodplant : Common Sorrel)

(b) 5th instar Green (Gr) larva. x 2.5
(Foodplant : Clustered Dock (Rumex obtusifolius))

Plate 3

(a) 5th instar Olive (OL) larva. x 2.5
(Foodplant : Clustered Dock).

(b) 5th instar Brown (Br) larva. x 2.5
(Foodplant : Clustered Dock).

Plate 4

(a) 5th instar Yellow-green (YG) larva. x 2.5
(Foodplant : Clustered Dock).

(b) 5th instar Yellow-brown (YB) larva. x 2.5

were kept at a low constant temperature of 4 ± 1°C. Three semples (ET3, ET4 and ETR2) were kept at a high constant temperature of 25 ± 1°C, and a further three (ET5, ET6 and ETR3) at a constant temperature of 14.5 ± 1°C. Of the remaining six samples, three (ET7, ET8 and ETR4) were kept at a temperature ranging between 4 ± 1°C and 12 ± 1°C where the cabinet thermostat was set at 12°C between 6 a.m. and 6 p.m., and at 4°C between 6 p.m. and 6 a.m. The final samples (ET9, ET10 and ETR5) were kept at a temperature ranging in a similar way, from 17 ± 1°C from 6 p.m. to 6 a.m., to 25 ± 1°C between 6 a.m. and 6 p.m.

Only the samples, ET1 - ET10 inclusive, were actually scored for colour, samples ETR1 - ETR5 inclusive, being reared simply as reserves, so that if any larvae in the ET samples died, they could be replaced with larvae taken at random from another sample which had been reared under the same conditions.

Results.

The colour scores given to 1st and 2nd instar larvae in these experiments are given in a simplified form in Appendix Table 3Bai. In this table, groups of five Mj hues are classed together in such a way that five hue groups, 2.5GY to 2.5G inclusive, result. Hues Mj 44 to Mj 68 fall into these five hue groups so that hues Mj 44 to Mj 48 are classed as 2.5GY, hues Mj 49 to Mj 53 as 5GY, and so on.

To compare the results statistically, the results of each pair of ET samples kept under the same set of conditions (e.g. samples ET1 and ET2) were added together, as it could be seen by inspection that samples kept under similar conditions did not

Table 3Bai.

Experiments to investigate the effect of temperature on larval colour. Colour analysis scores of samples ET1 - 10 inclusive, adjusted for statistical analysis. All instars.

Instar	Hue type	Colour type	ET1 and ET2 samples				ET3 and 4 samples				ET5 and 6 samples				ET7 and 8 samples				ET9 and 10 samples				Total			
First			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		8	12	12		9	11	11		9	12	10		8	12	12		9	12	12		43	59	57
	10GY	EG		7	6	5		7	6	6		9	5	5		8	5	5		6	6	5		37	28	26
Second			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		7	14	11		8	12	11		9	11	11		8	12	10		8	11	12		40	60	55
	10GY	EG		6	7	5		5	8	6		8	6	5		7	7	6		7	8	4		33	36	26
Third		Br	1				1				1				2				1				6			
		Y	0				0				0				0				1				1			
			shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
	2.5GY	OL		1	4			2	3			1	4			2	3			1	4			7	18	
			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	3IG		6	8	4		7	6	5		6	8	3		7	8	4		5	8	3		31	38	19
	10GY	3IG		8	9	9		8	10	8		9	8	10		7	9	8		10	10	7		42	46	42
Fourth		Br	5				5				5				5				6				26			
		Y	1				1				2				0				1				5			
			shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
	2.5GY	OL		8	14			9	14			8	15			8	13			8	15			41	71	
			shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1
	7.5GY	Gr		2	5	2		3	4	2		2	5	1		2	5	3		2	4	3		11	23	11
	10GY	Gr		8	4	1		7	4	1		7	3	2		9	5	0		7	4	0		38	20	4
Fifth			shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1
	7.5YR	Br		12	8	5		9	9	4		11	8	3		10	10	5		11	10	4		53	45	21
	10YR	Br		5	2	X		4	3	X		5	3	X		4	1	X		7	1	X		25	10	X
		Y	1				2				0				1				0				4			
			shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
	2.5GY	OL		2	6			5	2			4	5			3	5			4	4			18	22	
			shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1
	10GY	Gr		4	3	2		6	3	3		3	4	4		5	4	2		4	3	2		22	17	13

differ significantly. Then, low scores for peripheral score groups, which were represented with irregular frequency, were added to the score of the nearest group which was represented fairly consistently, a shade of the same hue being considered closer than a hue of the same shade. This method resulted in six score groups centred on 7.5GY/-2, 7.5GY/-1, 7.5GY/0, 10GY/-2, 10GY/-1 and 10GY/0, and the results of this treatment are shown in Table 3Bai.[1]

3rd instar results (Appendix Table 3Baii) were also treated in this way, but with a number of additional score groups included to cater for the larvae which fell outside the confines of the early green limits in the colour analysis tests. This produced the same six green score groups as in the statistics for 1st and 2nd instar scores, together with 2 groups, 2.5GY/-3 and 2.5GY/-2, from the olive main colour type limits, and four score groups bounded by the Br, PY, YB or YG main colour type limits. In fact, yellow groups were not considered in the statistics, as only one 3rd instar PY larva occurred. In this, and other statistical tests on environmental factors, yellow score groups were classed together under the notation Y, as relatively small numbers were involved

The same score groups were used for 4th instar results (Appendix Table 3Baiii), except that the green score groups were adapted to cater for the Gr main colour type distribution, rather than the EG colour type, thereby giving six green score

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[1] Results of 3rd, 4th and 5th instar colour scores treated for statistical analysis are also given in Table 3Bai.

groups for the statistics, centred on 7.5GY/-1, 7.5GY/0, 7.5GY/+1, 10GY/-1, 10GY/0 and 10GY/+1.

5th instar results (Appendix Table 3Baiii) were treated in the same way as 4th instar results, except that the number of green score groups was reduced to three for statistical purposes, these being centred on 10GY/-1, 10GY/0 and 10GY/+1, and the brown main colour type was split into five score groups, centred on 5YR/-3, 5YR/-2, 5YR/-1, 7.5YR/-3 and 7.5YR/-2.

A Chi-squared test comparing the results of ET1 - ET10 inclusive, showed that there was no significant difference between the samples in any of the instars,

(for 1st instar scores, $X^2_{20} = 1.559$, $p > 0.99$;
for 2nd instar scores, $X^2_{20} = 2.533$, $p > 0.99$;
for 3rd instar scores, $X^2_{36} = 4.272$, $p > 0.99$;
for 4th instar scores, $X^2_{36} = 8.767$, $p > 0.99$;
for 5th instar scores, $X^2_{40} = 13.603$, $p > 0.99$)

Similar tests, comparing the sum totals of the ten ET samples with the results from the colour analysis broods, CAB1 to CAB17 inclusive, for each instar, were carried out to check that the conditions of rearing, using the Fisons growth cabinets did not affect the colour of the larvae significantly, when compared with larvae reared in the insectary. For this the colour analysis scores were assigned to score groups in the same way as the ET sample results. The results of this treatment are given in Table 3Baii. Again, there is no significant difference in the scores being tested,

Table 3Baii.

Tests to determine the nature of larval colour variation. Colour analysis scores adjusted for comparison with the results of environmental experiments. All instars.

Instar	Hue type	Colour type				
First			shade	-2	-1	0
	7.5GY	EG		168	236	229
	10GY	EG		154	110	103
Second			shade	-2	-1	0
	7.5GY	EG		159	250	210
	10GY	EG		132	150	99
Third		Br	23			
		Y	8			
			shade	-3	-2	
	2.5GY	OL		31	68	
			shade	-2	-1	0
	7.5GY	3IG		119	160	77
	10GY	3IG		171	174	169
Fourth		Br	101			
		Y	23			
			shade	-3	-2	
	2.5GY	OL		166	269	
			shade	-2	-1	0
	7.5GY	Gr		46	91	44
	10GY	Gr		151	82	27
Fifth			shade	-3	-2	-1
	7.5 YR	Br		594	495	247
	10YR	Br		322	145	X
		Y	68			
			shade	-3	-2	
	2.5GY	OL		232	300	
			shade	-1	0	1
	10GY	Gr		225	204	166

(for 1st instar scores, $X^2_5 = 0.076$, $p > 0.99$;
for 2nd instar scores, $X^2_5 = 0.275$, $p > 0.99$;
for 3rd instar scores, $X^2_9 = 0.680$, $p > 0.99$;
for 4th instar scores, $X^2_9 = 1.172$, $p > 0.99$;
for 5th instar scores, $X^2_{10} = 2.427$, $p > 0.99$)

Discussion.

Apart from showing that the special rearing conditions did not affect colour variation, the homogeneity between the colour analysis scores and the summed ET sample scores, indicated by the results of the chi-squared tests comparing the two, imply that the method used to select random samples was effective.

Because of the homogeneity of the ET sample scores, it may be concluded that temperature control of larval colour variability was highly improbable. It was also noted that the rate of growth of the ET samples was very variable. The maximum length of the larval stage in ET1 was 28 weeks 2 days, in ET3 - 3 weeks 4 days, in ET5 - 9 weeks 2 days, in ET7 - 14 weeks 1 day, and in ET9 - 4 weeks 6 days. Thus, these experiments also indicate that the larval colour variation under review is not influenced by rate of growth, at least when this is temperature controlled.

(b) The effect of larval density.

To study the effect of overcrowding on larval colour variation, 51 boxes with varying numbers of larvae were placed in Fisons growth cabinets, set at a temperature of $8 \pm 1^{\circ}C$, between the hours of 6 p.m. and 6 a.m., when the cabinets were unlit, and at a temperature of $16 \pm 1^{\circ}C$ between 6 a.m. and 6 p.m.

s:-

ADDENDUM TO PAGE 69. Insert before Discussion.

One major criticism of this analysis and similar chi-squared tests in this section on the effects of environment on larval colour is that the expected frequencies for some of the classes with expected values of less than 5 are removed by adding them to another class. In the data under consideration, if this course were to be taken, difficulties arise relating to which classes should be totalled. For example, in the ET 3rd instar data, to obtain expected values greater than 5, all non-green larvae would have to be added together. This would obscure much of the major discontinuity in the colour score analysis. Undoubtedly, the best way to overcome these problems would be to repeat the experiments with larger samples, so that the expected values of all classes were greater than 5. However, to achieve this aim, the number of larvae used would have to be approximately 25 times greater than that under consideration in this data.

The only other reasonable course is to re-assess the data classing small groups together, in realization that much of the colour variation will thus be obscured. If this course were to be taken, it is suggested that the classes are adapted along the following lines:-

1st instar classes - Unchanged

2nd instar classes - Unchanged

3rd instar classes - Green classes unchanged except that 7.5GY/0 be classed with 7.5GY/-1. Br, Y, 2.5GY/-3 and 2.5GY/-2, should be classed together as non-green.

4th instar classes - Green classes reduced to three, these being centred upon 7.5GY/0, 10GY/-1 and 10GY/0. Olive classes unchanged, and Yellow and Brown larvae being classed together to produce the sixth class.

5th instar classes - Green classes reduced to one. Olive classes reduced to one and including Yellow larvae. Brown classes reduced to three, these being centred on 7.5YR/-3, 7.5YR/-2 and 10YR/-3.

To give an example of the way these adaptations would affect the results of the chi-squared tests, comparing the results of samples ET1 - 10 inclusive, using the adapted classes gave the following results:-

for 3rd instar larvae, $X^2_{20} = 2.287$, p 0.99;

for 4th instar larvae, $X^2_{16} = 1.445$, p 0.99;

for 5th instar larvae, $X^2_{16} = 2.780$, p 0.99.

The probability values for these tests are the same as those from comparable chi-squared tests using the original classes (see page 67).

when the cabinets were illuminated by four 18 inch, 15 watt bulbs. The boxes used were the same as those described in chapter 2, small round boxes being used for 1st, 2nd and 3rd instar larvae, and the larger rectangular boxes being used for 4th and 5th instar larvae.

Of the 51 samples, 19 were kept simply as reserves, so that if larvae died in the other 32 test samples to be scored, they could be replaced with larvae which had been treated in the same way. The samples were named the "environment overcrowding samples" (EO) and the "environment overcrowding reserve samples" (EOR). 30 samples, (EO1 - EO20 inclusive and EOR1 - EOR10 inclusive), had only one larva each. 6 samples (EO21 - EO24 inclusive, EOR11 and EOR12) each consisted of 5 larvae. 3 samples (EO25, EO26 and EOR13) had 10 larvae, and then 2 samples had each of the following number of larvae - 20 (EO27 and EOR14), 40 (EO28 and EOR15), 60 (EO29 and EOR16), 80 (EO30 and EOR17), 100 (EO31 and EOR18), and 200 (EO32 and EOR19).

Results.

The scoring of the EO samples was based on the score groups used in the temperature experiments. The results of the three sets of samples, EO1 to EO20, EO21 to EO24, and EO25 and EO26, were added together to give three sets of results each with 20 larvae. The results are given in Appendix Tables 3Bbi - iii inclusive.

For statistical analysis, the results were then treated in the same way as the temperature results, so that low scores from peripheral score groups were added to the score of the most similar colour score group. The brown values in the 3rd instar

were included with the 2.5GY shade -3 scores in the 3rd instar, and the yellow values were included with the 2.5GY shade -2 values in the 3rd, 4th and 5th instars. The results of this treatment are given in Table 3Bbi.

Chi-squared tests between the resulting scores of samples in each instar gave the following results,

(for 1st instar scores, $X^2_{40} = 3.5$, $p > 0.99$;
for 2nd instar scores, $X^2_{40} = 3.729$, $p > 0.99$;
for 3rd instar scores, $X^2_{72} = 3.923$, $p > 0.99$;
for 4th instar scores, $X^2_{72} = 8.46$, $p > 0.99$;
for 5th instar scores, $X^2_{80} = 9.402$, $p > 0.99$)

This shows that the samples are homogeneous for all five instars.

A further test comparing the sum score of all the EO samples and the results from the colour analysis broods, CAB1 to CAB17 inclusive, for each instar, showed that the scores being tested were homogeneous for all five instars,

(for 1st instar scores, $X^2_5 = 3.334$, $0.7 > p > 0.5$;
for 2nd instar scores, $X^2_5 = 1.396$, $0.95 > p > 0.9$;
for 3rd instar scores, $X^2_9 = 1.118$, $p > 0.99$;
for 4th instar scores, $X^2_9 = 0.992$, $p > 0.99$;
for 5th instar scores, $X^2_{10} = 1.821$, $p > 0.99$)

Discussion.

The results of the chi-squared tests indicate that on the basis of the tests carried out, larval colour variation is not affected by larval density. This result is perhaps a little surprising in the light of Long's experiments on the effects of population density on Lepidoptera larval colour, in which four of the five species of Hadeninae, a sub-family closely related to the

Table 3Bti.

Experiments to investigate the effect of larval density on colour variation. Colour analysis scores of samples E01 - 32 inclusive, adjusted for statistical analysis. All instars.

Instar	Hue type	Colour type	E01 - 20 inclusive				E021 - 24 inclusive			
First			shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		3	5	4		3	5	4
	10GY	EG		4	2	2		3	3	2
Second			shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		3	5	4		3	6	4
	10GY	EG		3	3	2		3	2	2
Third		Br	0				0			
		Y	0				0			
	2.5GY	OL	shade	-3	-2		shade	-3	-2	
				1	1			1	1	
	7.5GY	3IG	shade	-2	-1	0	shade	-2	-1	0
	10GY	3IG		2	3	2		2	4	1
				4	3	4		4	4	3
Fourth		Br	2				2			
		Y	1				0			
	2.5GY	OL	shade	-3	-2		shade	-3	-2	
				3	5			3	5	
	7.5GY	Gr	shade	-1	0	1	shade	-1	0	1
	10GY	Gr		1	2	1		1	2	1
				4	1	0		3	2	1
Fifth			shade	-3	-2	-1	shade	-3	-2	-1
	7.5YR	Br		4	2	2		3	3	2
	10YR	Br		2	1	X		3	1	X
		Y	1				0			
	2.5GY	OL	shade	-3	-2		shade	-3	-2	
				1	2			2	2	
	10GY	Gr	shade	-1	0	1	shade	-1	0	1
				2	1	1		2	1	1

Instar	Hue type	Colour type	E025 - 26 inclusive				E027				E028				E029			
First			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		3	5	5		2	5	5		6	11	8		10	12	14
	10GY	EG		3	2	2		4	2	2		8	4	3		11	7	6
Second			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		4	5	4		3	5	4		6	11	8		9	16	12
	10GY	EG		2	3	2		3	4	1		5	7	3		8	10	5
Third		Br	1				1				1				1			
		Y	0				0				0				0			
	2.5GY	OL	shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
				0	2			1	2			1	3			2	4	
	7.5GY	3IG	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	10GY	3IG		2	3	2		2	3	1		4	6	3		6	11	4
				3	4	3		3	3	4		7	7	8		10	11	11
Fourth		Br	2				3				5				6			
		Y	0				0				1				1			
	2.5GY	OL	shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
				3	6			4	4			6	11			10	17	
	7.5GY	Gr	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	10GY	Gr		1	2	1		0	2	1		2	4	1		3	6	2
				2	2	1		4	2	0		6	3	1		10	4	1
Fifth			shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1
	7.5YR	Br		4	4	2		3	4	1		8	7	4		13	10	6
	10YR	Br		2	1	X		3	1	X		4	1	X		6	2	X
		Y	0				0				1				1			
	2.5GY	OL	shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
				2	1			1	3			3	4			5	5	
	10GY	Gr	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1
				1	2	1		2	1	1		3	2	3		5	4	3

Instar	Hue type	Colour type	E030				E031				E032				Total (sum of all E0 samples)			
First			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		12	18	16		17	22	20		32	45	45		88	128	121
	10GY	EG		15	10	9		20	10	11		38	20	20		106	60	57
Second			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		10	23	17		15	26	21		30	50	45		83	147	119
	10GY	EG		11	13	6		12	17	9		24	32	19		71	91	49
Third		Br	2				2				5				13			
		Y	1				1				1				3			
	2.5GY	OL	shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
				3	5			3	7			6	15			18	40	
	7.5GY	3IG	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	10GY	3IG		8	13	6		12	16	8		21	31	16		59	90	43
				13	15	14		17	17	17		33	36	36		94	100	100
Fourth		Br	9				10				22				61			
		Y	2				1				6				12			
	2.5GY	OL	shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
				13	21			18	27			30	54			90	151	
	7.5GY	Gr	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	10GY	Gr		4	8	3		4	10	5		9	19	10		25	55	24
				12	6	2		15	8	2		28	18	4		84	46	12
Fifth			shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1
	7.5YR	Br		15	13	7		21	17	8		39	32	18		110	93	50
	10YR	Br		8	4	X		10	5	X		19	9	X		57	25	X
		Y	2				1				6				12			
	2.5GY	OL	shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
				7	7			9	10			17	21			47	55	
	10GY	Gr	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1
				7	6	4		9	7	3		16	13	10		47	37	27

Amphipyrinae, tested, showed an appreciable colour response to overcrowding in their later instars (Long, 1953). It is perhaps unfortunate that Long only carried out an investigation into the possibility of different genetic strains controlling the response on one species of moth. This species was Autographa gamma, which is a member of the Plusiinae and not so closely related to the Amphipyrids. Although his tests were inconclusive, there was some indication that some genotypes do show a variability in response. Possibly colour variation in P. meticulosa larvae is not affected by larval density, because in the later instars of this species the colours of larvae are genetically controlled, (see chapter 3C). On the other hand, if as Fox and Vevers (1960) speculate, the darkening in coloration caused by overcrowding in other species is the result of increased metabolic rate due to mutual disturbance, then, one might expect high larval density to affect the genetically controlled colours to some extent, which clearly is not the case in P. meticulosa. In any case, it must be remembered that Long (1953) noted that increased larval activity was not always associated with colour change, Pieris brassicae showing no response.

One other point should be noted, Long indicates that the darkening in coloration that he observed was in some cases due to melanization of the cuticle. The method of colour scoring employed in the experiments on P. meticulosa was specifically designed to give an indication of the ground colour of the larvae, and not the colour or extent of the stripes, dots and v-shaped

marks that occur. However, despite this, it may be said that no correlation between larval density and extent of larval markings was observed, although controlled experiments in which some method of quantifying the extent of the larval pattern would have to be carried out before this could be confirmed.

c. The effect of light.

As mentioned in chapter 1, Poulton and other workers have noted that larval and pupal colour of some Lepidoptera may be influenced by the colour of the larvae surroundings, or by the conditions of light at certain critical periods in their development. This seems to be particularly true in the case of many of the so-called "stick caterpillars", (Poulton 1885, 1886, 1887, 1892, 1893; Gould, 1892).

Larvae of P. meticulosa are not of the "stick" type, being, like the majority of Noctuid larvae, eruciform. The investigation into the effect of various "light" conditions on P. meticulosa thus seemed particularly pertinent, as it would not only serve to aid the investigation into the control of P. meticulosa larval colour variation as a whole, but might also give an indication as to whether Noctuid larvae of the normal type are affected by their surroundings in the same way as many "stick" caterpillars. It should be mentioned here, that the few observations of Poulton and Gould on eruciform Noctuid larvae (e.g. Mamestra brassicae L.) indicated that there was no response, but these observations were very inconclusive.

My own experiments were designed to investigate the

effect of three aspects of the "light" factor in the environment.

c/1 The effect of background colour on larval colour.

c/2 The effect of light duration upon larval colour.

c/3 The effect of light wavelength on larval colour.

The experiments for each of these investigations will be dealt with individually.

"c/1" To investigate the effect of background colour on larval colour, methods adapted from those used by Poulton (1892) were employed. 60 samples, named the "environmental light samples A" (ELA), were reared in Fisons growth cabinets under the same temperature and light duration conditions as used in the overcrowding experiments.

The samples were split into six groups, numbered 1, 2, 3, 4, 1R and 2R, of 10 samples and were marked with the group number, the sample type, (ELA), and the sample number, (1 - 10 for each group).

The variation of conditions involved placing tissue paper of various colours in the boxes with the foodplant, foodplant and tissue paper being placed in in roughly equal quantities. Alternatively, tissue paper was glued in a chequer pattern around the outside of the boxes, so that half of the area of the outside of each was covered. This latter treatment was carried out to ensure that if larval colour was shown to be affected by background colour, then, the possibility that this effect might be due to either the texture of the tissue paper, or to reduced humidity due to water absorption by the tissue paper,

could be investigated. It was noted that when tissue paper was placed in the boxes, it was never eaten by larvae.

Samples of a particular number in groups 1, 2 or 1R were reared under the same conditions. Similarly, samples of a particular number in groups 3, 4 or 2R were all reared under similar conditions. The 20 different treatments and the samples submitted to each are shown in Table 3Bc/1i.

The samples in groups 1R and 2R were not scored, but were kept as reserves to replace larvae of other samples which died.

Results and analysis of results.

The scoring of the ELA samples was based on the score groups used in the environmental temperature experiments. By observation, it could be seen that the scores for a particular sample number in groups 1 and 2 were not significantly different and, similarly, those of groups 3 and 4 were similar to one another. Therefore, the scores from groups 1 and 2 for each sample were added together, as were those of groups 3 and 4. The results are shown in Appendix Tables 3Bc/1i - v inclusive. For statistical analysis, the results were then treated in the same way as the temperature results, so that low scores from peripheral score groups were added to the most similar other score groups. 3rd instar yellow larvae were included with 3rd instar brown larvae. The results of this treatment are given in Tables 3Bc/1ii - v inclusive.

Chi-squared tests comparing the scores of the Group 1 samples added to the Group 2 sample results for each treatment showed that there was no significant difference in the colour

Table 3Bc/1i.

Experiments to investigate the effect of background colour on larval colour.

Treatments of the ELA samples

Treatments involving tissue paper being mixed with foodplant inside the boxes.			Treatments involving tissue paper being glued to the outside of the boxes		
Colour of tissue paper	Samples submitted Scored	Reserve	Colour of tissue paper	Samples submitted Scored	Reserve
None	1ELA1 and 2ELA1	1RELA1	None	3ELA1 and 4ELA1	2RELA1
White	1ELA2 and 2ELA2	1RELA2	White	3ELA2 and 4ELA2	2RELA2
Violet	1ELA3 and 2ELA3	1RELA3	Violet	3ELA3 and 4ELA3	2RELA3
Blue	1ELA4 and 2ELA4	1RELA4	Blue	3ELA4 and 4ELA4	2RELA4
Green	1ELA5 and 2ELA5	1RELA5	Green	3ELA5 and 4ELA5	2RELA5
Yellow	1ELA6 and 2ELA6	1RELA6	Yellow	3ELA6 and 4ELA6	2RELA6
Orange	1ELA7 and 2ELA7	1RELA7	Orange	3ELA7 and 4ELA7	2RELA7
Red	1ELA8 and 2ELA8	1RELA8	Red	3ELA8 and 4ELA8	2RELA8
Brown	1ELA9 and 2ELA9	1RELA9	Brown	3ELA9 and 4ELA9	2RELA9
Black	1ELA10 and 2ELA10	1RELA10	Black	3ELA10 and 4ELA10	2RELA10

Table 3Bc/1ii.

Experiments to investigate the effect of background colour on larval colour.

Colour analysis scores of the ELA samples, adjusted for statistical analysis.

First and Second larval instars.

Hue type	Colour type	First instar Samples	Shade -2	-1	0	Samples	Shade -2	-1	0	Second instar Samples	Shade -2	-1	0	Samples	Shade -2	-1	0
7.5GY	EG	1ELA1+	7	12	14	3ELA1+	9	12	13	1ELA1+	8	11	11	3ELA1+	7	12	11
10GY	EG	2ELA1	7	7	3	4ELA1	5	7	4	2ELA1	5	8	7	4ELA1	6	7	7
7.5GY	EG	1ELA2+	9	11	15	3ELA2+	9	10	16	1ELA2+	10	15	14	3ELA2+	9	10	11
10GY	EG	2ELA2	5	6	4	4ELA2	8	2	5	2ELA2	9	1	1	4ELA2	6	8	6
7.5GY	EG	1ELA3+	6	10	13	3ELA3+	8	14	14	1ELA3+	8	16	11	3ELA3+	7	15	5
10GY	EG	2ELA3	7	11	3	4ELA3	7	5	2	2ELA3	5	6	4	4ELA3	7	5	11
7.5GY	EG	1ELA4+	9	12	12	3ELA4+	12	13	8	1ELA4+	9	11	10	3ELA4+	7	12	9
10GY	EG	2ELA4	8	4	5	4ELA4	6	7	4	2ELA4	7	7	6	4ELA4	9	5	8
7.5GY	EG	1ELA5+	4	13	10	3ELA5+	9	8	13	1ELA5+	5	18	13	3ELA5+	10	10	5
10GY	EG	2ELA5	7	10	6	4ELA5	4	10	6	2ELA5	7	2	5	4ELA5	6	8	11
7.5GY	EG	1ELA6+	10	11	10	3ELA6+	11	8	15	1ELA6+	11	13	8	3ELA6+	10	11	15
10GY	EG	2ELA6	7	6	6	4ELA6	3	5	8	2ELA6	3	5	10	4ELA6	5	5	4
7.5GY	EG	1ELA7+	5	14	14	3ELA7+	10	12	15	1ELA7+	5	8	11	3ELA7+	10	14	16
10GY	EG	2ELA7	9	3	5	4ELA7	7	5	1	2ELA7	10	10	6	4ELA7	2	5	3

Table 3Bc/1ii. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis scores of the ELA samples, adjusted for statistical analysis.

First and Second larval instars.

Hue type	Colour type	First instar Samples	Shade -2	-1	0	Samples	Shade -2	-1	0	Second instar Samples	Shade -2	-1	0	Samples	Shade -2	-1	0
7.5GY	EG	1ELA8+	7	12	14	3ELA8+	5	15	12	1ELA8+	4	15	12	3ELA8+	8	10	10
10GY	EG	2ELA8	8	5	4	4ELA8	8	2	8	2ELA8	7	7	5	4ELA8	6	6	10
7.5GY	EG	1ELA9+	12	12	7	3ELA9+	3	10	14	1ELA9+	13	6	8	3ELA9+	10	9	11
10GY	EG	2ELA9	6	7	6	4ELA9	12	6	5	2ELA9	4	11	8	4ELA9	8	10	2
7.5GY	EG	1ELA10+	8	11	17	3ELA10+	8	12	12	1ELA10+	8	15	11	3ELA10+	8	9	13
10GY	EG	2ELA10	2	7	5	4ELA10	7	9	2	2ELA10	4	6	6	4ELA10	7	6	7
7.5GY	EG	Total	77	118	126	Total	84	114	132	Total	81	128	109	Total	86	112	106
10GY	EG		66	66	47		67	58	45		61	63	58		62	65	69

Table 3Bc/1iii.

Experiments to investigate the effect of background colour on larval colour. Colour analysis scores of the ELA samples, adjusted for statistical analysis. Third instar.

Hue type	Colour type	Sample					Sample				
	Br Y	1ELA1 and 2ELA1	1) 0) shade	1			3ELA1 and 4ELA1	1) 1) shade	2		
				-3	-2				-3	-2	
2.5GY	OL			1	3				2	3	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			6	7	2			3	9	6
10GY	3IG			8	12	10			7	9	9
	BR Y	1ELA2 and 2ELA2	2) 0) shade	2			3ELA2 and 4ELA2	1) 0) shade	1		
				-3	-2				-3	-2	
2.5GY	OL			2	2				1	4	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			5	7	10			9	4	4
10GY	3IG			7	6	9			7	9	11
	Br Y	1ELA3 and 2ELA3	1) 0) shade	1			3ELA3 and 4ELA3	1) 1) shade	2		
				-3	-2				-3	-2	
2.5GY	OL			2	2				2	2	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			10	3	6			8	6	3
10GY	3IG			9	10	7			8	10	9
	Br Y	1ELA4 and 2ELA4	2) 0) shade	2			3ELA4 and 4ELA4	1) 1) shade	2		
				-3	-2				-3	-2	
2.5GY	OL			3	3				2	3	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			4	12	3			7	7	4
10GY	3IG			6	6	11			3	13	9
	Br Y	1ELA5 and 2ELA5	2) 0) shade	2			3ELA5 and 4ELA5	2) 1) shade	3		
				-3	-2				-3	-2	
2.5GY	OL			2	2				1	3	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			7	8	4			5	11	3
10GY	3IG			8	10	7			12	5	7
	Br Y	1ELA6 and 2ELA6	1) 1) shade	2			3ELA6 and 4ELA6	2) 0) shade	2		
				-3	-2				-3	-2	
2.5GY	OL			0	4				2	4	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			7	9	6			6	8	10
10GY	3IG			11	6	5			4	9	5

Table 3Bc/1iii. (Continued)

Experiments to investigate the effect of background colour on larval colour. Colour analysis scores of the ELA samples, adjusted for statistical analysis. Third instar.

Hue type	Colour type	Sample					Sample				
	Br	1ELA7	0)	0			3ELA7	1)	1		
	Y	and	0)				and	0)			
		2ELA7	shade	-3	-2		4ELA7	shade	-3	-2	
2.5GY	OL			1	4				2	4	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			6	8	5			7	7	2
10GY	3IG			5	7	14			5	9	13
	Br	1ELA8	1)	2			3ELA8	0)	0		
	Y	and	1)				and	0)			
		2ELA8	shade	-3	-2		4ELA8	shade	-3	-2	
2.5GY	OL			2	3				1	3	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			8	7	1			7	8	6
10GY	3IG			6	9	12			7	4	14
	Br	1ELA9	2)	3			3ELA9	3)	3		
	Y	and	1)				and	0)			
		2ELA9	shade	-3	-2		4ELA9	shade	-3	-2	
2.5GY	OL			1	4				3	3	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			7	10	6			2	10	4
10GY	3IG			5	9	5			6	10	9
	Br	1ELA10	1)	2			3ELA10	1)	1		
	Y	and	1)				and	0)			
		2ELA10	shade	-3	-2		4ELA10	shade	-3	-2	
2.5GY	OL			2	2				0	4	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			9	7	2			10	4	4
10GY	3IG			7	11	8			7	14	6
	Br	Total	13)	17			Total	13)	17		
	Y		4)					4)			
			shade	-3	-2			shade	-3	-2	
2.5GY	OL			16	29				16	33	
			shade	-2	-1	0		shade	-2	-1	0
7.5GY	3IG			69	78	45			64	74	46
10GY	3IG			72	86	88			66	92	92

Table 3Bc/1iv.

Experiments to investigate the effect of background colour on larval colour. Colour analysis scores of the ELA samples, adjusted for statistical analysis. Fourth instar.

Hue type	Colour type	Sample					Sample				
	Br Y	1ELA1 and 2ELA1	4 2 shade	-3	-2		3ELA1 and 4ELA1	5 2 shade	-3	-2	
2.5GY	OL			8	14				9	15	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			3	0	3			2	6	2
10GY	Gr			8	8	0			8	1	0
	Br Y	1ELA2 and 2ELA2	5 1 shade	-3	-2		3ELA2 and 4ELA2	6 1 shade	-3	-2	
2.5GY	OL			9	15				10	11	
			shade	-1	0	1			-1	0	1
7.5GY	Gr			2	5	1			3	2	1
10GY	Gr			8	3	1			7	9	0
	Br Y	1ELA3 and 2ELA3	5 1 shade	-3	-2		3ELA3 and 4ELA3	9 1 shade	-3	-2	
2.5GY	OL			7	16				9	9	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			4	4	1			6	1	1
10GY	Gr			3	8	1			8	6	0
	Br Y	1ELA4 and 2ELA4	7 0 shade	-3	-2		3ELA4 and 4ELA4	6 0 shade	-3	-2	
2.5GY	OL			6	19				10	13	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			3	7	1			4	3	2
10GY	Gr			6	1	0			7	5	0
	Br Y	1ELA5 and 2ELA5	6 2 shade	-3	-2		3ELA5 and 4ELA5	3 2 shade	-3	-2	
2.5GY	OL			10	14				9	14	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			2	3	2			3	4	0
10GY	Gr			7	4	0			8	6	1

Table 3Bc/1iv. (Continued)

Experiments to investigate the effect of background colour on larval colour. Colour analysis scores of the ELA samples, adjusted for statistical analysis. Fourth instar.

Hue type	Colour type	Sample					Sample				
	Br	1ELA6	5				3ELA6	6			
	Y	and	1				and	2			
		2ELA6	shade	-3	-2		4ELA6	shade	-3	-2	
2.5GY	OL			9	12				11	12	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			5	8	0			0	6	0
10GY	Gr			6	1	3			8	4	1
	Br	1ELA7	6				3ELA7	8			
	Y	and	2				and	1			
		2ELA7	shade	-3	-2		4ELA7	shade	-3	-2	
2.5GY	OL			8	9				9	13	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			7	4	1			3	3	1
10GY	Gr			12	1	0			10	2	0
	Br	1ELA8	3				3ELA8	4			
	Y	and	2				and	1			
		2ELA8	shade	-3	-2		4ELA8	shade	-3	-2	
2.5GY	OL			11	14				9	14	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			3	3	1			4	3	3
10GY	Gr			3	10	0			5	7	0
	Br	1ELA9	6				3ELA9	5			
	Y	and	1				and	2			
		2ELA9	shade	-3	-2		4ELA9	shade	-3	-2	
2.5GY	OL			10	12				10	11	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			5	2	1			2	4	0
10GY	Gr			5	4	4			7	5	4
	Br	1ELA10	7				3ELA10	6			
	Y	and	1				and	1			
		2ELA10	shade	-3	-2		4ELA10	shade	-3	-2	
2.5GY	OL			9	13				15	8	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			5	1	0			3	3	1
10GY	Gr			10	2	2			7	5	1
	Br	Total	54				Total	58			
	Y		13					13			
			shade	-3	-2			shade	-3	-2	
2.5GY	OL			87	138				101	120	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			39	37	11			30	35	11
10GY	Gr			68	42	11			75	50	7

Table 3Bc/1v.

Experiments to investigate the effect of background colour on larval colour. Colour analysis scores of the ELA samples, adjusted for statistical analysis. Fifth instar.

Hue type	Colour type	Sample					Sample				
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA1		9	8	5	3ELA1		7	8	7
10YR	Br	and		6	3	X	and		6	2	X
		2ELA1					4ELA1				
	Y		2					2			
			shade	-3	-2			shade	-3	-2	
	OL			4	4				5	4	
			shade	-1	0	1		shade	-1	0	1
	Gr			3	4	2			2	4	3
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA2		10	5	3	3ELA2		14	6	3
10YR	Br	and		4	5	X	and		4	2	X
		2ELA2					4ELA2				
	Y		1					1			
			shade	-3	-2			shade	-3	-2	
	OL			2	7				8	3	
			shade	-1	0	1		shade	-1	0	1
	Gr			3	7	3			3	4	2
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA3		9	7	5	3ELA3		10	5	8
10YR	Br	and		7	3	X	and		4	3	X
		2ELA3					4ELA3				
	Y		1					1			
			shade	-3	-2			shade	-3	-2	
	OL			5	4				5	8	
			shade	-1	0	1		shade	-1	0	1
	Gr			2	4	3			3	2	1
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA4		13	4	3	3ELA4		9	7	6
10YR	Br	and		9	1	X	and		7	3	X
		2ELA4					4ELA4				
	Y		0					0			
			shade	-3	-2			shade	-3	-2	
	OL			1	9				9	3	
			shade	-1	0	1		shade	-1	0	1
	Gr			2	7	1			1	3	2

Table 3Bc/1v. (Continued)

Experiments to investigate the effect of background colour on larval colour. Colour analysis scores of the ELA samples, adjusted for statistical analysis. Fifth instar.

Hue type	Colour type	Sample					Sample				
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA5		11	9	4	3ELA5		11	6	1
10YR	Br	and		5	3	X	and		6	4	X
		2ELA5					4ELA5				
	Y		2					2			
			shade	-3	-2			shade	-3	-2	
	OL			5	3				2	8	
			shade	-1	0	1		shade	-1	0	1
	Gr			3	3	2			2	6	2
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA6		8	8	5	3ELA6		10	9	4
10YR	Br	and		8	4	X	and		4	2	X
		2ELA6					4ELA6				
	Y		1					2			
			shade	-3	-2			shade	-3	-2	
	OL			4	4				4	6	
			shade	-1	0	1		shade	-1	0	1
	Gr			3	3	2			3	4	2
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA7		6	9	9	3ELA7		11	10	4
10YR	Br	and		4	6	X	and		4	1	X
		2ELA7					4ELA7				
	Y		2					1			
			shade	-3	-2			shade	-3	-2	
	OL			6	1				3	4	
			shade	-1	0	1		shade	-1	0	1
	Gr			3	2	2			3	4	5
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA8		8	6	4	3ELA8		5	12	7
10YR	Br	and		8	3	X	and		9	0	X
		2ELA8					4ELA8				
	Y		2					1			
			shade	-3	-2			shade	-3	-2	
	OL			4	6				4	7	
			shade	-1	0	1		shade	-1	0	1
	Gr			1	5	3			2	1	2

Table 3Bc/1v. (Continued)

Experiments to investigate the effect of background colour on larval colour. Colour analysis scores of the ELA samples, adjusted for statistical analysis. Fifth instar.

Hue type	Colour type	Sample					Sample				
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA9		11	7	9	3ELA9		11	8	3
10YR	Br	and		5	4	X	and		4	2	X
		2ELA9					4ELA9				
	Y		1					2			
			shade	-3	-2			shade	-3	-2	
	OL			8	2				6	4	
			shade	-1	0	1		shade	-1	0	1
	Gr			2	1	0			2	7	1
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	1ELA10		6	11	5	3ELA10		11	6	6
10YR	Br	and		10	0	X	and		4	2	X
		2ELA10					4ELA10				
	Y		1					1			
			shade	-3	-2			shade	-3	-2	
	OL			2	7				6	5	
			shade	-1	0	1		shade	-1	0	1
	Gr			3	3	2			4	1	4
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	Total		91	74	52	Total		99	77	49
10YR	Br			66	32	X			52	21	X
	Y		13					13			
			shade	-3	-2			shade	-3	-2	
	OL			41	47				52	52	
			shade	-1	0	1		shade	-1	0	1
	Gr			25	39	20			25	36	24

scores in any of the instars,

(for 1st instar scores, $X^2_{45} = 29.689$, $0.975 > p > 0.95$;
for 2nd instar scores, $X^2_{45} = 52,557$, $0.25 > p > 0.1$;
for 3rd instar scores, $X^2_{72} = 53.111$, $0.95 > p > 0.90$;
for 4th instar scores, $X^2_{81} = 91.321$, $0.25 > p > 0.1$;
for 5th instar scores, $X^2_{90} = 71.464$, $0.95 > p > 0.90$).

Similar tests on the samples from groups 3 and 4 again gave non-significant results,

(for 1st instar scores, $X^2_{45} = 46.203$, $0.5 > p > 0.3$;
for 2nd instar scores, $X^2_{45} = 37.865$, $0.8 > p > 0.7$;
for 3rd instar scores, $X^2_{72} = 61.912$, $0.9 > p > 0.75$;
for 4th instar scores, $X^2_{81} = 74.079$, $0.75 > p > 0.5$;
for 5th instar scores, $X^2_{90} = 65,846$, $0.99 > p > 0.95$).

Furthermore, a series of chi-squared tests comparing the sum scores of the group 1 and 2 samples with the sum scores of the group 3 and 4 samples, also gave non-significant results for all instars,

(for 1st instar scores, $X^2_5 = 1.08$, $0.98 > p > 0.95$;
for 2nd instar scores, $X^2_5 = 2.738$, $0.8 > p > 0.7$;
for 3rd instar scores, $X^2_8 = 1.112$, $p > 0.99$;
for 4th instar scores, $X^2_9 = 5.596$, $0.8 > p > 0.7$;
for 5th instar scores, $X^2_{10} = 6.228$, $0.8 > p > 0.7$).

Finally, tests comparing the sum scores of all the ELA samples with the colour analysis scores (Table 3Baii), also gave non-significant results for all instars apart from the 4th, from which chi-squared gave a probability which was significant, although not highly so.

(for 1st instar scores, $X^2_5 = 4.902$, $0.5 > p > 0.3$;
for 2nd instar scores, $X^2_5 = 9.596$, $0.1 > p > 0.05$;
for 3rd instar scores, $X^2_9 = 6.542$, $0.7 > p > 0.5$;
for 4th instar scores, $X^2_9 = 19.089$, $0.05 > p > 0.02$;
for 5th instar scores, $X^2_{10} = 17.531$, $0.1 > p > 0.05$).

The main difference between the ELA 4th instar scores and the colour analysis 4th instar scores seems to be a decrease in the number of larvae scored in the paler colour groups in the ELA scores; however, this trend does not apply throughout, as the palest colour group, that containing yellow larvae, shows an increase. Thus, in the absence of a positive explanation for the difference in scores of the ELA samples and colour analysis samples in the 4th instar, it is suggested that this difference is due to chance.

Discussion.

The results of these chi-squared tests indicate that the colour of P. meticulosa larvae is not consistently affected by the general background colour of their surroundings, and thus they bear out Poulton and Gould's speculation that non stick-like Noctuid larvae probably do not respond to the colour of their surroundings in the same way as do the stick-like larvae of many of the Geometridae.

Moreover, the results show that there is no significant difference in the scores of larvae reared in conditions where coloured tissue paper was placed inside the boxes, compared with larvae reared in boxes which contained only foodplant, but which were partly covered with tissue paper. The larvae in the former samples were often seen to walk across the tissue as

they moved about in the boxes, and the conditions in the boxes containing tissue were generally the drier because the tissue tended to take up any moisture inside the boxes, particularly that from larval frass. Obviously, no conclusive statement can be made about the effect of either substrate texture or rearing humidity conditions on larval colour, based on these experiments; however, the observations do give some indication that neither of these factors have an effect.

"c/2" To determine whether the duration of light that a larva receives in a 24 hour period affects larval colour at any stage, 24 samples of 25 larvae were reared in boxes placed in five thermostatically controlled glass tanks. These samples were named the "environmental light samples B" (ELB) and the "environmental light reserve samples B" (ELBR). The temperature of the tanks was maintained at $8 \pm 2^{o}C$ between the hours of 6 p.m. and 6 a.m., and at $16 \pm 2^{o}C$ between 6 a.m. and 6 p.m.

The five tanks, which were labelled A - E inclusive, were illuminated for 0, 6, 12, 18 or 24 hours per day respectively, each tank being lit by two 100 watt bulbs set into the tank covers. 12 of the samples, (ELB1 - 10 and ELBR1 - 2), were kept in Tank A, and each of the other tanks had three samples, (tank B - ELB11, ELB12 and ELBR3; tank C - ELB13, ELB14 and ELBR4; tank D - ELB15, ELB16 and ELBR5; and tank E - ELB17, ELB18 and ELBR6).

Eggs from females of the CAB1 - 17 progeny were placed

in the tanks as soon as the samples had been made up, so that the treatment started at an early stage in the development. The samples from tanks B - E inclusive, were scored in each instar in the normal way, all scoring taking place during periods when the samples were illuminated. (The reserve samples were again not scored, the larvae in these samples being used to replace larvae in other samples which died.) Obviously, the samples in Tank A could not be scored during periods when these samples were illuminated as they were reared in darkness. Because of this, a special procedure for scoring these samples had to be developed. (This procedure is known as the "darkness scoring method" and will be referred to again in the experiments on the effect of foodplant on larval colour). When the larvae of samples in tank A were judged to be in the 1st instar, one box was removed from the tank and the contents of the box were spread out in a flat bottomed dish in another completely dark room. A flash photograph of the contents of the dish was then taken. The process was repeated for a second box. Up to this point, the whole procedure was carried out in total darkness, except for the two photographic flashes. The larvae from these two samples, (ELB1 and 2), were then scored normally. After scoring, the larvae were again photographed using flash. The photographs were taken to compare the colours of larvae before and after scoring, to check that bringing larvae into the light for scoring did not induce any immediate change in colour. These broods were then reared using the basic rearing methods and no unexpected colour changes occurred subsequently, which

might have been attributable to the flash.

The same procedure was repeated for two more samples, (ELB3 and 4), when larvae were judged to be in the 2nd instar, and similarly for two more samples in each of the 3rd (ELB5 and 6), 4th (ELB7 and 8), and 5th instars (ELB9 and 10). If larvae of any samples had died, these were replaced by larvae from the two reserve samples which were found by touch in the dark.

The difficulties of rearing larvae in complete darkness were to some extent overcome by using large rectangular boxes 470 mm long, 205 mm wide and 110 mm deep for these larvae. The foodplant used was sorrel, leaves being placed in the boxes by touch each 24 hours. A thick layer of blotting paper was placed on the bottom of each box to take up some of the moisture resulting from the decaying foodplant, and the considerable amount of frass that accumulated. Even though these precautions were taken, the accumulation of rotting leaves, frass, and in some cases dead larvae, resulted in high mortality rates in the 4th and 5th instars. In fact, all the larvae of two of these samples, ELB7 and ELB10, were found to have succumbed to a viral disease and were dead. Moreo ver, 9 larvae in ELB8 and 12 in ELB9, had also died. The larvae in these latter two samples were replaced from the ELBR1 and 2 samples, but as the mortality rate was so high, the results from these instars should be considered with a certain amount of caution, because, if one of the colour forms is hardier or more resistant to disease and bad conditions than the others, then biased colour scores will result.

Results and analysis.

The results of these samples are given in Appendix Table 3Bc/2i - iv. Once again, inspection of the results showed that samples reared under the same conditions were homogeneous; therefore, the two scored samples reared in each of the tanks, B - E inclusive, were added together. Similarly, the results from scoring two samples reared in tank A, in each of the first three instars, were added together. In the case of 4th and 5th instar samples reared in tank A, only one sample was scored due to the loss of the other samples through disease.

For statistical purposes, the score groups were adjusted in the same way as those from the environmental temperature experiments, with the exception that 3rd instar Y larvae were included with the 3rd instar Br larvae, due to their rare, but significant, occurrence. The results of this treatment are tabulated in Table 3Bc/2i.

Chi-squared tests comparing the scores of samples submitted to the different treatments, gave non-significant results for each instar,

(for 1st instar scores, $X^2_{20} = 21.884$, $0.5 > p > 0.3$;
for 2nd instar scores, $X^2_{20} = 18.198$, $0.7 > p > 0.5$;
for 3rd instar scores, $X^2_{32} = 23.047$, $0.9 > p > 0.8$;
for 4th instar scores, $X^2_{36} = 25.081$, $0.9 > p > 0.8$;
for 5th instar scores, $X^2_{36} = 30.486$, $0.9 > p > 0.8$)

Furthermore, comparison of the results of samples submitted to 6 and 18 hours light, (ELB11 and 12, and ELB15 and 16 respectively), which give the closest approximation of the

Table 3Bc/2i.

Experiments to investigate the effect of duration of light on larval colour. Colour analysis scores for the ELB samples, adjusted for use in statistical analysis. All instars.

Instar	Hue type	Colour type																											
First			Samples ELB1 & 2						Samples ELB11 & 12						Samples ELB13 & 14						Samples ELB15 & 16						Samples ELB17 & 18						Total					
			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0		
	7.5GY	EG			6	15	10				8	11	8				9	11	14				14	9	8				8	17	7				45	63	47	
	10GY	EG			3	7	9				10	5	8				6	6	4				10	4	5				4	8	6				33	30	32	
Second			Samples ELB3 & 4						Samples ELB11 & 12						Samples ELB13 & 14						Samples ELB15 & 16						Samples ELB17 & 18						Total					
			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0		
	7.5GY	EG			11	12	8				7	15	12				9	11	12				4	16	7				11	10	10				42	64	49	
	10GY	EG			8	6	5				5	4	7				6	8	4				10	6	7				5	3	11				34	27	34	
Third			Samples ELB5 & 6						Samples ELB11 & 12						Samples ELB13 & 14						Samples ELB15 & 16						Samples ELB17 & 18						Total					
		Br	2						1						2						0						3						8					
				3						1						3						0						3						10				
		Y	1						0						1						0						0						2					
			shade	-3	-2				shade	-3	-2				shade	-3	-2				shade	-3	-2				shade	-3	-2				shade	-3	-2			
	2.5GY	OL			2	2					1	3					3	1					1	4					3	1					10	11			
			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0			shade	-2	-1	0		
	7.5GY	3IG			5	6	7				5	7	8				10	12	6				6	8	10				7	10	5				33	43	36	
	10GY	3IG			6	11	8				8	10	7				4	6	5				9	5	7				6	7	8				33	39	35	
Fourth			Samples ELB8						Samples ELB11 & 12						Samples ELB13 & 14						Samples ELB15 & 16						Samples ELB17 & 18						Total					
		Br	3						6						6						4						7						26					
		Y	0						2						0						1						1						4					
			shade	-3	-2				shade	-3	-2				shade	-3	-2				shade	-3	-2				shade	-3	-2				shade	-3	-2			
		OL			3	7					12	10					8	16					8	13					12	9					43	55			
			shade	-1	0	1			shade	-1	0	1			shade	-1	0	1			shade	-1	0	1			shade	-1	0	1			shade	-1	0	1		
	7.5GY	Gr			2	2	0				3	3	1				2	4	3				8	5	1				5	3	2				19	17	7	
	10GY	Gr			4	3	1				5	6	2				8	2	1				4	5	1				9	2	0				30	18	5	
Fifth			Samples ELB9						Samples ELB11 & 12						Samples ELB13 & 14						Samples ELB15 & 16						Samples ELB17 & 18						Total					
			shade	-3	-2	-1			shade	-3	-2	-1			shade	-3	-2	-1			shade	-3	-2	-1			shade	-3	-2	-1			shade	-3	-2	-1		
	7.5YR	Br			3	4	2				9	7	2				6	11	4				12	6	1				10	7	2				40	35	11	
	10YR	Br			4	1	X				4	4	X				6	1	X				5	2	X				6	1	X				25	9			
		Y	1						2						0						1						1						5					
			shade	-3	-2				shade	-3	-2				shade	-3	-2				shade	-3	-2				shade	-3	-2				shade	-3	-2			
	2.5GY	OL			2	2					4	5					7	3					5	6					2	9					20	25			
			shade	-1	0	1			shade	-1	0	1			shade	-1	0	1			shade	-1	0	1			shade	-1	0	1			shade	-1	0	1		
	10GY	Gr			2	4	0				8	2	3				4	6	2				5	4	3				9	2	1				38	18	9	

natural mid-winter and mid-summer light conditions, and are thus most relevant to the natural environment of the larvae, also gave non-significant results,

(for 1st instar scores, $X^2_5 = 2.640$, $0.8 > p > 0.7$;
for 2nd instar scores, $X^2_5 = 4.212$, $0.7 > p > 0.5$;
for 3rd instar scores, $X^2_8 = 3.244$, $0.95 > p > 0.9$;
for 4th instar scores, $X^2_9 = 5.232$, $0.9 > p > 0.8$;
for 5th instar scores, $X^2_{10} = 3.492$, $0.98 > p > 0.95$)

The photographs taken before and after the samples reared in tank A were scored, showed that taking the larvae into the light for scoring did not induce an immediate change in larval colour.

These tests, then, indicate that the number of hours of light to which larvae are submitted each day, does not significantly affect larval colour.

"c/3" To determine the effect of light of different wavelengths on larval colour, 18 samples of 25 larvae were reared in boxes placed in six thermostatically controlled glass tanks. The temperature of these tanks was again maintained at $8 \pm 2^{o}C$, between the hours of 6 p.m. and 6 a.m., and at $16 \pm 2^{o}C$, between 6 a.m. and 6 p.m. The tanks were illuminated between 6 a.m. and 6 p.m. by a single 150 watt frosted light bulb, set in the lid of each tank. Spectrum filters, designed to blackout all light, except that of a small wavelength band, were placed in five of the tanks between the bulbs and the sample boxes. The tanks were each kept separately in complete darkness, apart from the illumination from their particular bulb, and for periods, (about 30 minutes per sample per instar), when the

larvae were being scored.

Three samples were placed in each tank. The sample numbers and the specifications of the wavelengths of the filter placed in each tank are given in Table 3Bc/3i. Only the samples ELC1 - 12 inclusive, were scored, the ELCR1 - 6 samples being used as reserves.

The larvae were scored using the method outlined in the environmental temperature experiments.

Results and analysis.

Inspection of the results showed that samples reared under the same conditions, were quite obviously homogeneous, and, therfore, the scores of the two scored samples from each tank were added together.

The results are tabulated in Appendix Tables 3Bc/3i - iii inclusive.

For statistical purposes, the score groups were adjusted in the same way as those from the ELB samples. The results of this treatment are given in Table 3Bc/3ii.

Once again, chi-squared tests comparing the six sets of scores for each instar, show that there is no significant difference in the samples, when taken as a whole,

(for 1st instar scores, $X^2_{25} = 25.881$, $0.5 > p > 0.3$;
for 2nd instar scores, $X^2_{25} = 26.362$, $0.5 > p > 0.3$;
for 3rd instar scores, $X^2_{40} = 31.368$, $0.9 > p > 0.75$;
for 4th instar scores, $X^2_{45} = 60.256$, $0.1 > p > 0.05$;
for 5th instar scores, $X^2_{50} = 57.588$, $0.25 > p > 0.1$)

Furthermore, a comparison of the scores of samples, ELC1 and 2, with those of the ELC9 and 10 samples, submitted

Table 3Bc/3i.

Experiments to investigate the effect of light of different wavelengths on larval colour. Sample numbers and specifications of the wavelengths of the filters placed in each tank.

Tank	Samples in tank	Wavelength of the filter (A)	Colour
1	ELC 1, ELC 2 and ELCR 1	3900 - 4400	Violet
2	ELC 3, ELC 4 and ELCR 2	4500 - 4900	Blue
3	ELC 5, ELC 6 and ELCR 3	5000 - 5500	Green
4	ELC 7, ELC 8 and ELCR 4	5600 - 6100	Yellow
5	ELC 9, ELC 10 and ELCR 5	6200+	Red
6	ELC 11, ELC 12 and ELCR 6	No filter used	White

Table 3Bc/3ii.

Experiments to determine the effect of light of different wavelengths on larval colour. Colour analysis scores for the ELC samples, adjusted for statistical analysis. (All instars).

Instar	Hue type	Colour type	Samples ELC1 and 2				Samples ELC3 and 4				Samples ELC5 and 6				Samples ELC7 and 8				Samples ELC9 and 10				Samples ELC11 and 12				Total (sum of all ELC samples)			
First			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		9	17	7		10	9	12		6	12	13		12	9	10		6	15	11		5	16	7		48	78	60
	10GY	EG		3	8	6		9	6	4		8	6	5		6	3	10		8	6	4		10	3	9		44	32	38
Second			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	EG		11	11	8		14	15	9		6	16	9		8	11	12		7	16	7		8	9	6		54	78	51
	10GY	EG		8	9	3		5	4	3		9	3	7		5	6	8		4	8	8		9	8	10		40	38	39
Third																														
		Br	2				4				1				0				2				0				9			
				3				4				1				1				2				0				11		
		Y	1				0				0				1				0				0				2			
			shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
	2.5GY	OL		3	1			2	2			3	3			1	4			0	2			1	4			10	16	
			shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0	shade	-2	-1	0
	7.5GY	3IG		6	11	8		9	8	5		4	6	9		8	10	6		6	5	6		4	7	11		37	47	45
	10GY	3IG		6	7	5		4	10	6		8	9	7		6	6	8		9	10	10		9	5	9		42	47	45
Fourth																														
		Br	5				2				9				3				6				6				31			
		Y	2				0				2				0				3				0				7			
			shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
	2.5GY	OL		13	8			10	15			6	17			8	13			10	14			8	8			55	75	
			shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1
	7.5GY	Gr		5	8	1		3	6	3		1	4	0		7	1	2		0	2	1		4	3	4		20	24	11
	10GY	Gr		2	3	3		8	2	1		7	2	2		11	4	1		8	4	2		12	5	0		48	20	9
Fifth			shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1	shade	-3	-2	-1
	7.5YR	Br		6	6	2		5	7	3		11	4	5		12	5	5		6	11	0		8	11	1		48	44	16
	10YR	Br		5	4	X		8	1	X		4	4	X		6	3	X		6	1	X		5	4	X		34	17	X
		Y	2				0				2				0				3				0				7			
			shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2		shade	-3	-2	
	2.5GY	OL		4	8			4	11			3	6			1	4			7	5			8	4			27	38	
			shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1	shade	-1	0	1
	10GY	Gr		4	7	2		6	2	3		7	3	1		6	6	2		3	3	5		4	4	1		30	25	14

to the shortest and longest wavelengths respectively, also gave a non-significant probability value for each instar,

(for 1st instar scores, $X^2_5 = 4.572$, $0.5 > p > 0.3$;
for 2nd instar scores, $X^2_5 = 5.545$, $0.5 > p > 0.3$;
for 3rd instar scores, $X^2_8 = 4.444$, $0.9 > p > 0.8$;
for 4th instar scores, $X^2_9 = 14.86$, $0.1 > p > 0.05$;
for 5th instar scores, $X^2_{10} = 6.908$, $0.8 > p > 0.7$).

From the results of these tests then, it appears that larval colour is not affected at any stage by the wavelength of light to which the ova and larvae are submitted.

(d) The effect of foodplant on larval colour.

Introduction.

In common with the larvae of many other Noctuid species, the larvae of Phlogophora meticulosa are polyphagous. In the wild state I have found that they feed mainly on docks and sorrels (Rumex spp.), plantains (Plantago spp.), and grasses, but South (1939) reports that they may be found on most herbaceous plants, and often attack geraniums in the greenhouse as well as outside. In captivity, the larvae will also feed readily on the foliage of many deciduous trees, such as oak, birch and sallow, although, in my experience, they rarely do so in the wild. When given a choice of fresh leaves in captivity, larvae show a preference for common sorrel (Rumex acetosa) and sheep's sorrel (Rumex acetosella) over other docks or plantains which are, in turn, preferred to grasses, oak, sallow, birch and other trees. The larvae will not only feed on the leaves

of plants, but will also readily accept the petals, sepals and seed pods of many plants as food. Therefore, a wide range of natural, coloured foods could be fed to larvae to investigate whether larval variation was affected by foodplant.

Initial experiments.

Materials and methods.

Initially, 150 larvae from each of six broods, named the "environmental foodplant broods", (EFB1 - 6 inclusive), from light-trapped females, were split into six groups of 25 larvae. Each sample from each brood was given a specific suffix to the brood number, these being i, ii or Ri when the samples were fed on sorrel, and iii, iv or Rii for samples fed on ribwort plantain (Plantago lanceolata). The Ri and Rii samples were kept as reserves.

Rearing was carried out in a Fisons growth cabinet set at a temperature of 8 $\pm$ 1°C from 6 p.m. to 6 a.m., when the cabinet was unlit, and at a temperature of 16 $\pm$ 1°C between 6 a.m. and 6 p.m. when the cabinet was lit by four 18 inch 15 watt cryselco bulbs. In these initial foodplant experiments, the leaves of the foodplant were not specifically selected for uniformity of colour as in some of the later tests. Colour scoring was carried out in the same way as in the temperature experiments (see chapter 3Bi).

Results and analysis of data.

The results of these tests are given in a condensed form in Appendix Tables 3Bd/1i - v inclusive. Inspection of the results showed that samples from the same brood, which were fed on the same foodplant, were quite obviously homogeneous in all

cases. Therefore, for statistical analysis of the results from samples fed on different foodplants, or from different broods, the results of the two samples from each brood which were fed on the same foodplant were added together. The score groups were adjusted with regard to peripheral values, where necessary, on similar lines to those used for the groups in the statistical tests on the temperature experiment data. For the 1st and 2nd instars, this led to eight score groups, all of which were green, these being centred on 7.5GY/-3, 7.5GY/-2, 7.5GY/-1, 7.5GY/0, - 10GY/-3, 10GY/-2, 10GY/-1 and 10GY/0. For 3rd instar scores, the same groups were used, together with a ninth group, which was defined by the limits of the Br main colour type. 4th and 5th instar results were classed in 14 groups, these being the 11 used in the classification of 5th instar results in the Temperature experiments, together with three additional green groups centred on 10GY/-1, 10GY/0 and 10GY/+1. The results of this treatment are given in Tables 3Bd/1i - v inclusive.

Chi-squared tests carried out to test for heterogeneity between the samples fed on sorrel for each of the first two instars, gave non-significant results, (for 1st instar scores, $X^2_{35} = 5.105$, $p > 0.99$; for 2nd instar scores, $X^2_{35} = 29.859$, $0.7 > p > 0.5$) indicating that the samples were homogeneous. Similarly, the samples fed on plantain were found to be homogeneous, (for 1st instar scores, $X^2_{35} = 12.958$, $p > 0.99$; for 2nd instar scores, $X^2_{35} = 13.289$, $p > 0.99$). On the other hand, when chi-squared tests were carried out on all the 1st or all the 2nd instar samples, the results were highly significant, (for 1st instar scores, $X^2_7 = 123.60$, $p < 0.001$; for 2nd instar scores, $X^2_7 = 140.438$, $p < 0.001$), showing that

Table 3Bd/1i.

Experiments to investigate the effect of foodplant colour on larval colour.

Colour analysis scores of the EFB samples, adjusted for statistical analysis.

Series 1. First instar.

Hue type	Fed on Sorrel Sample	shade -3	-2	-1	0	Fed on Plantain Sample	shade -3	-2	-1	0
7.5GY	EFB1si and 1sii	2	6	13	11	EFB1siii and 1siv	6	14	8	2
10GY		1	6	6	5		4	8	8	0
7.5GY	EFB2si and 2sii	1	7	11	11	EFB2siii and 2siv	6	16	9	3
10GY		1	6	7	6		3	6	6	1
7.5GY	EFB3si and 3sii	1	8	12	12	EFB3siii and 3siv	6	16	7	2
10GY		1	6	6	4		4	8	7	0
7.5GY	EFB4si and 4sii	2	7	11	12	EFB4siii and 4siv	9	15	9	1
10GY			7	5	6		3	8	5	0
7.5GY	EFB5si and 5sii	2	7	12	11	EFB5siii and 5siv	8	15	7	2
10GY		1	7	5	5		5	7	6	0
7.5GY	EFB6si and 6sii	1	7	10	14	EFB6siii and 6siv	6	13	10	1
10GY		1	6	6	5		6	5	8	1
7.5GY	TOTAL	9	42	69	71	TOTAL	41	89	50	11
10GY		5	38	35	31		25	42	40	2

Table 3Bd/1ii.

Experiments to investigate the effect of foodplant colour on larval colour.

Colour analysis scores of the EFB samples, adjusted for statistical analysis.

Series 1. Second instar.

Hue type	Fed on Sorrel Sample	shade -3	-2	-1	0	Fed on Plantain Sample	shade -3	-2	-1	0
7.5GY	EFB1si and 1sii	1	6	13	10	EFB1siii and 1siv	8	17	7	1
10GY		1	11	3	5		4	6	7	0
7.5GY	EFB2si and 2sii	1	7	10	12	EFB2siii and 2siv	5	17	10	1
10GY		0	7	7	6		3	6	7	1
7.5GY	EFB3si and 3sii	0	8	8	16	EFB3siii and 3siv	5	22	7	0
10GY		2	7	7	2		5	4	7	0
7.5GY	EFB4si and 4sii	3	7	11	12	EFB4siii and 4siv	7	15	9	2
10GY		0	6	3	8		4	6	7	0
7.5GY	EFB5si and 5sii	1	8	15	8	EFB5siii and 5siv	4	19	11	1
10GY		1	3	9	5		4	4	6	1
7.5GY	EFB6si and 6sii	1	9	12	13	EFB6siii and 6siv	6	19	7	1
10GY		1	5	5	4		3	6	8	0
7.5GY	TOTAL	7	45	69	71	TOTAL	35	109	51	6
10GY		5	39	34	30		23	32	42	2

Table 3Bd/1iii.

Experiments to investigate the effect of foodplant colour on larval colour. Series 1. Colour analysis scores of the EFB samples, adjusted for statistical analysis. Third instar.

Hue type	Colour type	Fed on Sorrel Sample					Fed on Plantain Sample				
			shade	-3	-2	-1	0	shade	-3	-2	-1	0
7.5GY	3IG	EFB 1si and 1sii		1	8	12	8	EFB 1siii and 1siv	10	13	7	2
10GY	3IG			1	6	7	7		6	8	3	1
			shade	-3	-2	-1	0	shade	-3	-2	-1	0
7.5GY	3IG	EFB 2si and 2sii		1	8	12	11	EFB 2siii and 2siv	10	12	8	3
10GY	3IG			1	4	7	6		4	8	4	1
	Br	EFB 3si and 3sii	25					EFB 3siii and 3siv	24			
			shade	-3	-2	-1	0	shade	-3	-2	-1	0
7.5GY	3IG			0	2	6	3		2	4	4	0
10GY	3IG			0	3	6	5		3	7	6	0

Table 3Bd/1iii. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour.
Series 1. Colour analysis scores of the EFB samples, adjusted for statistical analysis. Third instar.

Hue type	Colour type	Fed on Sorrel Sample						Fed on Plantain Sample					
			shade	-3	-2	-1	0		shade	-3	-2	-1	0
7.5GY	3IG	EFB 4si and 4sii		1	9	11	13	EFB 4siii and 4siv		9	10	10	2
10GY	3IG			0	5	7	4			6	8	5	0
			shade	-3	-2	-1	0		shade	-3	-2	-1	0
7.5GY	3IG	EFB 5si and 5sii		0	8	10	11	EFB 5siii and 5siv		9	14	10	1
10GY	3IG			1	6	7	7			4	7	5	0
			shade	-3	-2	-1	0		shade	-3	-2	-1	0
7.5GY	3IG	EFB 6si and 6sii		1	7	10	10	EFB 6siii and 6siv		0	10	7	7
10GY	3IG			1	8	8	5			2	5	12	7
	Br	Totals	25					Totals	24				
			shade	-3	-2	-1	0		shade	-3	-2	-1	0
7.5GY	3IG			4	42	61	56			40	63	46	15
10GY	3IG			4	32	42	34			25	43	35	9

Table 3Bd/1iv.

Experiments to investigate the effect of foodplant colour on larval colour. Series 1.

Colour analysis scores of the EFB samples, adjusted for statistical analysis. Fourth instar.

Hue type	Colour type	Fed on Sorrel Sample					Fed on Plantain Sample				
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr	EFB1si and 1sii		4	14	3	EFB1siii and 1siv		2	16	1
10GY	Gr			15	11	3			18	9	4
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	EFB2si and 2sii		5	3	0	EFB2siii and 2siv		2	4	0
10YR	Br			2	0	X			2	0	X
			shade	-3	-2			shade	-3	-2	
2.5GY	OL			12	18				10	19	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			1	3	0			2	5	0
10GY	Gr			4	2	0			3	3	0
			shade	-3	-2	-1		shade	-3	-2	-1
7.5GY	Br	EFB3si and 3sii		16	27	1	EFB3siii and 3siv		12	30	2
10GY	Br			5	1	X			3	3	X
			shade	-3	-2			shade	-3	-2	
2.5GY	OL	EFB4si and 4sii		12	15		EFB4siii and 4siv		8	18	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			2	7	1			0	5	3
10GY	Gr			5	6	2			8	7	1

Table 3Bd/1iv. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour. Series 1.

Colour analysis scores of the EFB samples, adjusted for statistical analysis. Fourth instar.

Hue type	Colour type	Fed on Sorrel Sample					Fed on Plantain Sample				
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	EFB5si and 5sii		0	0	0	EFB5siii and 5siv		0	0	0
10YR	Br			0	0	X			0	0	X
	PY		1					4			
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			2	11	5			5	14	3
10GY	Gr			16	13	2			13	9	2
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	EFB6si and 6sii		0	0	0	EFB6siii and 6siv		0	0	0
10YR	Br			0	0	X			0	0	X
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			6	17	4			2	13	5
10GY	Gr			11	11	1			17	9	4
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	Totals		21	30	1	Totals		14	34	2
10YR	Br			7	1	X			5	3	X
	PY		1					4			
			shade	-3	-2			shade	-3	-2	
2.5GY	OL			24	33				18	37	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			15	52	13			11	53	12
10GY	Gr			51	43	8			59	37	11

Table 3Bd/1v.

Experiments to investigate the effect of foodplant colour on larval colour. Series 1.
Colour analysis scores of the EFB samples, adjusted for statistical analysis. Fifth instar.

Hue type	Colour type	Fed on Sorrel Sample					Fed on Plantain Sample				
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr	EFB1si and 1sii		3	12	4	EFB1siii and 1siv		4	15	2
10GY	Gr			16	12	3			19	7	3
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	EFB2si and 2sii		16	12	3	EFB2siii and 2siv		20	9	4
10YR	Br			7	2	X			6	3	X
			shade	-3	-2			shade	-3	-2	
2.5GY	OL			2	5				2	3	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			0	0	0			0	0	0
10GY	Gr			1	2	0			2	1	0
			shade	-3	-2	-1		shade	-3	-2	-1
7.5GY	Br	EFB3si and 3sii		23	14	2	EFB3siii and 3siv		17	18	6
10GY	Br			8	3	X			7	2	X
			shade	-3	-2			shade	-3	-2	
2.5GY	OL	EFB4si and 4sii		13	16		EFB4siii and 4siv		10	18	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			2	4	1			3	7	2
10GY	Gr			8	5	1			6	4	0

Table 3Bd/1v. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour. Series 1.

Colour analysis scores of the EFB samples, adjusted for statistical analysis. Fifth instar.

Hue type	Colour type	Fed on Sorrel Sample					Fed on Plantain Sample				
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	EFB5si and 5sii		18	12	5	EFB5siii and 5siv		18	15	4
10YR	Br			8	6	X			7	2	X
	PY		1					4			
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			0	0	0			0	0	0
10GY	Gr			0	0	0			0	0	0
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	EFB6si and 6sii		12	5	1	EFB6siii and 6siv		10	7	2
10YR	Br			4	1	X			5	3	X
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			3	6	2			2	6	3
10GY	Gr			10	5	1			7	5	0
			shade	-3	-2	-1		shade	-3	-2	-1
7.5YR	Br	Totals		69	43	11	Totals		65	49	16
10YR	Br			27	12	X			25	10	X
	PY		1					4			
			shade	-3	-2			shade	-3	-2	
2.5GY	OL			15	21				12	21	
			shade	-1	0	1		shade	-1	0	1
7.5GY	Gr			8	22	7			9	28	7
10GY	Gr			35	24	5			34	17	3

sorrel-fed samples were different from the plantain-fed samples and that foodplant does affect larval colour in these instars.

Comparison of the results of the six pairs of samples, fed on sorrel during both the 4th and 5th instar quite obviously gives significant heterogeneity, as can be seen by comparing the results of samples EFB1si plus 1sii and EFB3si plus 3sii, the former containing 50 Gr larvae and the latter 50 Br larvae. The same situation occurs in both the 4th and 5th instar plantain-fed samples.

However, when chi-squared tests were carried out to compare the scores from sorrel-fed samples with those from plantain-fed samples, the result was not significant, (for 4th instar scores, $X^2_{13} = 9.350$, $0.8 > p > 0.7$; for 5th instar scores, $X^2_{13} = 6.262$, $0.95 > p > 0.9$), showing that whilst there was great variation between the colours from different broods, this was not affected by foodplant.

Again, similar tests were carried out on 3rd instar scores, and all three chi-squared tests gave highly significant probability values, (for comparison of 3rd instar sorrel-fed samples, $X^2_{40} = 146.898$, $p < 0.001$; for comparison of 3rd instar plantain-fed samples, $X^2_{40} > 120$, $p < 0.001$; for comparison between the sum scores of sorrel-fed samples and and the sum scores of plantain-fed samples, $X^2_8 = 91.442$, $p < 0.001$), which indicates that, in the 3rd instar, not only is there variation between the colours of larvae from different broods, but also that foodplant does affect larval colour in this instar.

Further analysis showed that samples fed on the same foodplant from four of the broods, (EFB1, EFB2, EFB4 and EFB5), were homogeneous, (for sorrel-fed samples, X^2_{21} = 3.507 , $p > 0.99$; for plantain-fed samples X^2_{21} = 6.134 , $p > 0.99$). These samples were also homogeneous to the sum of 2nd instar results of samples from the same broods fed on the same foodplant, (for sorrel-fed samples, X^2_7 = 11.510 , $0.2 > p > 0.1$; for plantain-fed samples, X^2_7 = 3.142 , $0.9 > p > 0.8$). A test comparing the 3rd instar results from the sorrel-fed samples, with the 3rd instar results of plaintain-fed samples from these four broods, gave highly significant results, (X^2_8 = 94.064 , $p < 0.001$), indicating that the samples were heterogeneous and that in these samples, foodplant affects larval colour in the 3rd instar, causing a result similar to that observed in 2nd instar larvae.

Samples from EFB3, when fed on sorrel, were different from sorrel-fed samples from the four aforementioned broods, ($X^2_8 > 80$, $p < 0.001$), and to samples fed on sorrel from EFB6, (X^2_8 = 36.963 , $p < 0.001$), the differences in both cases being highly significant. Similarly, plantain-fed samples from EFB3 were different from plantain-fed samples from the EFB1, 2, 4 and 5 broods, ($X^2_8 > 76.8$, $p < 0.001$), and to the plantain-fed samples from EFB6, (X^2_8 = 44.247 , $p < 0.001$), the differences again being highly significant. On the other hand, the EFB3 samples, fed on sorrel, whilst being significantly different to the EFB3 samples fed on plantain, were not highly so, (X^2_8 = 16.11 , $0.05 > p > 0.02$). Unlike the EFB1, 2, 4 and 5 samples, the EFB3 3rd instar scores were heterogeneous

to the 2nd instar scores from larvae fed on the same foodplant, (for sorrel-fed samples, $X^2_7 > 25$, $p < 0.001$; for plantain-fed samples, $X^2_6 > 24$, $p < 0.001$). These tests, then, show that whilst foodplant probably does affect 3rd instar larval colour in samples from EFB3, the result of this influence is different from that produced by foodplant in any of the other broods, or in EFB3 2nd instar larvae.

When similar tests were carried out on the results of the EFB6 samples, chi-squared values showed that samples fed on sorrel were homogeneous to those of the EFB1, 2, 4 and 5 broods which were fed on the same foodplant, ($X^2_7 = 1.697$, $0.98 > p > 0.95$), whilst those fed on plantain were different from those of EFB1, 2, 4 and 5 fed on the same foodplant, ($X^2_7 = 44.849$, $p < 0.001$). Furthermore, the EFB6 samples fed on sorrel were homogeneous with EFB6 samples fed on plantain, ($X^2_7 = 4.746$, $0.8 > p > 0.7$), and perhaps more importantly, plantain-fed samples from EFB6 were homogeneous with the sum of sorrel-fed samples from broods EFB1, 2, 4 and 5, ($X^2_7 = 9.443$, $0.3 > p > 0.2$). Moreoever, the 3rd instar sorrel-fed samples from EFB6 were homogeneous with the 2nd instar sorrel-fed samples from the same brood, ($X^2_7 = 2.319$, $0.95 > p > 0.9$), whilst comparison of the results from the 2nd and 3rd instar plantain-fed samples of this brood showed them to be heterogeneous, ($X^2_7 = 21.384$, $0.01 > p > 0.001$). The 3rd instar larval colour of the EFB6 samples, then, seems to be unaffected by foodplant, or at least, if there is an effect, it is not evident in these experiments.

Discussion.

From these results it was deduced that foodplant affects larval colour in the 1st and 2nd instars, but not in the 4th or 5th instars. In the 3rd instar, larval colour may be affected by foodplant, but the homogeneity of sorrel-fed samples from EFB6 with those from the same brood fed on plantain show that this is not always the case. Breeding experiments (see chapter 3C) indicated that the reason for this was that the mechanism controlling colour in the 3rd instar involves a switch-gene as well as foodplant, and because of this, the full mechanism of colour control in the 3rd instar will be explained in chapter 3C.

Further experiments.

Once evidence of a connection between foodplant and larval colour had been established, at least for the early instars, two further sets of experiments were carried out. These were designed to determine the extent to which foodplant could affect larval colour.

Firstly, a series of 16 samples of 25 larvae, taken at random from the main stock were reared. These were named the "second environmental foodplant samples" (SEF) and the "second environmental foodplant reserve samples" (SEFR). Eight of them, (SEF1 - 6 inclusive, SEFR1 and SEFR2), were reared on sorrel, the other eight samples, (SEF7 - 12, SEFR3 and SEFR4), being reared on plantain. The conditions of rearing were identical to those used for the initial foodplant experiments, except that the leaves given to the larvae of

samples SEF1 - 3, SEF7 - 9, SEFR1 and SEFR3 were changed every 12 hours and were carefully selected for uniformity of colour, and all the stems, petioles and main-veins of the leaves were removed. The leaves given to the other samples were not treated in this way. This, then, gave four treatments, with three samples to be scored being submitted to each (samples, SEFR1 - 4 inclusive, were kept as reserves and were not scored).

Results.

The colour score groups used, were the same as those in the environmental temperature experiments, and were again adjusted, where appropriate, for statistical tests. Again, the results of samples submitted to exactly the same treatment could be seen by inspection to be homogeneous, and so the results from such samples have been added together. The results are shown in Appendix Table 3Bd/2i, and the results adjusted for statistical purposes are given in Table 3Bd/2i.

Comparison of the four sets of results for each instar gave highly significant chi-squared values for 1st, 2nd and 3rd instar scores,

(for 1st instar scores, $X^2_{21} = 132.402$, $p < 0.001$;
for 2nd instar scores, $X^2_{21} = 134.500$, $p < 0.001$;
for 3rd instar scores, $X^2_{30} = 87.017$, $p < 0.001$)

whilst the chi-squared values for 4th and 5th instar scores showed homogeneity, (for 4th instar scores, $X^2_{27} = 4.614$, $p > 0.99$; for 5th instar scores, $X^2_{30} = 4.111$, $p > 0.99$).

Comparing the sum scores of the three samples submitted

Table 3Bd/2i.

Experiments to investigate the effect of foodplant colour on larval colour. Colour analysis scores for the SEF samples, adjusted for statistical analysis. All instars.

Instar	Hue type	Colour type	Samples SEF 1 - 3 Fed on Sorrel leaves picked for uniformity of colour					Samples SEF 4 - 6 Fed on Sorrel leaves picked randomly					Samples SEF 7 - 9 Fed on Plantain leaves picked for uniformity of colour					Samples SEF 10 - 12 Fed on Plantain leaves picked randomly					Totals				
			shade	-3	-2	-1	0	shade	-3	-2	-1	0	shade	-3	-2	-1	0	shade	-3	-2	-1	0	shade	-3	-2	-1	0
First	7.5GY			0	6	26	24		2	10	18	18		6	35	6	1		12	23	11	3		20	74	61	46
	10GY			0	3	13	3		1	9	8	9		3	18	6	0		7	10	9	0		11	40	36	12
Second	7.5GY		shade	-3	-2	-1	0	shade	-3	-2	-1	0	shade	-3	-2	-1	0	shade	-3	-2	-1	0	shade	-3	-2	-1	0
	10GY			0	4	29	23		2	11	19	17		5	38	6	0		11	26	12	1		18	79	66	41
				0	4	10	5		1	9	9	7		3	16	7	0		6	8	10	1		10	37	36	13
Third		Br	2					2					2					2					8				
		Y	1					2					0					1					4				
	2.5GY	OL	shade	-3	-2			shade	-3	-2			shade	-3	-2			shade	-3	-2			shade	-3	-2		
				3	6				3	5				2	5				3	4				11	20		
	7.5GY	3IG	shade	-2	-1	0	1	shade	-2	-1	0	1	shade	-2	-1	0	1	shade	-2	-1	0	1	shade	-2	-1	0	1
	10GY	3IG		0	7	26	4		0	10	12	6		7	28	7	2		10	15	12	5		17	60	57	17
				1	6	13	6		2	9	11	13		3	14	4	1		6	10	6	1		11	40	34	21
Fourth		Br	9					9					9					9					36				
		Y	2					2					2					2					8				
	2.5GY	OL	shade	-3	-2			shade	-3	-2			shade	-3	-2			shade	-3	-2			shade	-3	-2		
				16	16				16	16				12	20				15	17				59	69		
	7.5GY	Gr	shade	-1	0	1		shade	-1	0	1		shade	-1	0	1		shade	-1	0	1		shade	-1	0	1	
	10GY	Gr		5	6	1			6	5	2			4	7	2			3	8	2			18	26	7	
				13	6	1			11	7	1			12	6	1			11	6	2			47	25	5	
Fifth	7.5YR	Br	shade	-3	-2	-1		shade	-3	-2	-1		shade	-3	-2	-1		shade	-3	-2	-1		shade	-3	-2	-1	
	10YR	Br		17	13	4			16	14	4			13	11	4			13	14	3			59	52	15	
				10	3	X			8	5	X			9	4	X			10	3	X			37	15	X	
		Y	2					2					2					2					8				
	2.5GY	OL	shade	-3	-2			shade	-3	-2			shade	-3	-2			shade	-3	-2			shade	-3	-2		
				6	6				5	7				7	9				6	8				24	30		
	10GY	Gr	shade	-1	0	1		shade	-1	0	1		shade	-1	0	1		shade	-1	0	1		shade	-1	0	1	
				7	4	3			6	5	3			7	5	4			7	5	4			27	19	14	

to each treatment with those of other results individually gave the following results,

(For 1st instar scores:

comparing SEF1 - 3 scores with SEF4 - 6 scores, $X^2_7 = 13.502$, $0.1 > p > 0.05$; comparing SEF1 - 3 scores with SEF7 - 9 scores, $X^2_7 = 79.464$, $p < 0.001$; comparing SEF1 - 3 scores with SEF10 - 12 scores, $X^2_7 = 58.88$, $p < 0.001$; comparing SEF4 - 6 scores with SEF7 - 9 scores, $X^2_7 = 50.384$, $p < 0.001$; comparing SEF4 - 6 scores with SEF10 - 12 scores, $X^2_7 = 38.278$, $p < 0.001$; comparing SEF7 - 9 scores with SEF10 - 12 scores, $X^2_6 = 11.438$, $0.1 > p > 0.05$.

For 2nd instar scores:

comparing SEF1 - 3 scores with SEF4 - 6 scores, $X^2_7 = 11.560$, $0.2 > p > 0.1$; comparing SEF1 - 3 scores with SEF7 - 9 scores, $X^2_7 = 86.368$, $p < 0.001$; comparing SEF1 - 3 scores with SEF10 - 12 scores, $X^2_7 = 64.348$, $p < 0.001$; comparing SEF4 - 6 scores with SEF7 - 9 scores, $X^2_7 = 50.134$, $p < 0.001$; comparing SEF4 - 6 scores with SEF10 - 12 scores, $X^2_7 = 33.880$, $p < 0.001$; comparing SEF7 - 9 scores with SEF10 - 12 scores, $X^2_7 = 12.796$, $0.1 > p > 0.05$.

For 3rd instar scores:

comparing SEF1 - 3 scores with SEF4 - 6 scores, $X^2_9 = 13.498$, $0.2 > p > 0.1$; comparing SEF1 - 3 scores with SEF7 - 9 scores, $X^2_{10} = 27.382$, $p < 0.001$; comparing SEF1 - 3 scores with SEF10 - 12 scores, $X^2_{10} = 33.424$, $p < 0.001$; comparing SEF4 - 6 scores with SEF7 - 9 scores, $X^2_{10} = 30.460$, $p < 0.001$; comparing SEF4 - 6 scores with SEF10 - 12 scores, $X^2_{10} = 25.484$, $p < 0.001$; comparing SEF7 - 9 scores with SEF10 - 12 scores, $X^2_{10} = 15.452$, $0.2 > p > 0.1$).

These results show that in every case when sorrel-fed samples were compared with plantain-fed samples, the results showed a highly significant difference. On the other hand, comparisons between samples fed on uniformly picked leaves and samples fed on the same foodplant, but not specifically selected for uniformity of colour, produced non-significant results in all cases. However, it should be noted that, the probability values from these tests are in all cases only just above the 0.05 level, the highest being less than 0.2. Furthermore, if the unadjusted scores are considered, (Appendix Tables 3Bd/2i), it is fairly evident that the samples given leaves which were selected for colour uniformity, vary less than those which were submitted to randomly picked leaves. It is my belief that, if the samples used had been larger, significant differences between the colour scores of samples fed on uniformally picked leaves and the colour scores of larvae fed on randomly picked leaves, would result; however, this is only a subjective view and further experiments are needed to substantiate it.

If this view were substantiated, the results would indicate that larval colour is greatly influenced by foodplant in the early instars. This is shown by the fact that colour is affected by foodplant not only when larvae are fed on different species of plant, but also when two samples of larvae are fed on leaves of the same plant, the only difference being that the variation in the foodplant colour fed to one sample is less than in that fed to the other. The difference in colour variation in this latter case is probably due to a

decrease in variability in those larvae fed on the more uniformally coloured foodplant.

The final set of tests were designed firstly, to investigate the extent to which larval colour could be influenced by foodplant in the 1st and 2nd instars by using a range of foodplants of different types and colours. Secondly, by rearing some samples in total darkness, it was hoped that the experiments would ascertain whether the effect of foodplant was caused wholely as a result of the larvae ingesting the foodplant, or whether the foodplant colour may also cause an effect by stimulating the ocelli or some other sensory receptor. The latter possibility seems less likely, as the results from the environment light experiments produced no evidence to suggest a connection between background colour and larval colour. However, this possibility can not be rejected completely on this evidence, as the total effect of foodplant on larval colour may be the result of an interaction involving the nature and colour of the ingested material, and visual influences caused by the larva's immediate surroundings.

The test design resulted in a total of 70 samples of 25 larvae, from the main stock resulting from the CAB broods, being reared. The samples were split into five groups of 14 samples, and each of the 14 samples in each group were fed on one of 14 foodplant types. Group A samples were reared under the same conditions as the EFB samples, apart from the differences in foodplant. Group C and D samples were treated in the same way, but were not illuminated at any time. These samples were reared and scored using similar methods to those

outlined in chapter 3Bc. Due to the nature of the darkness - scoring method, sample C was scored in the 1st instar and sample D in the 2nd instar. Samples from groups B and E were not scored being kept as reserves for group A or group C and D samples respectively. Samples from group B were reared under the same conditions as those from group A, and group E samples were reared in the same way as those from groups C and D.

The 14 foodplants used were numbered 1 - 14, as shown in Table 3Bd/3i, which also shows their approximate colour according to the Munsell colour chart. The 42 scored samples are referred to as the "third environment foodplant samples (TEF) and were numbered using TEF, followed by the appropriate group letter and foodplant number.

In many cases, the unusual foodplants fed to larvae led to very slow development, and high mortality. For this reason, larvae were generally only kept on those foodplants for the 1st and 2nd instars, and were only scored in these instars.

Results.

Due to the wide variety of colours produced in larvae by these tests, many of the larvae could not be scored using the Majerus colour chart, and therefore, all the larvae were scored using the Munsell colour chart. Because of the comparatively wide divisions between colours in this chart, the "matches" were often very approximate. The results are given in Table 3Bd/3ii. Plates 5 - 9 inclusive show the range of larval colours which are obtainable by feeding larvae on a

Table 3Bd/3i.

Foodplants used in the third set of foodplant experiments, fed to the TEF samples.

Foodplant number	Foodplant (flower petals unless stated)	Colour	Munsell colour score
1	Broom	Yellow	2.5Y 8/10
2	Broom	Yellow part	2.5Y 7/10
		Red part	7.5R 4/8
3	Rose	Dark crimson	5R 5/12
4	Rose	Flame red	2.5YR 6/8
5	Rose	Pink	2.5R 5/10
6	Rose	Pale pink	2.5Y 9/2
7	Rose	Yellowish-pink	2.5Y 9/4
8	Rose	Very pale pink-white	10R 9/1
9	Pansy	Navy blue	7.5B 6/6
10	Pansy	Pale blue part	2.5PB 6/4
		Purple part	7.5PB 6/8
11	Rhodedendron	Mauve	5RP 4/8
12	Dock seeds	Red part	2.5R 6/10
		Green part	7.5GY 8/6
13	Geranium leaves (lacking chlorophyll)	White	2.5G 9/1
14	Synthetic diet (lacking plant pigments)	Transluscent white	-

Table 3Bd/3ii.

Experiments to investigate the effect of foodplant colour on larval colour.

Series 3. Colour analysis scores for the TEF samples. First and Second instar larvae.

Colours (Munsell indexing)	Dominant Colour	First instar results		Total	Second instar results		Total	Notes
		Sample TEFA1	Sample TEFC1		Sample TEFA1	Sample TEFD1		Frass varies from pale yellow to dark brown. Outer tissues very pale green.
2.5Y8/12	Yellow	14	10	24	11	13	24	
2.5Y9/6	Yellow	6	8	14	7	4	11	
5Y9/8	Yellow	3	3	6	6	4	10	
2.5GY8/4	Yellow/Green	0	0	0	0	2	2	
5GY10/4	Green	2	4	6	1	2	3	
		Sample TEFA2	Sample TEFC2		Sample TEFA2	Sample TEFD2		
5RP4/2	Purple/Grey	1	0	1	0	0	0	Frass, various shades of red, pink, orange and brown. Colour palest in posterior segments. Head capsule pale mauve or pale red.
5R3/2	Crimson/Grey	2	1	3	2	3	5	
7.5R3/2	Crimson/Grey	10	14	24	16	13	29	
7.5R4/2	Crimson/Grey	4	3	7	3	3	6	
2.5Y8/12	Yellow	3	4	7	3	4	7	Frass varies from pale yellow to brown. Outer tissues very pale green. Larvae scored in Y or GY colour groups are thought to have been feeding on the yellow parts of the petals only.
2.5Y9/6	Yellow	2	1	3	1	1	2	
5Y9/8	Yellow	2	2	4	0	1	1	
2.5GY8/4	Yellow/Grey	1	0	1	0	0	0	

Table 3Bd/3ii. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour.

Series 3. Colour analysis scores for the TEF samples. First and Second instar larvae.

Colours (Munsell indexing)	Dominant Colour	First instar results		Total	Second instar results		Total	Notes
		Sample TEFA3	Sample TEFC3		Sample TEFA3	Sample TEFD3		Frass varies from orange to brown. Outer tissues pale green. Very difficult to score due to the contrast of the central and outer tissue colours. The head capsule of larvae scored in the R colour groups was pale pink.
5R3/2	Crimson/Grey	6	8	14	9	7	16	
5R4/2	Crimson/Grey	15	11	26	10	14	24	
7.5R4/2	Crimson/Grey	3	2	5	3	2	5	
10R3/2	Crimson/Grey	1	4	5	3	1	4	
2.5GY4/4	Yellow/Green	0	0	0	0	1	1	
		Sample TEFA4	Sample TEFC4		Sample TEFA4	Sample TEFD4		Frass, various shades of pink, orange, red and brown. Outer tissues rather translucent in appearance, and tinged with various shades of green. Head capsule reddish-orange. Again, larvae were very difficult to score with any degree of accuracy.
5R4/10	Red	2	0	2	1	2	3	
7.5R5/8	Red	3	3	6	2	4	6	
7.5R6/8	Red	2	1	3	0	1	1	
10R4/10	Red	3	7	10	6	3	9	
10R5/10	Red	9	11	20	13	10	23	
10R6/8	Red	5	3	8	2	4	6	
5RY6/6	Orange	0	0	0	1	1	2	
10RY9/4	Ochre	1	0	1	0	0	0	

Table 3Bd/3ii. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour.

Series 3. Colour analysis scores for the TEF samples. First and Second instar larvae.

Colours (Munsell indexing)	Dominant Colour	First instar results		Total	Second instar results		Total	Notes
		Sample TEFA5	Sample TEFC5		Sample TEFA5	Sample TEFD5		Frass varies from orange to brown. The head capsule is pink, with or without a distinct greenish tinge. Outer tissues rather translucent and usually pale pink or pinkish-orange.
2.5R4/10	Pink/Red	3	6	9	2	5	7	
2.5R5/10	Pink/Red	5	6	11	9	10	19	
5R5/10	Red	6	7	13	9	4	13	
5R6/8	Red	4	3	7	3	4	7	
5R7/8	Red	4	2	6	1	2	3	
7.5R8/6	Red	2	1	3	1	0	1	
10R8/2	Red/Orange	1	0	1	0	0	0	
		Sample TEFA6	Sample TEFC6		Sample TEFA6	Sample TEFD6		Frass varies from yellow through orange to brown. Outer tissue, pale green. Occasionally, the anterior end of the larvae was of a more reddish colour, and the anal segments were often of a greenish hue. Head capsule, pale pinkish-orange.
2.5YR7/6	Orange	1	0	1	1	1	2	
2.5YR8/4	Orange	2	4	6	2	3	5	
5YR7/6	Orange	4	4	8	5	6	11	
5YR8/4	Orange	16	12	28	13	15	28	
5YR9/2	Pale Ochre	2	5	7	4	0	4	

Table 3Bd/3ii. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour.

Series 3. Colour analysis scores for the TEF samples. First and Second instar larvae.

Colours (Munsell indexing)	Dominant Colour	First instar results		Total	Second instar results		Total	Notes
		Sample TEFA7	Sample TEFC7		Sample TEFA7	Sample TEFD7		Frass, various shades of pink and pale to mid-brown. Anterior segments usually darker than the rest. Anal segments always pale green.
5YR8/4	Pale Pink/Yellow	1	3	4	2	0	2	
7.5YR8/4	Pale Pink/Yellow	2	3	5	1	4	5	
10YR9/2	Pale Yellow	2	4	6	3	7	10	
2.5Y9/2	Pale Yellow	5	2	7	3	5	8	
5Y9/2	Pale Yellow	10	11	21	13	8	21	
7.5Y9/2	Pale Yellow	5	2	7	3	1	4	
		Sample TEFA8	Sample TEFC8		Sample TEFA8	Sample TEFD8		Frass, various shades of pink and pale to mid-brown. Anterior segments usually darker than the rest. Anal segments always pale green.
7.5YR9/2	Pale Pink/Yellow	4	5	9	3	2	5	
10YR9/2	Pale Yellow	9	6	15	2	6	8	
2.5Y9/2	Pale Yellow	11	10	21	15	15	30	
2.5Y9/4	Pale Yellow	1	4	5	5	2	7	

Table 3Bd/3ii. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour.

Series 3. Colour analysis scores for the TEF samples. First and Second instar larvae.

Colours (Munsell indexing)	Dominant Colour	First instar results		Total	Second instar results		Total	Notes
		Sample TEFA9	Sample TEFC9		Sample TEFA9	Sample TEFD9		Frass green. Anterior few segments often tinged with blue.
7.5Y6/4	Yellow	2	1	3	0	0	0	Head capsule usually of a pale
7.5Y8/4	Yellow	4	2	6	3	1	4	blue-green colour, but occasionally
10Y5/6	Yellow/Green	1	4	5	1	3	4	blue. Development on this food-
10Y6/4	Yellow/Green	2	4	6	5	2	7	plant was very slow. Mortality
10Y8/6	Yellow/Green	3	1	4	3	5	8	was high.
2.5YG6/6	Yellow/Green	6	6	12	7	6	13	
5YG6/6	Yellow/Green	6	4	10	3	5	8	
7.5YG7/10	Green/Yellow	1	3	4	3	3	6	
		Sample TEFA10	Sample TEFC10		Sample TEFA10	Sample TEFD10		Frass green. Head capsule and anterior two or three segments often
7.5Y8/4	Yellow	2	1	3	0	0	0	much darker than the rest, and of a
7.5Y9/2	Pale Yellow	3	2	5	4	2	6	bluish colour. Development was
10Y6/6	Yellow	3	5	8	1	4	5	very slow on this foodplant.
10Y8/4	Yellow	5	2	7	4	4	8	
10Y9/2	Pale Yellow	6	5	11	7	4	11	
2.5YG8/4	Yellow/Green	4	5	9	6	9	15	
5YG9/2	Lemon Yellow	2	5	7	3	2	5	

Table 3Bd/3ii. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour. Series 3. Colour analysis scores for the TEF samples. First and Second instar larvae.

Colours (Munsell indexing)	Dominant Colour	First instar results		Total	Second instar results		Total	Notes
		Sample TEFA11	Sample TEFC11		Sample TEFA11	Sample TEFD11		Frass, various shades of red and brown. Outer tissues translucent of a green colour with a pinkish tinge. Posterior two or three segments of a pale pink colour.
7.5G4/4	Green	3	1	4	3	1	4	
10G4/4	Green	7	12	19	11	6	17	
10G6/4	Green	8	5	13	4	8	12	
2.5BG4/4	Green	4	1	5	4	5	9	
2.5BG6/4	Green	2	4	6	2	4	6	
5BG4/4	Turquoise	1	2	3	1	1	2	
		Sample TEFA12	Sample TEFC12		Sample TEFA12	Sample TEFD12		Frass, pink, green or brown. Very difficult to score due to variability in colour.
5R8/6	Pale Red/Grey	3	4	7	2	4	6	Thought to have been feeding mainly on the red parts of the pods. Head capsule, anterior two or three segments and anal segments usually much paler than the rest. 12th and occasionally 11th segment, often much brighter than rest.
5R8/8	Pale Red/Grey	9	13	22	9	4	13	
7.5R8/6	Pale Red/Grey	4	1	5	1	2	3	
7.5GY5/6	Green/Yellow	3	4	7	3	6	9	Thought to have been feeding mainly on the green part of the pods. Outer tissues pale grey-green.
10GY6/10	Green	3	1	4	7	3	10	
10GY7/8	Green	3	2	5	3	6	9	

Table 3Bd/3ii. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour.

Series 3. Colour analysis scores for the TEF samples. First and Second instar larvae.

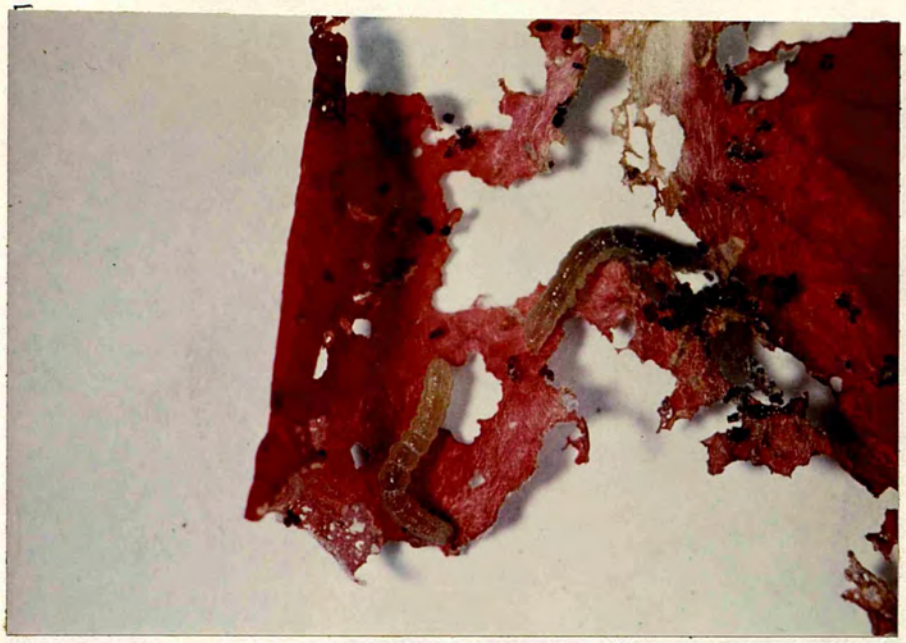
Colours (Munsell indexing)	Dominant Colour	First instar results		Total	Second instar results		Total	Notes
		Sample TEFA13	Sample TEFC13		Sample TEFA13	Sample TEFD13		Frass yellow, ochre or pale brown. Head capsule of a very pale yellow colour. The anterior few segments were generally of a greyish-yellow colour. Outer tissues rather translucent with a pale yellow tinge.
10YR9/2	Pale Ochre	3	3	6	1	4	5	
2.5Y9/2	Pale Yellow	16	20	36	21	12	33	
2.5Y9/4	Pale Yellow	1	0	1	1	3	4	
5Y9/2	Pale Yellow	5	2	7	2	8	10	
		Sample TEFA14	Sample TEFC14		Sample TEFA14	Sample TEFD14		Frass yellow, ochre or pale brown. Head capsule of a very pale yellow colour. The anterior few segments were generally of a greyish-yellow colour. Outer tissues rather translucent with a pale yellow tinge.
10YR9/2	Pale Ochre	8	10	18	12	10	22	
10YR9/4	Pale Yellow	5	5	10	2	0	2	
2.5Y9/2	Pale Yellow	9	6	15	6	8	14	
2.5Y9/4	Pale Yellow	3	4	7	5	7	12	

Plate 5

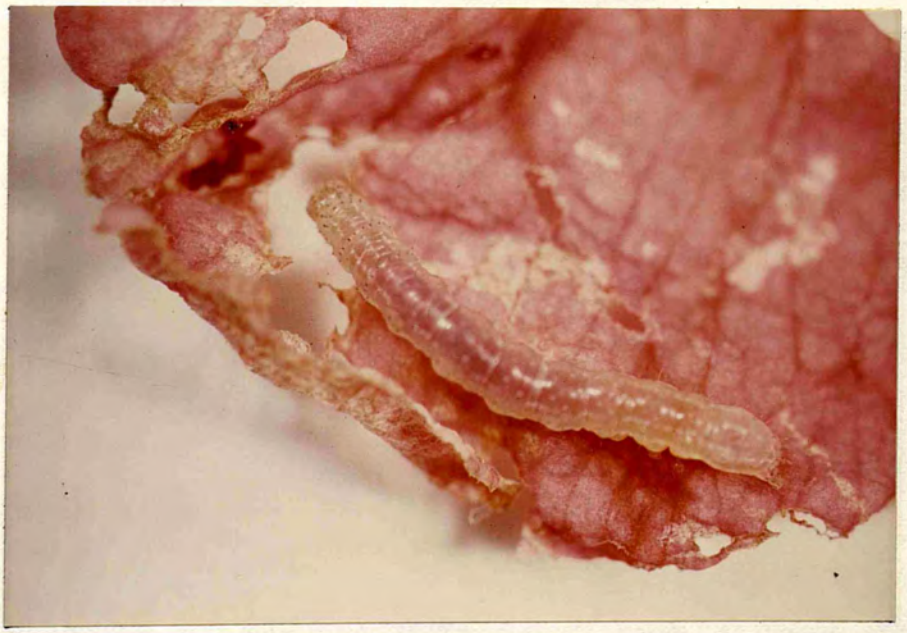
(a) 2nd instar larva. x 12
From TEFA1, fed on yellow broom petals.

(b) 2nd instar larva. x 12
From TEFA2, fed on yellow and red broom petals.

Plate 6



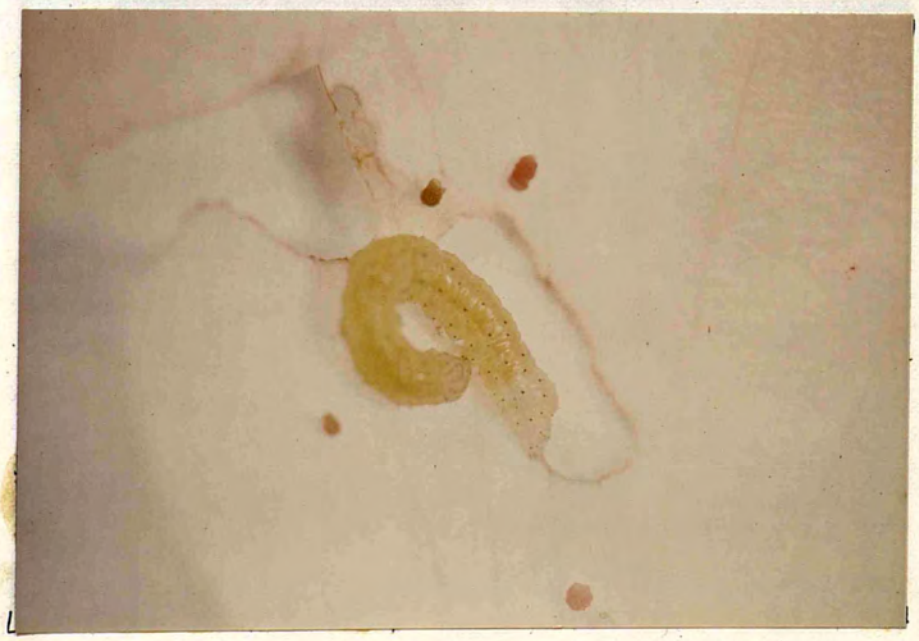
(a) 2nd instar larvae. x 4
From TEFA3, fed on dark crimson rose petals.



(b) 2nd instar larva. x 12
From TEFA5, fed on pink rose petals.

Plate 7

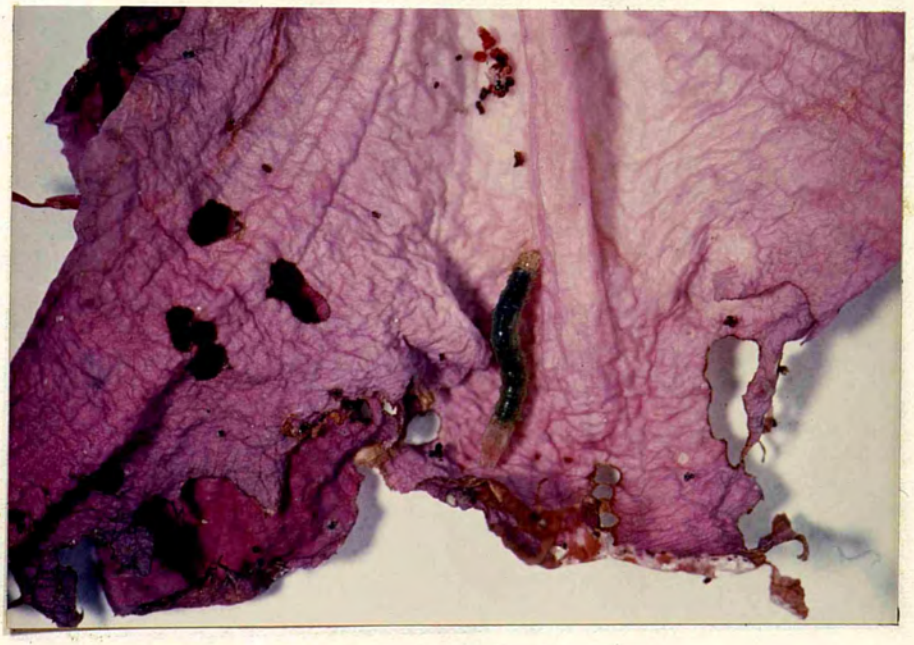
(a) 2nd instar larva. x 12
From TEFA7, fed on yellowish pink rose petals.



(b) 2nd instar larva. x 12
From TEFA8, fed on very pale pink-white rose petals.

Plate 8

(a) 2nd instar larva. x 12
From TEFA10, fed on blue and purple pansy petals.



(b) 2nd instar larva. x 4
From TEFA11, fed on mauve rhodedendron petals.

Plate 9

(a) 2nd instar larva. x 12
From TEFA12, fed on red and green dock seed pods.

(b) 2nd instar larva. x 12
Fed on common sorrel leaves.

variety of foodplants.

With the exceptions of those fed on pansy, rhodedendron, very pale pink/white rose, geranium and synthetic diet, the majority of the larvae in each sample assumed a colour similar to that of their food. The colour of the frass varied considerably, but in most cases seemed to be influenced by the colour of the foodplant.

Larvae fed on mauve rhodedendron assumed a dark green or turquoise colour, whilst those fed on pansy became pale green or yellow-green in colour. Those fed on very pale pink/white rose petals, chlorophyll deficient geranium leaves, or the synthetic diet assumed a very pale-ochre or yellow colour. Microscopic examination indicated that this was due to cuticular and epidermal pigments.

The results of the group A samples were compared with those from C and D, in the appropriate instar, for each foodplant, by a series of chi-squared tests. The results of these tests are given in Appendix Table 3Bd/3i. Only one of these tests gave a significant result, and this was only just significant. It therefore seems that the effect of foodplant on the colour of larvae is not influenced by any sort of visual stimulation.

Finally, a number of larvae from other stocks were fed on sorrel during the 1st instar, and transferred on to other foodplants about 24 hours after their 1st larval ecdysis. These larvae changed colour assuming a colour similar to that of the new foodplant soon after commencing to feed on it. The colour changes in these larvae began at the anterior end

of the insect and proceeded down to the posterior end. This feature, coupled with the fact that fresh frass in most cases was more or less similar to that of the foodplant, indicates that larval colour in early instars is due mainly to the colour of the gut contents. Obviously, the overall appearance of the larvae will also be affected by the colour of the cuticular, epidermal and other pigments, but these are usually pale in colour, and at least partly translucent.

Conclusions.

In conclusion, then, the results of the experiments to investigate the effect of temperature, larval density, light and foodplant on larval colour, show that the only factor of these which does have an effect is foodplant. There is evidence of a very close correlation between foodplant and larval colour in the 1st and 2nd instars. This correlation also occurs in the 3rd instar, but not in all cases. Foodplant seemingly has little effect on 4th and 5th instar larval colour.

It seems then, that colour in these later instars must be controlled either genetically or by some other, as yet untested, environmental factor; the former possibility being indicated as the more likely by the results of the EFB samples, where variation in the later instars, between broods kept under identical conditions, was considerable. In any case, the colour variation in the later instars is not controlled in the same way as that in the early instars, the change in the nature of the controlling factor seeming to take place at the start of the 3rd or 4th instar.

If the conclusions of the colour variation analysis are considered (chapter 3A), it may be seen that, to a large extent, the hypothesis that 1st and 2nd instar variation is controlled environmentally or by a polygenic system is supported as far as it goes by the environmental experiment results. It has, in fact, been shown that 1st and 2nd instar variation is foodplant controlled. Similarly, the theory that 4th and 5th instar variation is controlled by a small number of major genes or by environmental factors under the influence of a threshold switch effect is not disputed in any way by the results of the environment experiments, although they do indicate that the former possibility is the more likely. However, the results of the foodplant environmental experiments led to a different view of the colour analysis results for 3rd instar larvae. The analysis of variation in 3rd instar larval colour led originally to the deduction that 3rd instar colour was controlled by similar factors to those controlling 4th and 5th instar variation. Evidence from the first foodplant experiments show that 3rd instar colour variation is in some cases foodplant controlled, whilst 4th and 5th instar variation is not. The differences in the distribution of 3rd and 4th instar colour analysis scores, and in particular the changes between the limits of the EG, 3IG and Gr colour types, leads to the deduction that the 3rd instar colour variation may be the result of a combination between 2nd and 4th instar variation control mechanisms. Then, the colour of the majority of larvae would be foodplant controlled, whilst

the colour of a smaller number of larvae, including all those in the OL, Br, PY, YG and YB main colour types, and some of the larvae in the 3IG colour type, would be controlled by a different mechanism, probably similar to that controlling 4th instar larval colour. Which of the two mechanisms controls variation in particular larvae is probably determined genetically.

C. The investigation into the possibility of larval colour variation being controlled by a genetic mechanism.

Methods and Tabulation of results.

To investigate the possibility of a genetic mechanism being involved in the control of larval colour variation in the later instars, 67 initial broods (IB1 - 67 inclusive) were reared. All these broods and a large number of subsequent broods were reared using the basic rearing methods outlined in chapter 2B. All the larvae were fed on sorrel. As these broods were all kept under similar conditions and yet still showed wide variation in the colour of the 3rd, 4th and 5th instar larvae, both within a single brood, and between broods, a genetic mechanism of control was indicated. Analysis of the results of the initial broods and subsequent control crosses, has led to an explanation of the larval colour variation in later instars which is based upon five unlinked major genes, and involves a complex system of epistatic interactions.

To explain the constitution of the genetic mechanism and the way in which it was worked out, only the results from initial broods (IB1 - 9 inclusive and IB46), together with 30

of the control crosses (CC1 - 12 inclusive, and YCC1 - 18 inclusive), will be discussed in detail.

The results of broods IB1 - 9 inclusive, and CC1 - 12 inclusive are tabulated in Table 3Ci, (p 137), those of IB46 and YCC1 - 18 inclusive, in Table 3Cii, (p 152), whilst the results of other main stock broods which are specifically mentioned in the text are tabulated in Appendix Table 3Ci. (In all over 600 broods have been bred, and the results of all those not mentioned in the text agree with the conclusions outlined in the following text.

The method of scoring larvae was as that described in chapter 2C, but the treatment of the results before tabulation needs some explanation. Quite simply, all larvae which fell within the limits of one or other of the colour types or main colour types defined and limited in chapter 3A, were assigned to that colour type. Thus, 1st and 2nd instar larvae were placed in one of two groups, either being assigned to the early green (EG) colour type, or, if they fell outside the limits of this group, they were classed as "others". 3rd, 4th and 5th instar larvae were placed in one of seven colour types. For 3rd instar larvae, these were 3rd instar green (3IG), olive (OL), brown (Br), plain yellow (PY), yellow-green (YG), yellow-brown (YB), and again a class called "others" which contained all larvae which did not fall into one of the former six classes. For 4th and 5th instar larvae the same classes were used with the exception of 3IG which was replaced by the green main colour type (Gr). From all the IB and CC broods reared, only 49 larvae were placed in the "others" class at any stage, and so results from these larvae are tabulated separately in Table 3Ciii.

Table 3Ci.

Colour type analysis of a number of the broods bred to determine the mechanism controlling the colours Green, Olive and Brown in the third, fourth and fifth instars.

Brood number	Origin of parent 1	Phenotype of parent 1	Genotype of parent 1 Alleles of genes: C	B	A	Origin of parent 2	Phenotype of parent 2	Genotype of parent 2 Alleles of genes: C	B	A	Number of eggs laid	Number of 1st instar larvae	Number of 2nd instar larvae	Third instar larvae of colour types: Gr	OL	Br	Fourth instar larvae of colour types: Gr	OL	Br	Fifth instar larvae of colour types: Gr	OL	Br
IB1	Wild	Unknown	(--)	'o	--	Wild	Unknown	(+-)	oo	--	201	189	187	86	49	44	-	92	82	-	86	81
IB2	Wild	Unknown	xx	''	--	Wild	Unknown	xx	''	--	183	165	164	159	-	-	156	-	-	155	-	-
IB3	Wild	Unknown	(++)	'o	++	Wild	Unknown	(xx)	'o	xx	53	52	51	46	-	-	10	26	9	-	-	43
IB4	Wild	Unknown	--	oo	xx	Wild	Unknown	--	oo	xx	217	213	202	-	-	193	-	-	192	-	-	187
IB5	Wild	Unknown	xx	''	+-	Wild	Unknown	xx	''	--	317	294	293	290	-	-	287	-	-	137	-	146
IB6	Wild	Unknown	(++)	''	--	Wild	Unknown	(xx)	oo	--	160	144	139	137	-	-	-	132	-	-	132	-
IB7	Wild	Unknown	++	oo	xx	Wild	Unknown	xx	oo	xx	62	60	59	56	-	-	-	-	50	-	-	49
IB8	Wild	Unknown	(++)	''	--	Wild	Unknown	(xx)	'o	--	169	162	160	157	-	-	72	75	-	66	69	-
IB9	Wild	Unknown	(++)	('o)	++	Wild	Unknown	(xx)	(oo)	xx	109	103	98	92	-	-	-	46	42	-	-	84

Brood number	Origin of Male parent	Phenotype of Male parent	Genotype of Male parent Alleles of genes: C	B	A	Origin of Female parent	Phenotype of Female parent	Genotype of Female parent Alleles of genes: C	B	A	Number of eggs laid	Number of 1st instar larvae	Number of 2nd instar larvae	Third instar larvae of colour types: Gr	OL	Br	Fourth instar larvae of colour types: Gr	OL	Br	Fifth instar larvae of colour types: Gr	OL	Br
CC1	IB1	3Gr4OL5OL	+-	'o	--	IB1	3Gr4OL5OL	+-	'o	--	76	71	66	52	8	4	14	31	16	14	31	16
CC2	IB2	3Gr4Gr5Gr	xx	''	--	IB3	3Gr4Gr5Br	xx	''	++	272	256	240	236	-	-	235	-	-	-	-	228
CC3	IB3	3Gr4Gr5Br	xx	''	++	IB2	3Gr4Gr5Gr	xx	''	--	148	138	137	136	-	-	133	-	-	-	-	130
CC4	IB2	3Gr4Gr5Gr	xx	''	--	IB3	3Gr4Gr5Br	xx	''	+-	78	76	72	72	-	-	72	-	-	34	-	37
CC5	CC2	3Gr4Gr5Br	xx	''	+-	CC3	3Gr4Gr5Br	xx	''	+-	64	61	60	60	-	-	56	-	-	12	-	43
CC6	CC3	3Gr4Gr5Br	xx	''	+-	CC4	3Gr4Gr5Br	xx	''	+-	166	165	160	157	-	-	153	-	-	44	-	108
CC7	CC1	3Gr4Br5Br	(++)	oo	xx	CC1	3Gr4Br5Br	(+x)	oo	xx	89	84	79	78	-	-	-	-	74	-	-	73
CC8	IB2	3Gr4Gr5Gr	(++)	''	--	IB4	3Br4Br5Br	(--)	oo	+-	174	164	153	149	-	-	-	147	-	-	74	69
CC9	CC8	3Gr4OL5OL	+-	'o	--	CC8	3Gr4OL5OL	+-	'o	--	138	136	134	111	15	7	32	68	31	29	67	31
CC10	CC9	3Br4Br5Br	--	oo	--	CC9	3OL4OL5OL	--	'o	--	158	148	148	-	62	70	-	59	66	-	56	65
CC11	IB3	3Gr4OL5Br	(++)	'o	+-	IB3	3Gr4OL5Br	(+x)	'o	+-	264	249	243	240	-	-	60	126	53	18	27	189
CC12	CC11	3Gr4Gr5Gr	(+x)	''	--	CC11	3Gr4OL5Br	(++)	'o	+-	184	177	171	168	-	-	80	85	-	40	43	79

Table 3Ciii.

Details of all larvae which were colour scored as"others".

Brood number	Number of larvae	Instar	Colour of larvae (Majerus score)	Nearest colour type
IB61	14	4th	Could not be scored	Given the notation GP
IB72	1	4th	Mj 24/+2	PY
CC41	2	4th	Mj 25/+2	PY
CC41	1	5th	Mj 25/+2	PY
CC41	1	5th	Mj 24/+2	PY
CC53	2	3rd	Mj 36/-1	OL
CC53	2	4th	MJ 36/-1	OL
CC98	2	2nd	Mj 69/+1	EG
CC98	1	2nd	Mj 61/+2	EG
CC109	2	1st	Mj 71/-1	EG
CC109	2	3rd	Mj 71/0	3IG
CC109	1	3rd	Mj 71/+1	3IG
CC113	2	5th	Mj 28/-2	Br
CC113	1	5th	Mj 29/-2	Br
MSG19	2	4th	Mj 67/-1	Gr
MSG21	2	5th	Mj 58/-4	Gr
MSG21	1	5th	Mj 59/-4	Gr
MSG29	1	3rd	Mj 70/0	Gr
MSG29	1	4th	Mj 48/+1	Gr
MSO37	4	5th	Mj 51/-4	OL
MSO51	1	5th	Mj 37/-2	OL
MSB3	2	1st	Mj 71/-1	EG
MSB34	2	4th	Mj 2/-2	Br
MSB34	1	4th	Mj 2/-3	Br
MSB34	2	5th	Mj 2/-2	Br
MSB41	1	5th	Mj 2/-3	Br

For further details of the broods from which these larvae came see Appendix Table 3Ci.

Of these 49 larvae, 35 had scores within one hue or shade of the defined limits of one or other of the other classes. The other 14 larvae were all green with a distinct pink tinge in the 4th instar, and could not be given a realistic colour score, using the Majerus colour chart. This colour-type was named green-pink (GP). These 14 larvae all came from the same brood, IB61, and all the larvae from this brood were brown in the 5th instar. Due to its rarity, the GP form is not considered to be an important part of the larval polymorphism being investigated. However, it is perhaps worth mentioning that only 12 of the 14 GP larvae attained the adult state. Of these 12 imagines, seven, (three males and four females), were severely crippled and would not mate, even using hand pairing techniques such as those described by Clarke and Sheppard, (1956), and Gorodenski, (1970). The other five imagines, (four males and one female), enlarged their wings fully, and two of these seemed to pair successfully. The female laid 41 ova, however, none of these eggs hatched. The possible control mechanisms of the GP form will be outlined later in this chapter, but it seems that the presence of the GP producing mechanism has strong adverse effects on some aspects of the physiology of the insects in which it occurs and GP is unlikely to be maintained in a population except by recurrent mutation.

Analysis of results.

It was decided that splitting up the seven main colour types, found in the latter three instars, into two groups would appreciably ease the explanation of the results, and,

as many broods contained no yellow larvae of any sort, the mechanism controlling the 3IG, Gr, OL, and Br colour types will be outlined first under the heading "the Gr, Br , OL complex", the system controlling the three yellow colour types, PY, YG, and YB, being discussed later.

"The Gr, Br , OL complex".

When viewing the results of the initial broods which had no yellow larvae in their progeny, a number of points were noted.

(i) There was no change of colour type between the 1st and 2nd instars, all larvae being assigned to the EG colour type in both these instars.

(ii) A number of broods, but not all, showed a change in colour type of some, or all larvae, between the 2nd and 3rd instars.

(iii) Some broods, again not all, showed a change in colour type of some, or all larvae, between the 3rd and 4th instars.

(iv) Some broods, again not all, gave changed in the colour type of some, or all larvae, between the 4th and 5th instars.

(v) Colour type changes within broods were often not confined to one ecdysis, in that changes occurred in some broods at all three of the later ecdyses. Furthermore, individual larvae sometimes changed colour twice, changing either at the 2nd or 3rd larval ecdysis, and again at the 4th; however, none of the larvae changed colour at both the 2nd and 3rd ecdysis.

(vi) No changes of colour from OL or Br to Gr were observed at any stage.

(vii) No changes of colour from Br to OL were observed at any stage.

(viii) All green to OL changes observed, occurred at either the 2nd or the 3rd larval ecdysis.

(ix) Changes from green to Br occurred at either the 2nd,3rd or 4th larval ecdysis.

(x) All OL to Br changes observed, occurred at the 4th larval ecdysis.

(xi) Eight phenotypes were observed, excluding those involving any of the yellow colour types. All of these were EG in both the 1st and 2nd instars. Each phenotype was given a particular notation:-

(a) Green in the 3rd, 4th and 5th instars - 3Gr 4Gr 5Gr[1]
(b) Green in the 3rd and 4th instars, brown in the 5th instar - 3Gr 4Gr 5Br
(c) Green in the 3rd instar, olive in the 4th and 5th instars - 3Gr 4OL 5OL
(d) Green in the 3rd instar, olive in the 4th instar, brown in the 5th instar - 3Gr 4OL 5Br
(e) Green in the 3rd instar, brown in the 4th and 5th instars - 3Gr 4Br 5Br
(f) Olive in the 3rd, 4th and 5th instars - 3OL 4OL 5OL
(g) Olive in the 3rd and 4th instars, brown in the 5th instar - 3OL 4OL 5Br
(h) Brown in the 3rd, 4th and 5th instars - 3Br 4Br 5Br

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[1] The abbreviation 3Gr is used for 3rd instar green larvae in this explanation because, as will be shown later, the green colour type in the 3rd instar is made up of larvae of two genotypes which are not phenotypically distinguishable from one another or, due to the overlapping of the 3IG and Gr colour types, from 4th instar green larvae.

A wide range of genetic mechanisms was considered while trying to explain the observed results of the initial broods and the control crosses.

I feel it is unnecessary to discuss in depth all the possible genetic mechanisms of control which were rejected. I will, therefore, confine myself to outlining only the more likely genetic mechanisms which were rejected, stating the nature of each mechanism and the evidence on which it was rejected.

One obvious possibility is that the whole variational system is governed by a multiple allelic switch-gene, where each colour is controlled by a different allele; however, this can immediately be rejected as a number of broods involving yellow larvae had up to five colour types in the progeny, (e.g. YCC9).

The next consideration was that the Gr, Br, OL complex could be controlled by a multiple allelic switch-gene, whilst the PY, YG, YB complex was in part or totally controlled by an independant system. Three such multiple allelic series were considered. The first of these involved a series of eight alleles in which one allele produced each of the observed Gr, Br, OL complex phenotypes. The 1:1:1:1 ratio between the four phenotypes in IB1 can only be obtained if there is a cyclical type of dominance, in which there is no universal dominant or recessive allele, but the dominance between any two alleles is complete. The mechanism was finally rejected due to the results of CC1, in which either a 3:1 ratio of 3Gr 4OL 5OL : 3Gr 4Br 5Br or a 3:1 ratio of 3Gr 4OL 5OL :

3Br 4Br 5Br would be expected, depending on the direction of the dominance cycle; however, the observed results differ significantly from both these expected ratios as approximately a quarter of the larvae were green in the 4th instar.

A multiple allelic series with six alleles, each one controlling the production of one colour at one particular instar and subsequent instars, was rejected due to the results of IB1, as no cross could be envisaged which would have given the 1:1:1:1 ratio of 3Gr 4OL 5OL : 3Gr 4Br 5Br : 3OL 4OL 5OL : 3Br 4Br 5Br, observed in the progeny of IB1.

A third multiple-allelic mechanism was considered, involving olive as a heterozygote with various alleles producing green or brown in various instars when homozygous. However, no system could be conceived which would have given all the eight Gr, Br, OL complex phenotypes that have been observed.

A number of complexes with two genes at unlinked loci were considered, in which one gene primarily controlled colour type, and the other, the stage at which larval colour changes would occur. Two of these complexes will be described here. One of these was a theoretical situation in which the colour controlling gene, K, was triallelic with one dominant allele which would produce a 5th instar colour change to brown. The other two alleles show incomplete dominance to one another, producing green or brown larvae in the 3rd, 4th or 5th instar when homozygous or in the 3rd or 4th instars only when paired with the dominant allele, and olive when heterozygous. These two alleles are hypostatic to the three alleles of the triallelic gene L, controlling the stage at which colour changes occur.

One of these alleles prevents any colour change other than that produced by the dominant allele of K. The other two alleles which are co-dominant produce 3rd and 4th instar colour changes, influenced by the two recessive alleles of gene K. This theoretical system was rejected as it did not cater for 4th instar olive larvae, which change to brown in the 5th instar.

The second system envisaged involved a biallelic gene K, in which the alleles, k' and k^o, showed incomplete dominance, the two homozygotes giving the potential for green or brown larvae. This gene was completely hypostatic to the second gene L, which had four alleles. One of these, l, gave no change in colour at any stage. A second allele, l', gave 3rd instar colour changes, the nature of the changes being determined by the alleles of K. A third allele, l^o, gave colour changes, again influenced by the K alleles in the 4th instar, and the fourth allele, l^+, produced a 5th instar change to brown. This system would give the observed results of IB1, if the parents of IB1 were $k'k^ol^ol^o$ x k^ok^oll', l^o was dominant to l, and l' was expressed phenotypically when present with l^o. The expected results of CC1, a cross between two 3Gr 4OL 5OL progeny from IB1 which were $k^ok'l^ol$, would then be as observed. The observed results from CC7 again comply with the expected if one of the 3Gr 4Br 5Br parents from the CC1 progeny was $k^ok^ol^ol^o$. Furthermore, the results from IB2 and IB4 would have been obtained, if the parents of these broods were $k'k'l^xl^x$ x $k'k'l^xl^x$, (where l^x may be any of the L alleles except l^+) and $k^ok^ol'l'$ x $k^ok^ol^yl^y$, (where l^y may be any of

the L alleles), respectively.

The results of CC8, however, cannot be explained, as the presence of l' in the parent of CC8 from IB4 should have meant that at least a quarter of the 3rd instar larvae were olive, and the results of CC8 show that this was not so, all larvae being green at this stage. Therefore, this theory must also be rejected.

All the other systems considered involving two genes, had to be rejected, either because they did not cater for all the phenotypes observed, or because the ratios of phenotypes expected in one or more of the initial broods and control cross broods, differed significantly from those observed.

A number of mechanisms involving three genes at unlinked loci were investigated and one of these led to the proposed theory of control of the Gr, Br, OL complex. The first of the genes to be named was A and this caused changes of colour between the 4th and 5th instars.

It has been noted that colour changes in the Gr, Br, OL complex between the 4th and 5th instars are from either green or olive to brown. Therefore, a biallelic gene was proposed to control this change, with one allele, a^+, producing brown larvae in the 5th instar and the second allele, a, causing no change in colour from the 4th instar. In CC2 and CC3 which were crosses between adults from 3Gr 4Gr 5Gr and 3Gr 4Gr 5Br larvae, all progeny were 3Gr 4Gr 5Br, indicating that a^+ is dominant to a, and in these two crosses the 3Gr 4Gr 5Br parent is homozygous for a^+. CC4, which was again a cross between adults from a 3Gr 4Gr 5Gr and a 3Gr 4Gr 5Br larva produced a

1:1 ratio of parental types, indicating that in this case the 3Gr 4Gr 5Br parent was the heterozygote $a^{+}a$.

All the progeny of CC2 - 4 inclusive, which were brown in the 5th instar should have been heterozygous, as the 3Gr 4Gr 5Gr parent must be homozygous recessive for A. Therefore, crosses between these 5Br larvae ought to give a 3:1 ratio of brown to green (non-brown) larvae in the 5th instar. CC5 and CC6 show that this does occur, (for CC5, $X_1^2 = 0.297$, $0.7 > p > 0.5$; for CC6, $X_1^2 = 1.263$, $0.3 > p > 0.2$). This hypothesis was then considered in relation to the other initial and control cross broods and none of the results from these broods produced observed, phenotypic ratios which differed significantly from those expected.

The control of colour changes between the 3rd and 4th instars in the Gr, Br, OL complex was, at first, thought to be under the control of a triallelic gene with the allele for olive dominant to the other two alleles of which one is completely dominant to the other. This dominance arrangement is the only one consistent with the observed results of 4th instar larvae in IB3. The mechanism was eventually rejected due to the IB1 and CC1 results. The results of IB1 may be explained by the mechanism, but as both parents of CC1 were olive in the 4th instar, then, at least three-quarters of the progeny of CC1 should have been olive, as the olive allele is dominant in the mechanism being considered. The other quarter should either be all green, or all brown. In the observed results, only approximately half of the larvae are olive, and of the

rest, about half were green and half were brown.

A biallelic gene B, with alleles b' and b^o and exhibiting incomplete dominance, was then considered. From the progeny of IB3 which approximates to a 1:2:1 ratio of green to olive to brown, (X^2_2 = 1.133 , 0.7 > p > 0.5), it may be deduced that green and brown will be produced by the two homozygotes b'b' and b^ob^o respectively, with olive being produced by the heterozygote $b'b^o$.

If the 4th instar Gr, Br, OL complex variation is controlled by this system, then crosses between two adults from 4Gr larvae should always give all 4Gr progeny, as only b' should be present; this is observed in broods CC2 - 6 inclusive. Similarly, crosses between adults from two 4Br larvae, which would be homozygous for b^o, should always produce all brown 4th instar progeny, as is observed in CC7. Crosses between adults from two 4OL larvae should give a 1:2:1 ratio of green to olive to brown 4th instar progeny as observed in CC1, CC9 and CC11, whilst crosses between adults from a 4OL larva,and a 4Gr larva or a 4Br larva,should give a 1:1 ratio of the parental types as in CC12 and CC10 respectively. Finally, crosses between 4Gr and 4Br larvae should always give all olive 4th instar progeny, as occurs in CC8.

Only three broods have been discovered which produced results that differed significantly from these expected ratios, unless yellow larvae occurred in the brood. One of these broods involved the GP form which has already been mentioned. The ratio of GP larvae to Gr larvae in the 4th instar of this

brood approximated to a 1:1 ratio, and it seems probable that the GP form is produced by a unifactorial dominant allele, which may be allelomorphic to b' and b^{o}. In both the other exceptions, CC74 and CC91, the differences were only just significant, (for CC74 in the 4th instar, X_1^2 = 4.5714, $0.05 > p > 0.02$; and for CC91, X_1^2 = 3.8571, $0.05 > p > 0.02$), and the broods had a high mortality rate in the early instars.

Colour changes between the 2nd and 3rd instars appear to be controlled by two biallelic genes, as the 3rd instar progeny of CC9 are 111 green, 15 olive and 7 brown, which approximates to a 13:2:1 ratio, (X_2^2 = 0.446, p = 0.8).

A situation where two genes, F and G, both biallelic with alleles f^+ and f, and g^+ and g respectively was tested initially. This could give the observed CC9 ratio under the following conditions:-

(i) g^+ is dominant to g.

(ii) f^+ and f show incomplete dominance to one another.

(iii) g^+ is epistatic to f^+ and f.

(iv) g^+ causes there to be no change in larval colour at the 2nd larval ecdysis, unless colour is affected by a change in foodplant.

(v) The alleles, f^+ and f, when homozygous, produce brown and green 3rd instar larvae respectively, the heterozygote, f^+f, giving olive.

(vi) Both of the parents of CC9 were heterozygous for genes F and G, as well as for B.

In CC10 the expected ratio, if this hypothesis is correct,

would be 1:1 olive to brown for all instars as observed, but would involve half the brown 3rd instar larvae turning olive at the 3rd ecdysis, and half the olive 3rd instar larvae turning brown for the 4th instar, as the two systems, the gene B on the one hand and the proposed genes F and G on the other hand, do not affect one another's action in the present hypothesis. Observation showed that all larvae in CC10 were the same colour in the 4th instar as they had been in the 3rd instar, and so the theory in question was rejected.

However, the evidence does indicate that at least two genes are involved in the control of colour changes between the 2nd and 3rd instars, and after testing and rejecting a number of other mechanisms involving two, three or four loci, a system was considered in which a biallelic gene C, showing complete dominance was acting in conjunction with gene B. The dominant allele, c^+, produces larvae whose colour in the 3rd instar is determined to a great extent by foodplant, (see chapter 3B). The recessive allele, c, causes the effect of B to be initiated at the start of the 3rd instar. Thus, $ccb'b^o$ larvae will produce 3OL 4OL larvae, whereas $c^+cb'b^o$ or $c^+c^+b'b^o$ give green 3rd instar larvae affected by foodplant, and olive 4th instar larvae.

If this hypothesis is correct then the progeny of IB4 must be homozygous recessive for C, as they are all brown in the 3rd instar. Then, the progeny of CC8 must be c^+c, as one of the parents (from IB4) is known to be cc, and yet all the progeny are green in the 3rd instar, although they are olive in the 4th instar. On this basis, the parents of CC9 which

came from CC8 progeny are known to have been $c^{+}cb'b^{o}$ and, therefore, in the 3rd instar larvae of CC9, three-quarters would be expected to contain c^{+}, thus, being green. 1/16th would be ccb'b' and, again, be green due to b' being homozygous; 2/16th would be $ccb'b^{o}$, producing olive due to $b'b^{o}$ being expressed phenotypically in the 3rd instar, and 1/16th would be $ccb^{o}b^{o}$, giving brown due to $b^{o}b^{o}$ being expressed in the 3rd instar. Thus a 13:2:1 ratio of green:olive:brown larvae would be expected in the 3rd instar of CC9, and this indeed is as observed. All the data from other broods bred for genetic analysis support this genetic mechanism.

This genetic mechanism for control of 3rd instar larval variation also explains the results of the foodplant experiments in relation to the 3rd instar. If the 3rd instar results from the EFB3 samples (Table 3Bd/1iii) are considered, then it appears that approximately half of them match the colour of the foodplant fairly accurately, whilst the other half are brown. This situation would be due to the genotype of the former half being $c^{+}cb^{o}b^{o}$, thus leaving the larval colour under the influence of foodplant due to the presence of c^{+}. In the latter group, the genotype could then be $ccb^{o}b^{o}$, and as c is homozygous recessive, the larvae will be brown in the 3rd instar, due to $b^{o}b^{o}$ being expressed at this stage. Similarly, the lack of correlation between foodplant colour seen in the results of the EFB6 samples fed on plantain could be due to the genotype of the larvae in these samples being ccb'b'. This would mean that while they remain green, the larvae are now under the influence of b'b' in the 3rd instar, and all relevant data indicates that the green colour produced by b' is neither affected by foodplant, nor by any of the

other environmental factors tested in the environmental experiments. The homogeneity between the EFB6 samples fed on sorrel and other samples fed on sorrel which were foodplant-influenced is almost certainly due to the similarity in the green colour produced by gene b' and the green colour induced by sorrel in 3rd instar larvae which are foodplant affected. (A summary of the Gr, Br, OL complex is given in Tables 3Di and 3Dii, pp. 159 - 162).

"The PY, YG, YB complex".

The mechanism controlling the PY, YG, YB complex proved much more difficult to formulate than that inducing the colours in the Gr, Br, OL complex, partly because of the complexity of the control mechanism's action, and partly because of the scarcity of the yellow types. Fortunately, one light-trapped female produced a brood of 172 eggs, and all the subsequent larvae were plain-yellow in the 3rd, 4th and 5th instars (3PY 4PY 5PY). This brood was IB46, (Table 3Cii). Eight crosses (broods YCC1 - 8 inclusive) were reared, one parent in each case being an adult from IB46, the other parent being from stocks which had been kept for two generations, without producing any yellow larvae. A further ten crosses were then bred, using progeny from the YCC1 - 8 broods as parents, these being broods YCC9 - 18, (Table 3Cii).

A large number of genetic mechanisms were considered when trying to explain the rather complicated results of these broods.

No yellow larvae occurred in broods YCC1 - 8 inclusive, so it appears that yellow is recessive to non-yellow. In the

Table 3C14.

Colour type analysis of a number of the broods bred to determine the mechanism controlling the colours, Plain Yellow, Yellow-green and Yellow-brown in the third, fourth and fifth larval instars.

Brood number	Origin of parent 1 (Male except in IB46)	Phenotype of parent 1 (Male except in IB46)	Genotype of parent 1 (Male except in IB46) Alleles of genes:- C	B	A	D	E	Origin of parent 2 (Female except in IB46)	Phenotype of parent 2 (Female except in IB46)	Genotype of parent 2 (Female except in IB46) Alleles of genes:- C	B	A	D	E	Number of eggs laid	Number of First instar larvae	Number of Second instar larvae	Number of third instar larvae of colour types:- Gr	OL	Br	PY	YG	YB	Number of fourth instar larvae of colour types:- Gr	OL	Br	PY	YG	YB	Number of fifth instar larvae of colour types:- Gr	OL	Br	PY	YG	YB
IB46	Wild	Unknown	xx	xx	xx	--	(oo)	Wild	Unknown	xx	xx	xx	--	('')	172	161	157	-	-	-	139	-	-	-	-	-	138	-	-	-	-	-	137	-	-
YCC1	MS63	3Gr 4Gr 5Gr	+-	oo	--	++ or ++		IB46	3PY 4PY 5PY	--	''	--	--	'o	133	131	127	67	59	-	-	-	-	-	119	-	-	-	-	-	117	-	-	-	-
YCC2	MS71	3Br 4Br 5Br	--	oo	--	++ or ++		IB46	3PY 4PY 5PY	--	'o	--	--	'o	194	191	188	-	95	90	-	-	-	-	92	89	-	-	-	-	91	87	-	-	-
YCC3	IB46	3PY 4PY 5PY	--	'o	--	--	'o	MS71	3Gr 4OL 5OL	+-	'o	--	++ or ++		165	155	147	94	37	16	-	-	-	31	74	36	-	-	-	30	73	36	-	-	-
YCC4	MS74	3OL 4OL 5OL	--	'o	--	++ or ++		IB46	3PY 4PY 5PY	--	''	--	--	'o	189	186	184	91	92	-	-	-	-	88	91	-	-	-	-	87	89	-	-	-	-
YCC5	IB46	3PY 4PY 5PY	--	oo	xx	--	'o	MS75	3Br 4Br 5Br	--	oo	xx	++ or ++		236	230	230	-	-	227	-	-	-	-	-	225	-	-	-	-	-	221	-	-	-
YCC6	MS76	3Gr 4Gr 5Gr	xx	''	--	++ or ++		IB46	3PY 4PY 5PY	--	''	--	--	'o	178	168	158	152	-	-	-	-	-	148	-	-	-	-	-	146	-	-	-	-	-
YCC7	IB46	3PY 4PY 5PY	--	'o	--	--	'o	MS76	3Gr 4OL 5Br	++	'o	+-	++ or ++		49	47	45	43	-	-	-	-	-	13	18	9	-	-	-	4	8	26	-	-	-
YCC8	MS79	3Gr 4Gr 5Gr	xx	''	--	++ or ++		IB46	3PY 4PY 5PY	--	''	--	--	'o	231	224	220	218	-	-	-	-	-	208	-	-	-	-	-	200	-	-	-	-	-
YCC9	YCC1	3OL 4OL 5OL	--	'o	--	+-	+o	YCC1	3OL 4OL 5OL	--	'o	--	+-	+o	220	213	210	47	104	45	2	10	-	46	102	45	2	9	-	41	97	44	2	9	-
YCC10	YCC1	3OL 4OL 5OL	--	'o	--	+-	+'	YCC1	3OL 4OL 5OL	--	'o	--	+-	+'	285	276	261	66	119	58	4	-	11	66	113	57	4	-	11	65	113	56	4	-	11
YCC11	YCC1	3OL 4OL 5OL	--	'o	--	+-	(+o)	YCC1	3OL 4OL 5OL	--	'o	--	+-	(+')	179	174	170	41	82	38	9	-	-	40	82	38	9	-	-	40	81	38	9	-	-
YCC12	YCC2	3OL 4OL 5OL	--	'o	--	+-	+'	YCC3	3OL 4OL 5OL	--	'o	--	+-	+'	367	352	347	77	163	75	5	-	15	75	160	75	5	-	15	73	157	74	5	-	14
YCC13	YCC4	3Gr 4Gr 5Gr	--	''	--	+-	(+o)	YCC5	3Br 4Br 5Br	--	oo	--	+-	(+')	160	153	151	-	136	-	9	-	-	-	135	-	9	-	-	-	134	-	9	-	-
YCC14	YCC4	3OL 4OL 5OL	--	'o	--	+-	(+o)	YCC5	3Br 4Br 5Br	--	oo	--	+-	(+')	189	185	184	-	84	82	12	-	-	-	82	82	11	-	-	-	82	80	11	-	-
YCC15	YCC6	3Gr 4Gr 5Gr	+-	''	--	+-	+o	YCC6	3Gr 4Gr 5Gr	+-	''	--	+-	+o	97	94	93	91	-	-	-	-	-	84	-	-	-	5	-	82	-	-	-	5	-
YCC16	YCC7	3Gr 4OL 5Br	+-	'o	+-	+-	+'	YCC7	3Gr 4OL 5Br	+-	'o	+-	+-	+'	314	309	307	237	43	20	1	-	3	69	143	67	5	-	14	19	38	220	1	-	18
YCC17	YCC7	3Gr 4Gr 5Br	+-	''	+-	+-	+'	YCC8	3Gr 4Gr 5Gr	--	''	--	+-	+'	157	156	156	146	-	-	6	-	-	137	-	-	11	-	-	67	-	69	6	-	5
YCC18	YCC6	3Gr 4Gr 5Gr	+-	''	--	+-	+o	YCC4	3OL 4OL 5OL	--	'o	--	+-	+o	294	291	288	200	72	-	8	-	-	128	131	-	17	-	-	127	130	-	17	-	-

broods YCC9 - 18 inclusive, yellow type larvae in every case represent approximately $1/16^{th}$ of the total number of larvae. This indicates firstly, that the parents of these broods may be heterozygous for the genes involved - as indeed they should be as one of their own parents was yellow - and, secondly, that yellow may be the result of a double homozygous recessive.

If the results of YCC9, YCC10 and YCC11 are considered, in each of these cases, the parents were 3OL 4OL 5OL progeny from YCC1, so the parental genotypes of these three broods must have been $ccb'b^{o}aa$. Although the total number of yellow larvae in these broods is always approximately $1/16^{th}$ of the total number of larvae, the frequencies of the three yellow types in these three crosses are quite distinct. YCC9 gives a ratio of yellow-green (YG) to PY which is not significantly different from 3:1, ($X_1^2 = 0.396$, $0.7 > p > 0.5$). Similarly the yellow larvae in YCC10 approximate to a 3:1 ratio of yellow-brown (YB) to PY, ($X_1^2 = 0.023$, $0.9 > p > 0.8$), whilst in YCC11, all yellow larvae are of the PY type.

If these ratios are considered in relation to the total number of larvae in each brood, then in YCC9, approximately $3/64^{th}$ of the larvae are YG and $1/64^{th}$ are PY, which indicates that at least three heterozygotes are involved, one of which may, or may not be, $b'b^{o}$. If it is assumed that two more biallelic genes, in addition to A, B and C, are present in the system, that these are named D and E with alleles d^{+} and d, and e^{+} and e respectively, and that these are heterozygotes in the parents of YCC9, the full parental genotype would be

$ccb'b^{o}aad^{+}de^{+}e$. If d^{+} is dominant to d, and e^{+} is dominant to e, with the dominant alleles producing no larval colour change, and the recessive alleles producing a change to yellow when present as a double homozygous recessive, then the observed ratio of 1:15, yellow to non-yellow larvae would be expected. However, this system does not explain the occurrence of two distinct types of yellow larvae in the brood. The situation is further complicated by the occurrence of $3/64^{th}$ YB and $1/64^{th}$ PY larvae in YCC10, and $1/16^{th}$ PY larvae with no other yellow type larvae in YCC11.

To explain the occurrence and frequencies of the three yellow colour types, one of the two additional genes must be tri-allelic. The proposed control mechanism for the PY, YG, YB complex, is therefore based on five unlinked genes, of which three - A, B and C - have already been described. The other two genes have been named D and E. D is biallelic with a dominant allele, d^{+}, which has no effect on larval colour, and a recessive allele, d, which gives rise to yellow pigment. Gene E is triallelic with a dominant allele e^{+} which has no effect on larval colour except to suppress the action of d when this allele is homozygous. The two other alleles, e' and e^{o}, are recessive to e^{+} and show incomplete dominance to one another. If e' is present in the genotype, either as a homozygote, or as a heterozygote with e^{o}, then it prevents the phenotypic expression of b'. Similarly, if e^{o} is present, b^{o} and a^{+} do not express themselves phenotypically. Whether the alleles e' and e^{o} cause green and brown pigment respectively to be broken down, or whether these alleles prevent the production of these pigments is at present a matter for conjecture.

If the alleles, e' and e^{o} are hypostatic to d^{+} and d

is hypostatic to e^+, then the results of YCC9, YCC10 and YCC11 can be explained. The non-yellow parent of YCC1 was known to be $c^+cb^ob^oaa$. The progeny of YCC1 produce a ratio of approximately 1:1, 3Gr 4OL 5OL:3OL 4OL 5OL. To give this ratio, the yellow parent from IB46 must have been ccb'b'aa. The progeny of YCC1 will, thus, be either $c^+cb'b^oaa$ or $ccb'b^oaa$. The latter type which was recognisable by being olive in the 3rd instar, was used in the YCC9, YCC10 and YCC11 broods. If the proposed control mechanism is correct, then none of the progeny of IB46 must contain either d^+ or e^+, and as b' is known to be present, then the genotype must be either dde'e' or $dde'e^o$, as e' must be present to prevent the phenotypic expression of b', thus giving all PY larvae.

Assuming that the insect from IB46 used as a parent of YCC1, had the genotype ccb'b'aaddе'e^o, and that the non-yellow parent of YCC1 was $c^+cb'b^oaad^+d^+e^+e^+$, then considering only genes D and E, a 1:1 ratio of d^+de^+e' to $d^+de^+e^o$ should be present in the progeny of this brood. In YCC9, if both parents were $d^+de^+e^o$, $1/16^{th}$ of the larvae should have been dde^oe^o, and thus, of a yellow colour. Of these, as both parents were olive, and therefore heterozygous for gene B, one quarter should have contained b'b', half should have contained $b'b^o$, and a quarter b^ob^o. The former two of these groups would give YG larvae, due to the presence of b' producing green pigment, dd producing yellow pigment, and e^o preventing the expression of b^o in the $b'b^o$ larvae. The third group, with b^ob^o, will produce PY larvae, due to the lack of b' to produce green pigment, and the presence of e^oe^o

preventing the expression of b^{o}.

The results of YCC10 may be explained in a similar way, if both parents are $d^{+}de^{+}e'$, so that $1/16^{th}$ of the larvae would be dde'e', and, again of these a quarter should have contained b'b', half $b'b^{o}$, and a quarter $b^{o}b^{o}$. In this case, the former group will give PY, due to the absence of any brown producing allele, and the presence of e'e' to prevent the expression of b'. The other two groups will both give YB larvae, due to the presence of b^{o} and dd producing brown and yellow pigments respectively.

The results of YCC11 are as would be expected if one parent was $d^{+}de^{+}e'$, and the other $d^{+}de^{+}e^{o}$, so that all progeny which are yellow must be $dde'e^{o}$. This would result in neither the green producing allele b', nor the brown producing alleles, b^{o} and a^{+}, being expressed phenotypically, as both e' and e^{o} are present in these larvae, and therefore, they would all be of the PY type.

Three further points may be deduced from the results of broods YCC9 - 18 inclusive and the other broods involving yellow larvae. Firstly, in several of the broods (e.g. YCC18), the number of yellow type larvae increases between the 3rd and 4th instars. In YCC18 this seems to be because c^{+} is present in the genotype of one of the parents, with the result that half the progeny of YCC18 would contain c^{+}. As the number of yellow larvae approximately doubled between the 3rd and 4th instars, it may be deduced that the genes D and E are both hypostatic to gene C in the 3rd instar - that is to say the alleles d, e' and e^{o} are expressed in the 3rd

instar only when the allele c is homozygous.

Secondly, the results of brood YCC17 show that some larvae change from being PY in the 4th instar, to YB in the 5th instar. This is because their genotype is b'b'a^+adde'e', so that in the 4th instar, they are PY due to suppression of the phenotypic expression of b' by e', and the lack of the brown producing allele b^o. In the 5th instar, a^+ expresses itself and adds brown pigment to the yellow produced by dd. It was, in fact, this unusual change which first led me to the idea of the alleles e' and e^o being epistatic to the alleles of B and allele a^+.

Finally, it would be expected that larvae with the genotype b'$b^o a^+ a^+$dd$e^o e^o$ or b'b'$a^+ a^+$dd$e^o e^o$, would change colour from being YG in the 4th instar, due to the presence of b' and the absence of e', to being PY in the 5th instar due to the absence of any gene to produce green in the 5th instar and the presence of e^o to prevent the phenotypic expression of a^+. However, the results of brood CC97, a cross between a 3Gr 4Gr 5Br adult and a 3Gr 4Br 5Br adult gave thirteen 4th instar yellow type larvae, out of a total of 214 larvae. All of these were YG in both the 4th and 5th instars. It must be assumed, therefore, that the allele e^o not only prevents the phenotypic expression of a^+ in the 5th instar, but also prevents the loss in this instar of green pigments produced by b' in the 4th instar.

D. Discussion.

A summary of the mechanisms which control larval colour in P. meticulosa is set out in Tables 3Di and 3Dii. Table 3Di

Table 3Di.

Summary of factors controlling each of the colour forms found in each larval instar.

Instar	Colour	Method of control
First	Early Green (EG)	Foodplant. (Other colours may be produced when larvae are fed on non-green food).
Second	Early Green (EG)	Foodplant. (Other colours may be produced when larvae are fed on non-green food).
Third	3rd instar green (3IG)	Made up of green larvae under two methods of control. (i) when c^+ is present, colour is under the control of foodplant. (ii) when c is homozygous, the green colour is caused by b' being homozygous.
	Olive (OL)	Produced when c is homozygous, b' and b^o, and either d^+ and/or e^+ are present in the genotype.
	Brown (Br)	Produced when both c and b^o are homozygous, and either d^+ and/or e^+ are present in the genotype.
	Plain Yellow (PY)	Produced in any one of three ways, all of which involve the alleles c and d being homozygous. Coupled with these two homozygotes, if both e' and e^o are present, or if e' and b' are homozygous, or if e^o and b^o are homozygous, PY larvae will result.
	Yellow-green (YG)	Produced when c, d and e^o are homozygous and b' is present.
	Yellow-brown (YB)	Produced when c, d and e' are homozygous and b^o is present.

Table 3Di. (Continued)

Summary of factors controlling each of the colour forms found in each larval instar.

Instar	Colour	Method of control
Fourth	Green (Gr)	Produced when b' is homozygous if either d^+ and/or e^+ are present.
	Olive (OL)	Produced when b' and b^o are present if either d^+ and/or e^+ are present.
	Brown (Br)	Produced when b^o is homozygous if either d^+ and/or e^+ are present.
	Plain Yellow (PY)	Produced in any one of three ways, either when d is homozygous and e' and e^o are present, or when d, e' and b' are homozygous, or when d, e^o and b^o are homozygous.
	Yellow-green (YG)	Produced when d and e^o are homozygous and b' is present.
	Yellow-brown (YB)	Produced when d and e' are homozygous and b^o is present.
Fifth	Green (Gr)	Produced when b' and a are homozygous if either d^+ and/or e^+ are present.
	Olive (OL)	Produced when a is homozygous and b' and b^o are present if either d^+ and/or e^+ are present.
	Brown (Br)	Produced when d^+ is present and/or b^o is homozygous and either d^+ and/or e^+ are present.
	Plain Yellow (PY)	Produced in any one of three ways; either when d is homozygous and e' and e^o are present, or when d, e' and b' and a are homozygous, or when d, e^o and b^o are homozygous.
	Yellow-green (YG)	Produced when d, e^o and b' are homozygous.
	Yellow-brown (YB)	Produced when d, and e' are homozygous and either b^o and/or a^+ are present.

Table 3Dii.

Basic effects of the eleven alleles of the five major genes which control larval colour, and the interaction between these alleles.

Allele	Instar in which the allele may act	Basic effect of the alleles and their interactions
a^+	5th	Causes larvae to be brown in the 5th instar. Dominant to a. Epistatic to alleles of gene B. Hypostatic to allele d when this is homozygous and e^+ is not present. Hypostatic to alleles e' and e^o, in any combination, when neither d^+ nor e^+ are present.
a	5th	Causes larvae to retain their 4th instar colour through the 5th instar. Recessive to a^+.
b'	3rd, 4th and 5th	Causes green pigment to be produced. Incompletely dominant to b^o. Hypostatic to allele d when this is homozygous and e^+ is not present. Hypostatic to alleles e' and e^o, in any combination, when neither d^+ nor e^+ are present. Hypostatic to allele c^+ (only in the 3rd instar). Hypostatic to allele a^+ unless both d and e^o are homozygous. (only in the 5th instar).

Table 3Dii. (Continued)

Basic effects of the eleven alleles of the five major genes which control larval colour, and the interaction between these alleles.

Allele	Instar in which the allele may act	Basic effect of the alleles and their interactions
b^o	3rd, 4th and 5th	Causes brown pigment to be produced. Incompletely dominant to b'. Otherwise as b' except that the interaction between a^+ and b^o is not known as both have similar effects.
c^+	3rd	Causes 3rd instar larval colour to be dependant on foodplant. Dominant to c. Epistatic to all alleles of genes B, D and E.
c	3rd	Causes 4th instar larval colour to arise in the 3rd instar. Recessive to c^+.
d^+	3rd, 4th and 5th	Causes larval colour to be dependant on alleles of genes A, B and C. Dominant to d . Hypostatic to c^+ (only in the 3rd instar). Epistatic to e' and e^o.
d	3rd, 4th and 5th	Causes yellow pigment to be produced. Recessive to d^+. Hypostatic to e^+. Hypostatic to c^+ (only in the 3rd instar).

Table 3Dii. (Continued)

Basic effects of the eleven alleles of the five major genes which control larval colour, and the interaction between these alleles.

Alleles	Instar in which the allele may act	Basic effect of the alleles and their interactions
e^+	3rd, 4th and 5th	Causes larval colour to be dependant on alleles of genes A, B and C. Dominant to e' and e^o. Hypostatic to c^+ (only in the 3rd instar). Epistatic to d.
e'	3rd, 4th and 5th	Prevents the phenotypic expression of b'. Recessive to e^+. Incompletely dominant to e^o. Hypostatic to d^+. Hypostatic to c^+ (only in the 3rd instar). Epistatic to b' in the 3rd instar if neither c^+ nor d^+ are present. Epistatic to b' in the 4th and 5th instars if d^+ is not present.
e^o	3rd, 4th and 5th	Prevents the phenotypic expression of b^o and a^+. Recessive to e^+. Incompletely dominant to e'. Hypostatic to d^+. Hypostatic to c^+ (only in the 3rd instar). Epistatic to b^o in the 3rd instar if neither c^+ nor d^+ are present. Epistatic to b^o in the 4th instar if d^+ is not present. Epistatic to b^o and a^+ in the 5th instar if d^+ is not present.

outlines the factors controlling each of the colour forms found in each instar, and Table 3Dii shows the interactions between the eleven alleles of the five major genes which are involved in the control of larval colour.

It is perhaps illustrative of the complexity of the system to note that a cross between two adults which are heterozygous for each of the five genes, (taking one parent to be $e^{+}e'$ and the other $e^{+}e^{o}$), would give the following ratio:-

135 (3Gr 4OL 5Br) : 90 (3Gr 4Gr 5Br) : 90 (3Gr 4Br 5Br) : 45 (3Gr 4OL 5OL) : 45 (3OL 4OL 5Br) : 30 (3Gr 4Br 5Br) : 30 (3Br 4Br 5Br) : 24 (3Gr 4PY 5PY) : 15 (3OL 4OL 5OL) : 8 (3PY 4PY 5PY).

The precise way in which the genetic system acts is open to several theoretical interpretations. As major colour changes occur without exception at larval ecdyses, it seems likely that the concentration or balance of the hormones, ecdyson and juvenile hormone are involved in the system - a possibility which is particularly relevant to the action of the alleles of gene C, and to the similarity in the phenotypic expression of $b^{o}b^{o}$ and a^{+}.

It must be remembered that the colour types under the control of genes A, B, C, D and E are all variable. Mather (1943) notes that polymorphic genes have a switching role only, the alternatives which they switch being determined by adjustment of other genes. If this is true, then the variation in the main colour types is probably caused by a polygenic system. The expression of a particular set of genes from this

system will be controlled by the presence, or absence of one or more of the alleles of the major genes involved.

When this is considered in relation to the situation in P. meticulosa, the possibility arises that some of the alleles, particularly b^o and a^+, may have identical actions on the polygenic system, except for the instar in which they act. This possibility is supported by the results of CC11, which was a cross between two IB3, 3Gr 4OL 5Br larvae with the genotypes $b'b^oa^+a^x$, (where a^x may be a^+ or a). At least $3/16^{th}$ of the progeny from this cross should have the genotype $b^ob^oa^+a^x$. This genotype would produce brown 4th and 5th instar larvae. Unless b^ob^o blocks the effect of a^+, then the colour of the 4th instar will be that produced by those genes in the polygenic system which are "switched on" by b^ob^o, whilst the 5th instar colour will be that produced by the genes "switched on" by a^+. Thus, if the genes "switched on" by b^ob^o and a^+ are not identical, the shade of brown in the 4th and 5th instars of some of the 4Br 5Br larvae from CC11 should vary. However, observation on CC11 and other similar broods indicated that there was no significant change in the shade of the brown between the 4th and 5th instars. So, it seems that the phenotypic expressions of b^o and a^+ are identical, either because they affect the same genes in the polygenic system, or because, although they each affect a different set of polygenes, the difference in the action of the two sets was too small to be detected with the methods being used to score colour variation. The fact that the allele e^o prevents both b^o and a^+ being expressed phenotypically also

indicates that b^{o} and a^{+} may have similar actions. The possibility of $b^{o}b^{o}$ blocking the expression of a^{+} is also unlikely as a discernable difference should then be noticeable in the 5th instar brown colours of 4Gr 5Br and 4Br 5Br larvae, unless the actions of $b^{o}b^{o}$ and a^{+} are identical. No such difference has been observed.

On the other hand, the similarity in the phenotypic expression of $b^{o}b^{o}$ and a^{+} could be due to these having similar effects on the hormonal system of moulting, and there is no reason to suppose that the effect on the hormonal system must involve polygenes. This is not to say that a polygenic system may not then cause slight variations in the hormonal system, independently of the major genes, and that these slight variations may, then, manifest themselves phenotypically as the variation in shade of the main colour types.

The variation could also be explained by environmental factors having an effect on the shade within colour types, and the similarities in the shades of colour types between the 3rd, 4th and 5th instars could be due to the effect being caused by the environment at a critical phase in the development, if this point precedes the 4th instar. However, it must be said that no evidence to support this possibility was obtained from the environmental experiments (chapter 3B). On the other hand, the possibility should not be ruled out completely, because such effects may involve several environmental factors acting in conjunction - a situation which would be very difficult to uncover - or they may involve environmental

factors which have not been considered in this study.

Finally, it may be that the variation within colour types is affected by the quantity and type of pigments in the larval foodplant, although the lack of a difference in the shades of 4th and 5th instars, fed on sorrel and plantain in the first environment foodplant experiments, opposes this view.

The mechanism of larval colour control in P. meticulosa is surprisingly complicated if the mechanism has evolved simply due to selection for colour.

The evolution of the system will be discussed in more depth in chapter 9; however, I think it appropriate to highlight one feature of the system here, so that the reader may keep it in mind during the ensuing chapters.

The analysis of the results from over 600 broods indicates that the system does not involve any linkage.

This is, perhaps, the most unexpected feature of the whole system for a number of authors, (Mather, 1955; Sheppard, 1959; Ford, 1964), have noted that, as in many cases the combined effects of several major-genes are often necessary to promote advantageous adjustments, the evolution of close linkage between them is favoured, so holding together the appropriate characters for which they are responsible. This linkage is often so close that "super-genes" evolve, and, in many cases, polymorphism is controlled by such super-genes - as in the case of shell colour and banding in Cepaea nemoralis (Cain and Sheppard, 1954). Indeed, Mather (1955) makes the comment that the tendency for increased linkage will be

particularly evident when there is strong disruptive selection as in mimicry.

If the larval colour control system in P. meticulosa has evolved through selection for colour with regard to crypsis - which is after all, a very similar phenomenon to mimicry, in that an edible insect matches or mimics an inedible object which is in effect acting as a model - then it seems to follow that this must have been due to disruptive selection, and so linkage would be expected. The question why linkage does not seem to have evolved in the system[1], was one of those considered most carefully during the work carried out after the mechanism controlling larval colour had been discovered.

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[1] Experiments on larval behaviour (chapter 4) show that gene C controls a behaviour and a colour character (chapter 3C), and that both alleles of gene B may cause a change in larval behaviour at the start of the 4th instar. It will be suggested in chapter 9E (page 396) that gene B may be a supergene.

CHAPTER 4 EXPERIMENTS TO DETERMINE THE EXISTENCE OF DIFFERENTIAL LARVAL BEHAVIOUR CONTROLLED BY THE ALLELES OF THE FIVE MAJOR GENES CONTROLLING LARVAL COLOUR.

A. Introduction

The system controlling larval colour is surprisingly complex if it has evolved directly through selection for colour, and it seems probable that the genes concerned have other functions. Ford (1955) notes that there are reports that the green and brown larval forms of P. meticulosa tend to rest upon those parts of the foodplant which they match. If any of the predators of the larvae use colour vision to find the larvae, then it follows that any system which causes a closer match between larval colour and resting substrate would increase the crypsis of the larvae and so be selectively advantageous.

It has already been shown that larvae in the 1st or 2nd instars, together with those 3rd instar larvae which have c^+ in their genotype, have developed a system which causes a very close match between larval and foodplant colour. This system involves larval colour being controlled by the colour of the food eaten. However, in larvae of later instars this system does not operate, as colour is under complete genetic control and is not affected by foodplant. If, then, larvae of P. meticulosa do habitually rest upon those parts of the foodplant that they match, a behavioural system which is controlled, at least in part, by one or more of the major genes controlling larval colour seems the most likely explanation of the habit.

To investigate this possibility, a series of experiments

were devised to test whether there were any behavioural differences correlated with larval colour type which might increase crypsis.

Some of the experiments were also devised with a view to testing whether the various methods available for collecting samples of larvae from the wild, which were used for investigations into gene frequencies of natural populations and for some of the predation experiments, would provide random samples.

In general, the behaviour of P. meticulosa larvae, in the later instars, is such that they rest close to the ground amongst the low herbage and plant debris by day, and begin to move up to the leaves of the foodplants at sunset, returning to the lower herbage layer about half an hour after sunrise.

Three main methods by which crypsis could be increased by behaviour were considered. Firstly, the larvae may "choose" specifically coloured backgrounds, which they match, to feed or rest upon. Secondly, the larvae may feed or rest in situations where they match the general background colour of their surroundings. Thirdly, there may be differences in the levels to which larvae of various colour types ascend and descend in the herbage strata; therefore, as the overall colour of the herbage varies at different levels, larvae may tend to spend more time at a level where they match the general colour of their surroundings than would be expected if there were no such differences between colour types.

Three different sets of experiments were designed to test these possibilities. Initially, only 4th and 5th instar larvae were considered.

B Initial behaviour experiments.

i The first behaviour experiment.

Experimental procedure.

The tests to determine whether larvae choose specifically coloured backgrounds to feed or rest upon, involved 22 samples of 50 larvae. These samples were named the "first behaviour experiment samples" (FBE). Each sample consisted of two groups of 25 larvae with known genotype. The genotypes in each sample are given in Table 4Bi. The samples FBE1 - FBE10 inclusive were used for experiments when in the 4th instar, whilst samples FBE11 - FBE22 inclusive were used when in the 5th instar. Until larvae were needed for experiments, they were reared using the basic breeding methods described in chapter 2, except that the foodplant used was plantain rather than sorrel.

The experiments were carried out in perspex boxes, 320 mm x 320 mm x 151 mm, which were kept outside so that they were submitted to normal daylight. (All experiments were carried out in March and April). Each box was split into four quarters by removable partitions. One quarter of the box was loosely filled with a foodplant of an appropriate colour, i.e. a colour similar to that of larvae of one of the two genotypes, a second quarter being filled with a foodplant appropriate to that of the colour of larvae of the second genotype. The types of foodplant used for each genotype are also given in Table 4Bi.

13 larvae of one genotype and 12 of the other, in a particular sample, were then put into each of the two empty sections of the box. The partitions separating the four quarters of the box

Table 4Bi.

First behaviour experiment. The genotypes and phenotypes of the FBE samples with the instar in which samples were scored, and the foodplant considered appropriate to each phenotype. (Fourth instar)

Sample Number	Genotype 1 C	B	A	D	E	Phenotype of genotype 1	Foodplant appropriate to genotype 1 (leaves in all cases)
FBE1	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE2	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE3	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE4	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE5	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE6	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE7	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE8	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE9	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE10	+-	oo	--	+ or +		3Gr 4Br 5Br	Purple rose

Table 4Bi. (Continued)

First behaviour experiment. The genotypes and phenotypes of the FBE samples with the instar in which samples were scored, and the foodplant considered appropriate to each phenotype. (Fourth instar)

Sample Number	Genotype 2 C	B	A	D	E	Phenotype genotype 2	Foodplant appropriate to genotype 2 (leaves in all cases)
FBE1	+-	oo	--	+ or +		3Gr 4Br 5Br	Purple rose
FBE2	+-	oo	++	+ or +		3Gr 4Br 5Br	Purple rose
FBE3	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE4	+-	'o	++	+ or +		3Gr 4OL 5Br	Plantain
FBE5	+-	''	++	+ or +		3Gr 4Gr 5Br	Sorrel
FBE6	+-	oo	++	+ or +		3Gr 4Br 5Br	Purple rose
FBE7	+-	oo	--	+ or +		3Gr 4Br 5Br	Purple rose
FBE8	+-	''	--	''	'o	3Gr 4PY 5PY	Young dock shoots
FBE9	+-	'o	--	''	'o	3Gr 4PY 5PY	Young dock shoots
FBE10	+-	oo	--	''	'o	3Gr 4PY 5PY	Young dock shoots

Table 4Bi. (Continued)

First behaviour experiment. The genotypes and phenotypes of the FBE samples with the instar in which samples were scored, and the foodplant considered appropriate to each phenotype. (Fifth instar)

Sample Number	Genotype 1 C	B	A	D	E	Phenotype genotype 1	Foodplant appropriate to genotype 1 (leaves in all cases)
FBE11	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE12	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE13	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE14	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE15	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE16	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE17	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE18	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE19	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE20	+-	''	--	+ or +		3Gr 4Gr 5Gr	Sorrel
FBE21	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE22	+-	oo	--	+ or +		3Gr 4Br 5Br	Purple rose

Table 4Bi. (Continued)

First behaviour experiment. The genotypes and phenotypes of the FBE samples with the instar in which samples were scored. and the foodplant considered appropriate to each phenotype. (Fifth instar)

Sample Number	Genotype 2 C	B	A	D	E	Phenotype genotype 2	Foodplant appropriate to genotype 2 (leaves in all cases)
FBE11	+-	oo	--	+ or +		3Gr 4Br 5Br	Purple rose
FBE12	+-	oo	++	+ or +		3Gr 4Br 5Br	Purple rose
FBE13	+-	'o	--	+ or +		3Gr 4OL 5OL	Plantain
FBE14	+-	'o	++	+ or +		3Gr 4OL 5Br	Purple rose
FBE15	+-	''	++	+ or +		3Gr 4Gr 5Br	Purple rose
FBE16	+-	oo	++	+ or +		3Gr 4Br 5Br	Purple rose
FBE17	+-	oo	--	+ or +		3Gr 4Br 5Br	Purple rose
FBE18	+-	'!	++	+ or +		3Gr 4Gr 5Br	Purple rose
FBE19	+-	'o	++	+ or +		3Gr 4OL 5Br	Purple rose
FBE20	+-	''	--	''	'o	3Gr 4PY 5PY	Young dock shoots
FBE21	+-	'o	--	''	'o	3Gr 4PY 5PY	Young dock shoots
FBE22	+-	oo	--	''	'o	3Gr 4PY 5PY	Young dock shoots

were removed at some time between 11 a.m. and 1 p.m. The number of larvae on each of the foodplants, was counted after 24 and again after 36 hours. This was done so that larvae were counted both in the day and at night with the result that data were collected both when larvae would naturally be resting low down amongst the herbage, and when they would be higher up the herbage strata, feeding.

Results and Method of analysis.

The results from this treatment are tabulated in Table 4Bii.

The statistical analysis of the data simply involved a series of chi-squared tests to compare the number of larvae of each genotype on each foodplant, taking the expected ratio between genotypes on each foodplant as 1:1 in all cases.

The chi-squared values from these tests are given in Appendix Table 4Bi.

Discussion.

None of the results of the chi-squared tests are significant at the 5% level. The evidence from these experiments suggests, then, that larvae do not rest or feed on leaves which they match to a greater extent than would be expected from random chance. As can be seen from the results, larvae in many cases did show a significant preference for one foodplant over another, e.g. sorrel preferred to rose in FBE18, ($X^2_1 = 5.12$, $0.05 > p > 0.02$); however, similar preferences occurred in all samples fed upon two particular plants, although in some cases the preference was not significant, and in every case, where a significant preference occurred, the two genotypes of a sample preferred the same plant. Thus, there is

Table 4Bii.

First behaviour experiment. Number of larvae of each of the two genotypes from each sample on each of the two foodplants used in each test.

Sample number	Foodplant 1 (Appropriate to colour of genotype 1)	Number of larvae of genotype 1 on foodplant 1 (day)	Number of larvae of genotype 2 on foodplant 1 (day)	Number of larvae of genotype 1 on foodplant 1 (night)	Number of larvae of genotype 2 on foodplant 1 (night)
FBE1	Sorrel	16	14	11	17
FBE2	Sorrel	14	16	17	17
FBE3	Sorrel	14	14	15	14
FBE4	Sorrel	13	14	17	13
FBE5	Plantain	10	13	9	12
FBE6	Plantain	13	14	12	17
FBE7	Plantain	15	13	13	13
FBE8	Sorrel	11	17	16	12
FBE9	Plantain	12	11	13	10
FBE10	Purple rose	9	10	10	8
FBE11	Sorrel	19	12	15	18
FBE12	Sorrel	13	16	18	13
FBE13	Sorrel	11	14	11	19
FBE14	Sorrel	14	17	14	18
FBE15	Plantain	13	14	14	14
FBE16	Plantain	17	10	16	13
FBE17	Plantain	13	14	13	18
FBE18	Sorrel	17	16	17	17
FBE19	Plantain	12	17	15	12
FBE20	Sorrel	16	11	13	14
FBE21	Plantain	11	12	10	12
FBE22	Purple rose	6	12	8	8
	Total	289	291	297	309

Table 4Bii. (Continued)

First behaviour experiment. Number of larvae of each of the two genotypes from each sample on each of the two foodplants used in each test.

Sample number	Foodplant 2 (Appropriate to colour of genotype 2)	Number of larvae of genotype 1 on foodplant 2 (day)	Number of larvae of genotype 2 on foodplant 2 (day)	Number of larvae of genotype 1 on foodplant 2 (night)	Number of larvae of genotype 2 on foodplant 2 (night)
FBE1	Purple rose	9	11	14	8
FBE2	Purple rose	11	9	8	8
FBE3	Plantain	11	11	10	11
FBE4	Plantain	12	11	8	12
FBE5	Sorrel	15	12	16	13
FBE6	Purple rose	12	11	13	8
FBE7	Purple rose	10	12	12	12
FBE8	Dock shoots	14	8	9	13
FBE9	Dock shoots	13	14	12	15
FBE10	Dock shoots	16	15	15	17
FBE11	Purple rose	6	13	10	7
FBE12	Purple rose	12	9	7	12
FBE13	Plantain	14	11	14	6
FBE14	Purple rose	11	8	11	7
FBE15	Purple rose	12	11	11	11
FBE16	Purple rose	8	15	9	12
FBE17	Purple rose	12	11	12	7
FBE18	Purple rose	8	9	8	8
FBE19	Purple rose	13	8	10	13
FBE20	Dock shoots	9	14	12	11
FBE21	Dock shoots	14	13	15	13
FBE22	Dock shoots	19	13	17	17
	Total	261	289	253	241

no evidence of a correlation between foodplant preference and the genetic system controlling colour.

ii The second behaviour experiment.

Experimental procedure.

To determine whether the larval resting or feeding position is affected by the general colour of the surroundings, tests were carried out using 22 samples of 50 larvae, named the "second behaviour experiment samples" (SBE). Again, each sample consisted of two groups of 25 larvae with known genotype, numbered in the same way as the FBE samples. The boxes used were similar in size to those used in the previous experiment, and sorrel was used as food for the larvae throughout. Pieces of brown or green paper were taped on to the top and sides of the box in a chequer pattern, so that one half of the box was covered in 20 mm squares of brown paper, with the spaces between the brown paper left clear. The other half of the box had a similar pattern with the brown paper replaced by green paper. Again, the larvae were reared using the basic methods described in chapter 2B until they were needed for the experiments. The foodplant and larvae were placed in the boxes in the same way as in the first behaviour experiments, and the number of larvae of each genotype in each half of each box was again counted after 24 hours and 36 hours.

Results and Method of data analysis.

The results of these counts are given in Table 4Biii.

The statistical analysis again involved a chi-squared test on the results of each of the samples, comparing the number of larvae of each genotype in each half of a box. The expected ratio

Table 43iii.

Second behaviour experiment. Number of larvae of each of the two genotypes from each sample in the brown and green halves of the boxes.

Sample	No. of larvae of genotype 1 in the green halves of the boxes (Day)	No. of larvae of genotype 1 in the brown halves of the boxes (Day)	No. of larvae of genotype 2 in the green halves of the boxes (Day)	No. of larvae of genotype 2 in the brown halves of the boxes (Day)
SBE1	8	17	9	16
SBE2	10	15	9	16
SBE3	9	16	10	15
SBE4	8	17	6	19
SBE5	11	14	8	17
SBE6	12	13	8	17
SBE7	7	18	10	15
SBE8	9	16	7	18
SBE9	9	16	8	17
SBE10	10	15	6	19
SBE11	8	17	12	13
SBE12	14	11	11	14
SBE13	10	15	9	16
SBE14	7	18	6	19
SBE15	8	17	10	15
SBE16	9	16	12	13
SBE17	10	15	9	16
SBE18	10	15	8	17
SBE19	6	19	4	21
SBE20	9	16	7	18
SBE21	7	18	10	15
SBE22	11	14	8	17
TOTAL:	202	348	187	363

Sample	No. of larvae of genotype 1 in the green halves of the boxes (Night)	No. of larvae of genotype 1 in the brown halves of the boxes (Night)	No. of larvae of genotype 2 in the green halves of the boxes (Night)	No. of larvae of genotype 2 in the brown halves of the boxes (Night)
SBE1	11	14	13	12
SBE2	13	12	14	11
SBE3	8	17	14	11
SBE4	14	11	11	14
SBE5	10	15	12	13
SBE6	8	17	9	16
SBE7	13	12	11	14
SBE8	14	11	13	12
SBE9	9	16	14	11
SBE10	16	9	9	16
SBE11	14	11	12	13
SBE12	15	10	10	15
SBE13	13	12	11	14
SBE14	8	17	10	15
SBE15	11	14	14	11
SBE16	9	16	13	12
SBE17	14	11	16	9
SBE18	13	12	14	11
SBE19	11	14	12	13
SBE20	13	12	10	15
SBE21	11	14	10	15
SBE22	12	13	13	12
TOTAL:	259	291	265	285

between genotypes in each of the two halves of a box was taken to be 1:1. The results of these chi-squared tests are given in Appendix Table 4Bii.

Discussion.

The probability values from the chi-squared tests show that there is no significant difference between the behaviour of larvae of any of the genotypes used, and, therefore, it may be deduced that larvae do not exhibit a behavioural response to background colour correlated with the genetic system controlling larval colour.

One other feature of the results of these experiments is worth noting here, as it led to further experiments on larval behaviour. The scores for larvae counted after 36 hours, i.e. at night, give approximately a 1:1 ratio of larvae in each half of the box irrespective of genotype. On the other hand, when the scores for larvae counted after 24 hours are considered, it is apparent that in every case the number of larvae in the half of the box covered with brown paper was greater than that in the other half of the box. As the frequency of larvae in the two halves of the box is approximately equal at night, and there is a predominance of larvae in one half of the box in the day, it seemed obvious to assume that this difference was due to a light effect. As 4th and 5th instar larvae of P. meticulosa appear to exhibit a negative phototactic response, in that they "hide" close to the ground amongst the dense herbage during the hours of daylight, it was considered possible that larvae may have favoured the brown half of the boxes because the brown paper let less light in than the green paper. Measurements of the light inside the box at the

ends, using a Sangamo-Weston Master IV light meter, showed that the brown ends of the boxes were darker than the green ends. It was concluded that the reason for the majority of larvae in the tests positioning themselves in the brown half of the boxes, was probably due to light intensity in this part of the boxes being lower than in the green halves, rather than the difference in light quality between the two halves.

To substantiate this conclusion, two additional samples (SBE23 and SBE24), of 50 larvae, with genotype ccb'b'aa were reared under the same conditions and treated in the same way as the other SBE samples, but with one half of each box being completely covered with thick black paper, and the other half totally uncovered. The SBE23 and SBE24 samples were used when the larvae were in the 4th and 5th instars respectively.

The count from sample SBE23 after 36 hours, (i.e. at night), gave 23 larvae in the covered half of the box, and 27 in the uncovered portion, a difference which is not significant, ($X^2_1 = 0.32$, $0.7 > p > 0.5$). On the other hand, the count from SBE23 after 24 hours gave a result of 47 larvae in the covered portion of the box, compared with 3 in the uncovered part. This difference is highly significant, ($X^2_1 = 38.72$, $p < 0.001$).

Similarly, for sample SBE24, the ratio of larvae in the covered and uncovered halves of the box was 28:22 at night, (a ratio which does not differ significantly from the expected 1:1 ratio; $X^2_1 = 0.72$, $0.5 > p > 0.3$), and 45:5 during the day, (a ratio which shows a highly significant departure from the expected 1:1 ratio; $X^2_1 = 32$, $p < 0.001$).

This evidence seems to substantiate the conclusion that 4th

and 5th instar larvae exhibit a negative phototactic response, based on light intensity rather than light wavelength.

iii The third behaviour experiment.

Experimental procedure.

The third set of experiments was designed to study the level in the herbage to which larvae of different genotypes move at dusk and dawn. These tests were carried out on a piece of rough grassland near Ringwood. Four plots of flat ground, each five metres square, were chosen for their similarity and for their uniformity of vegetation. The vegetation of the plots consisted of grasses, docks, plantains and other low growing herbaceous plants.

The plots were prepared over a period of four months. This preparation involved the removal of all larvae already present on the plots and the removal of any potential larval predators. Each plot was surrounded by a two foot high fence of corrugated iron to keep out mammal predators, and small mammal traps were set up in the plots to ensure that no small mammals were already within the confines of the plots. The plots were thoroughly swept and searched for larvae four months before the tests were to be carried out, and any larvae found were removed. Black muslin was then stretched across the plots to prevent any further Angleshades females laying eggs on the plots. The plots were kept under observation until the experiments had been completed, so that any imagines which had emerged from eggs, larvae, or pupae, which had not been noticed in the initial search and sweep could be removed.

During the experiments, each of the plots was divided into

five strips by 0.306m high strips of sheet metal. Each strip measured five metres by one metre and the five strips in each plot were marked A - E inclusive. Four samples of larvae were prepared. These were named the "third behaviour experiment samples" (TBE1 - TBE4 inclusive). Each sample consisted of 500 larvae. Two of the samples (TBE1 and TBE2) had 125 larvae of each of the four genotypes, $c^+c^Xb'b'aa(d^+d^X$ or $e^+e^X)$, $c^+c^Xb'b^Oaa(d^+d^X$ or $e^+e^X)$, $c^+c^Xb^Ob^Oaa(d^+d^X$ or $e^+e^X)$ and $c^+c^Xb'b^Oaadde'e^O$, so that the four colour types Gr, OL, Br and PY were each represented in the sample by 125 larvae. These two samples were used for experiments when in the 4th instar. The other two samples (TBE3 and TBE4) consisted of 125 larvae of the same four genotypes as in the first samples, with the exception that the $c^+c^Xb^Ob^Oaa(d^+d^X$ or $e^+e^X)$ larvae were replaced by $c^+c^Xb'b'a^+a^+(d^+d^X$ or $e^+e^X)$ larvae. These samples were used when the larvae were in the 5th instar.

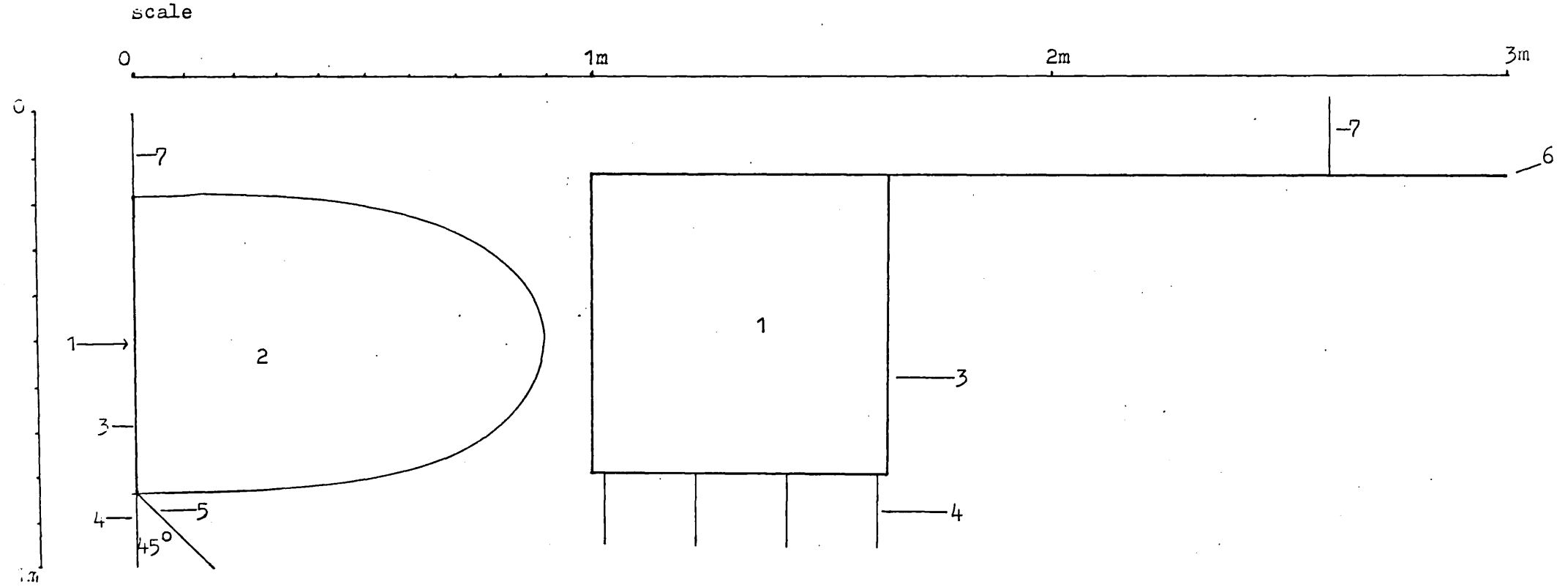
Each of the four samples was put out on one of the plots, (which were given the same number as the sample assigned to them), in such a way that 25 larvae of each of the four genotypes in a sample, were scattered at random over each strip. Samples were put out between 9.30 a.m. and 1.30 p.m. The larvae on plots TBE1 and TBE3 were collected again approximately 24 hours after they were put out, and those on plots TBE2 and TBE4, about 36 hours after they were put out, so that one sample of 4th instar larvae and one sample of 5th instar larvae were re-collected by day, the others being re-collected by night.

Five methods of re-collecting larvae were designed, and each of these was assigned to one of the strips in each plot. The

A strips were sampled using a conventional sweep-net, sweeping being carried out so that the lower edge of the net opening scraped along the ground as much as possible. The B strips were sampled using a specifically constructed sweep-net. This consisted of a net attached to a square rim of side 650 mm, attached along one side to a two metre pole which ran parallel to that side. Eight solid metal rods were attached to the side of the frame, opposite the pole side, and at right angles to it. Four of these rods protruded out 100 mm from the edge of the rim in the same plane as the net opening, the other four protruding approximately 141 mm from the edge at an angle of 45° to the first four. (See Fig. 4i). The net was used in such a way that the angle of sweep was parallel to the ground, and the first set of metal rods kept the bottom edge of the rim at least 100 mm from the ground. The net opening was vertical at the beginning of each sweep, and it was maintained in this plane by the second set of rods which were kept in contact with the ground by an anti-clockwise wrist pressure on the net handle. The C strips were sampled using the same method, but with the 100 mm rods and the 141 mm rods replaced by 250 mm and 354 mm rods respectively.

The D strips were sampled by lifting the turf of the whole strip to a depth of about 40 mm with a turf spade. The resulting sods of turf were placed in closed boxes and later were systematically dissected, to collect the larvae. The turfs were checked repeatedly to ensure that every larvae had been collected.

The final group of strips, the E strips, were searched systematically on hands and knees, a process which took over five hours to complete for each strip.



1. Net opening. 2. Net. 3. Net frame.
4. Metal rods to keep the net opening a set distance above the ground.
5. Metal rods to help keep the net opening vertical.
6. Handle. 7. Additional handle to aid anti-clockwise wrist pressure.

Fig. 4i. Side and front diagrams of sweep-net designed to sample only those larvae situated 100 mm or more above the ground. (For example, used to re-collect larvae from the B strips in the 3rd behaviour experiment).

When collecting samples from strips A, B and C at night, a small torch was used so that the boundaries of the strips could be seen. Samples from the D and E strips were collected at night, using bright electric lights which had been erected specifically for that purpose. The strips were sampled in alphabetical order in all cases, so that the A, B and C samples had been collected before the strong lights were switched on.

Results and analysis of data.

The results of these tests are given in Table 4Biv.

To analyse the results, a number of chi-squared tests were carried out, and the results of these are given in Appendix, Table 4Biii. The statistical analysis of the results shows that in the experiments, larvae in the 4th instar behave in the same way as the 5th instar larvae, in that significant departures from the expected, which occur in tests on 5th instar larvae, also occur in similar tests on the 4th instar.

The number of larvae re-collected from each sample shows considerable variation. In the case of the A strips, there was no significant difference in the number of larvae taken by day and the number taken at night. On the other hand, for strips B and C, the samples taken at night were significantly greater in number than those taken by day. These results were expected as it was already known that larvae remain in the very low levels of the herbage in the day, and only climb to the higher herbage strata at night.

The samples from the D strips showed no significant differences, all samples being of 99 or 100 larvae. Again this was expected as the method of sampling the D strips was designed

Table 4Biv.

Third behaviour experiments. Number of larvae of each genotype from each sample re-collected from each of the five strips in plots 1 - 4 inclusive.

Sample Number	Plot Number	Time	Genotype C	B	A	D	E	No. of larvae re-collected from each strip A	B	C	D	E	Total
TBE1	1	Day	+x	''	--	+ or +		17	3	4	25	22	71
			+x	'o	--	+ or +		9	6	5	25	21	66
			+x	oo	--	+ or +		12	8	3	25	17	65
			+x	'o	--	--	'o	15	6	2	25	22	70
							Total	53	23	14	100	82	272
TBE2	2	Night	+x	''	--	+ or +		18	14	6	25	19	82
			+x	'o	--	+ or +		16	16	11	25	13	81
			+x	oo	--	+ or +		21	17	9	25	16	88
			+x	'o	--	--	'o	13	12	9	24	17	75
							Total	68	59	35	99	65	326
TBE3	3	Day	+x	''	--	+ or +		14	7	4	25	21	71
			+x	'o	--	+ or +		17	6	2	25	25	75
			+x	''	++	+ or +		11	10	2	25	21	69
			+x	'o	--	--	'o	13	3	3	25	20	64
							Total	55	26	11	100	87	279
TBE4	4	Night	+x	''	--	+ or +		15	17	10	25	15	82
			+x	'o	--	+ or +		20	14	11	24	19	88
			+x	''	++	+ or +		17	14	6	25	20	82
			+x	'o	--	--	'o	16	18	11	25	18	88
							Total	68	63	38	99	72	340

so that as many larvae were collected as possible. The fact that two of the re-collected samples only had 99 larvae, rather than the 100 which were put out, was probably due either to sampling error, or to some type of predator, possibly a carabid beetle, being missed during the preparation of the plot.

The day-collected samples from the E strips were numerically greater than the night-collected samples, this difference however was not significant. The difference, if a non-random one, is probably due to day-light being of a greater intensity than the light from the electric bulbs used at night, so that more larvae were missed when sampling the E strips by visual means at night, than during the day. The denser shadows produced by the electric lights may also have had some effect.

When considering the ratios of the genotypes in the collected samples, heterogeneity tests show that in no case is there a significant departure from the 1:1:1:1 ratio, which would be expected if there is no correlation between larval behaviour with respect to the position of larvae in the herbage stratas, and the larval genotypes, (for chi-squared values, see Appendix Table 4Biii).

The results from the three sets of behaviour experiments, then, show that in the 4th and 5th instars, there is no evidence of any correlation between larval genotype, with respect to the major genes which control the main larval colour-types in these instars, and the larval behaviour with respect to (a) the colour of the substrate that larvae place themselves upon, (b) the general background colour around the point at which larvae rest or feed, and (c) the level in the herbage strata to which larvae move during the day or night.

C. Further behaviour experiments.

i. Introductory remarks.

At one time I had only intended to carry out the three sets of behavioural experiments already described in this chapter. However, observations in other parts of the study induced me to extend the range of the behavioural experiments.

When rearing larvae from eggs, I noted that in the 1st, 2nd and to a lesser extent 3rd instars, the majority of larvae tended to congregate in the portion of the boxes which were best illuminated during the day, indicating that the young larvae may be positively phototactic. If this possibility were to be verified, then as 4th and 5th instar larvae have been shown to be negatively phototactic, (see second behaviour experiments), there must be a point at which the larval behaviour pattern changes from one of positive phototaxis to one of negative phototaxis. As the phototactic response of larvae will obviously have some bearing on larval camouflage, it was considered necessary to discover the exact stage at which this behavioural change takes place before research was carried out on the significance of larval colour type in relation to visual selection.

The second observation, which led to further research, was noted during tests designed to give estimates of the allelic frequencies of those alleles which have been shown to control larval colour, (see chapters 3C and 5). Two methods were employed to obtain data from which to estimate allelic frequencies. One of these involved rearing large numbers of larvae from eggs laid by light-trapped females. The other involved the sampling of larvae in natural populations by sweep-netting both during the day and at night. The phenotypic frequencies from the two methods were reasonably similar for all colour types except those in the 3rd

instar. The proportions of OL, Br and the three yellow colour types in this instar were much lower in the samples produced by rearing stocks from light-trapped females, whilst the proportion of 3IG larvae was proportionately higher.

Two possible explanations for this feature seem plausible. On one hand, the dominant allele c^+, may have been heavily selected against compared with the c allele during the 3rd and subsequent larval instars, so that the number of adults with the c^+ allele would have been much lower than the number of 3rd instar larvae with it. Alternatively, a differential behaviour characteristic, controlled by gene C, could have led to inaccuracies in sampling by sweep-netting, or indeed by light-trapping females, as the effects of c^+ and c need not necessarily be confined to the larval stage of the life cycle. The first, second and third behaviour experiments suggest that there is no correlation between behaviour and genotype in the 4th or 5th instars, but they do not rule out the possibility of such a correlation occurring in the 3rd instar.

Of these two possible explanations, the former may be deemed unlikely, if not disproved altogether, by analysing the phenotypic frequencies from the samples obtained by sweep-netting. If the proportions of the phenotypes in the night collected samples are considered, they prove to be very similar to those observed in the light-trapped female data. The discrepancy in the phenotypic frequencies of the 3rd instar is due to a disproportionately low number of all the 3rd instar colour types, except 3IG, being taken in the daytime sweep-netted samples, (see chapter 5 for full data and analysis). This could obviously not be due to selection acting

against the c^+ allele, between the 3rd larval instar and the imago stage. On the other hand, the behaviour theory now seems to hold more credence.

Hypothetically, both the change from positive to negative phototaxis, and the differences in the proportions of the 3rd instar phenotypes, could be explained on the basis of a behavioural characteristic controlled by gene C. If it is assumed that 4th and 5th instar larvae are always negatively phototactic, then it may be that the recessive allele, c, when homozygous, not only causes 4th instar larval colour, controlled by alleles of the genes B, D and E, to arise in the 3rd instar, but may also induce larvae to be negatively phototactic in the 3rd instar. On the other hand, larvae with c^+ in their genotype would have a phototactic response similar to that shown by 1st and 2nd instar larvae, which would appear, from uncontrolled observations, to be a positive response.

ii Fourth behaviour experiment.

Experimental procedure.

To investigate this hypothesis, 16 samples of 50 larvae of known genotype were reared from the ova to pupa in rectangular perspex boxes 750 mm x 500 mm x 50 mm with white muslin stretched over the top. The samples were named the "fourth behavioural experiment samples" (4BE1 - 4BE16 inclusive). Each sample was monogenotypic and the genotype for each sample is given in Table 4Ci. The boxes were prepared for use by covering one half of each with thick black paper. Boxes were kept outside in direct sunlight. The number of larvae in the covered and uncovered halves of the boxes were counted every six hours at 3 a.m., 9 a.m., 3 p.m., and

Table 4Ci.

Fourth behaviour experiment. Investigation into the phototactic response of larvae. Mean number of larvae from each sample in the covered and uncovered portions of the boxes at each count time. (All instars)

UNCOVERED PORTION OF BOXES

Sample number	Genotype of sample Alleles of genes: C	B	A	D	E	Phenotype of sample for instars: 1	2	3	4	5	First instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night	Second instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night	Third instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night	Fourth instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night	Fifth instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night
4BE1	+x	''	--	+ or +		EG	EG	3IG	Gr	Gr	37.6	48.2	48.4	41.6	33.6	46.5	47.7	40.2	33.3	45.3	46.0	36.8	23.0	3.3	1.3	21.6	22.4	2.3	0.8	22.3
4BE2	+x	''	+x	+ or +		EG	EG	3IG	Gr	Br	35.8	46.4	49.2	38.8	37.4	48.2	48.4	39.4	34.2	46.8	47.0	38.3	21.3	3.1	0.9	19.8	23.3	2.0	0.5	22.4
4BE3	+x	'o	--	+ or +		EG	EG	3IG	OL	OL	40.2	48.2	49.4	42.6	35.6	48.6	48.4	38.6	32.7	46.7	46.7	37.7	20.8	2.8	1.4	21.0	23.3	1.9	0.4	22.9
4BE4	+x	'o	+x	+ or +		EG	EG	3IG	OL	Br	36.2	47.0	47.2	40.2	34.8	47.4	48.6	41.0	33.5	47.8	48.0	36.7	23.6	3.6	1.6	20.4	24.1	2.0	0.6	23.3
4BE5	+x	oo	--	+ or +		EG	EG	3IG	Br	Br	38.3	49.4	48.2	42.6	34.9	46.9	47.8	40.2	33.2	45.5	45.3	37.5	24.3	6.0	0.8	19.4	24.5	2.9	1.0	20.9
4BE6	+x	oo	+x	+ or +		EG	EG	3IG	Br	Br	39.6	47.4	48.6	43.0	36.8	47.6	48.4	38.4	32.5	45.7	46.2	37.0	23.1	3.3	1.8	22.5	24.0	1.8	0.6	23.3
4BE7	+x	'o	--	--	'o	EG	EG	3IG	PY	PY	32.8	44.6	46.2	38.6	37.2	49.2	49.0	38.8	34.2	47.2	47.3	36.2	26.1	2.4	1.4	21.9	24.0	3.0	0.4	22.8
4BE8	+x	'o	+x	--	'o	EG	EG	3IG	PY	PY	40.0	49.2	49.0	41.2	35.0	48.6	49.2	39.4	36.3	46.3	46.8	37.8	22.8	5.6	1.3	21.8	22.8	1.8	0.6	22.4
4BE9	--	''	--	+ or +		EG	EG	3IG	Gr	Gr	34.5	48.4	48.6	43.0	39.3	46.7	47.4	38.3	23.2	4.3	3.0	19.3	25.0	2.8	0.9	22.8	21.9	2.3	0.8	22.6
4BE10	--	''	+x	+ or +		EG	EG	3IG	Gr	Br	38.8	46.0	47.8	38.2	36.2	49.0	48.6	39.6	21.7	4.2	2.7	20.5	22.9	5.1	1.0	23.0	26.3	2.4	0.3	22.5
4BE11	--	'o	--	+ or +		EG	EG	OL	OL	OL	36.4	46.6	47.2	39.6	37.2	47.4	49.0	37.2	22.0	3.8	2.5	18.8	24.8	3.0	1.4	22.8	25.0	2.6	1.1	22.8
4BE12	--	'o	+x	+ or +		EG	EG	OL	OL	Br	40.1	49.2	49.6	42.1	34.6	47.8	47.8	40.7	22.5	4.3	3.2	18.8	24.0	4.9	1.5	22.6	24.1	1.8	0.9	20.8
4BE13	--	oo	--	+ or +		EG	EG	Br	Br	Br	38.6	48.6	49.0	43.4	36.8	48.2	48.6	39.6	22.7	3.7	2.0	20.0	23.3	3.1	0.8	19.8	24.4	2.5	0.8	22.6
4BE14	--	oo	+x	+ or +		EG	EG	Br	Br	Br	44.3	48.8	48.6	40.4	36.3	46.3	46.7	38.7	21.8	3.8	4.0	19.7	24.5	2.6	1.3	21.4	24.1	1.9	1.0	21.3
4BE15	--	'o	--	--	'o	EG	EG	PY	PY	PY	38.6	49.0	49.6	39.2	35.8	46.6	48.0	37.2	21.3	4.7	3.3	19.5	22.0	2.8	1.1	20.4	22.6	3.0	0.4	21.0
4BE16	--	'o	+x	--	'o	EG	EG	PY	PY	PY	39.2	47.8	48.2	39.4	36.4	48.8	49.0	40.4	22.7	4.0	2.2	18.7	21.5	3.4	1.4	22.0	23.3	1.9	0.8	23.3

COVERED PORTION OF BOXES

Sample number	Genotype of sample Alleles of genes: C	B	A	D	E	Phenotype of sample for instars: 1	2	3	4	5	First instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night	Second instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night	Third instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night	Fourth instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night	Fifth instar 3 a.m. Night	9 a.m. Day	3 p.m. Day	9 p.m. Night
4BE1	+x	''	--	+ or +		EG	EG	3IG	Gr	Gr	12.4	1.8	1.6	8.4	15.6	2.9	1.7	9.2	14.7	2.7	2.0	11.2	24.0	43.8	45.8	25.4	26.6	44.8	46.3	24.8
4BE2	+x	''	+x	+ or +		EG	EG	3IG	Gr	Br	14.2	3.6	0.8	11.2	12.6	1.8	1.6	10.6	15.8	3.2	3.0	11.7	27.8	45.9	48.1	29.3	24.8	46.0	47.5	25.6
4BE3	+x	'o	--	+ or +		EG	EG	3IG	OL	OL	9.8	1.8	0.6	7.4	14.4	1.4	1.6	11.4	16.5	2.5	2.5	11.5	28.3	46.3	47.6	28.0	25.8	47.1	48.6	27.1
4BE4	+x	'o	+x	+ or +		EG	EG	3IG	OL	Br	13.8	3.0	2.8	9.8	15.2	2.6	1.4	9.0	16.5	2.2	2.0	13.3	26.4	46.4	48.4	29.6	25.9	48.0	49.4	26.8
4BE5	+x	oo	--	+ or +		EG	EG	3IG	Br	Br	11.3	0.2	1.4	7.0	14.1	2.1	1.2	8.8	15.8	3.5	3.3	11.5	24.8	43.0	48.3	29.6	24.5	46.1	48.0	27.1
4BE6	+x	oo	+x	+ or +		EG	EG	3IG	Br	Br	10.4	2.6	1.4	7.0	14.0	2.2	1.2	11.4	16.5	3.3	2.8	12.0	25.9	45.8	47.3	26.5	24.0	46.3	47.4	24.8
4BE7	+x	'o	--	--	'o	EG	EG	3IG	PY	PY	17.2	5.4	3.8	11.4	12.8	0.8	1.0	11.2	15.8	2.8	2.7	13.8	23.9	47.6	48.6	28.1	26.0	47.0	49.6	27.3
4BE8	+x	'o	+x	--	'o	EG	EG	3IG	PY	PY	10.0	0.8	1.0	8.8	15.0	1.4	0.8	10.6	13.3	3.3	2.8	11.8	28.3	43.4	47.8	27.3	24.3	45.3	46.4	24.6
4BE9	--	''	--	+ or +		EG	EG	3IG	Gr	Gr	15.3	1.4	1.2	6.8	12.9	1.5	0.8	9.9	24.8	43.7	45.0	28.7	23.0	45.3	47.1	25.3	26.1	45.8	47.3	25.4
4BE10	--	''	+x	+ or +		EG	EG	3IG	Gr	Br	11.2	4.0	2.2	11.8	13.8	1.0	1.4	10.4	28.3	45.8	47.3	29.5	26.1	42.9	48.0	26.0	22.8	46.6	48.8	26.5
4BE11	--	'o	--	+ or +		EG	EG	OL	OL	OL	13.6	3.4	2.8	10.4	12.8	2.6	1.0	12.8	28.0	46.2	47.5	31.2	25.3	47.0	48.6	27.3	23.6	46.0	47.5	25.8
4BE12	--	'o	+x	+ or +		EG	EG	OL	OL	Br	9.8	0.7	0.3	7.8	14.4	1.2	1.2	8.3	26.5	44.7	45.8	30.2	24.8	43.9	47.3	26.3	23.9	46.3	47.1	27.3
4BE13	--	oo	--	+ or +		EG	EG	Br	Br	Br	11.4	1.4	1.0	6.6	14.2	1.8	1.4	10.4	26.5	45.3	47.0	29.0	25.8	45.9	48.3	29.3	24.6	46.5	48.3	26.4
4BE14	--	oo	+x	+ or +		EG	EG	Br	Br	Br	5.1	0.6	0.8	9.0	12.7	2.7	2.3	10.5	27.2	45.2	45.0	29.3	24.5	46.4	47.8	27.6	24.9	47.1	48.0	27.8
4BE15	--	'o	--	--	'o	EG	EG	PY	PY	PY	11.4	1.0	0.4	10.8	14.2	3.4	2.0	12.8	26.7	43.3	44.7	28.5	26.0	45.3	46.9	27.6	25.4	45.0	47.6	27.0
4BE16	--	'o	+x	--	'o	EG	EG	PY	PY	PY	10.8	2.2	1.8	10.6	13.6	1.2	1.0	9.6	27.3	46.0	47.8	31.3	28.5	46.6	48.6	28.0	26.8	48.1	49.3	25.8

9 p.m. The experiments were carried out during September and October, so that the 3 a.m. and 9 p.m. counts were carried out in darkness and the 9 a.m. and 3 p.m. counts in daylight. Larvae which died during the experiments were not replaced.

When newly hatched, the larvae, having consumed the major part of the empty egg case, are highly photopositive for between 12 and 18 hours, and during this period they do not feed at all. If there is no light for the larvae to respond to during this period they do not move, (personal observation).

Results and analysis of data.

The results of these tests are given in Table 4Ci. The values given show the mean number of larvae in each of the two halves of the boxes at each of the 4 count times. The instars are treated individually for each sample. The mean value is given because no significant difference occurred in the counts at a particular time, for any of the samples, from one day to the next, if the instar of the larvae did not change. The mean value for 1st instar larvae was calculated from the time that larvae began feeding.

It may be seen from the results that the vast majority of larvae from all the samples are photopositive in the 1st and 2nd instars, virtually all larvae occurring in the uncovered part of the boxes in the daytime. Larvae in these instars continued to move around and feed at night, and some of the larvae moved into the covered area during this period although in every case, more than half the larvae stayed in the uncovered portion. In the case of 4th and 5th instar larvae, the vast majority show a negative response to light during the day, most of the larvae being found

in the covered portions of the boxes at 9 a.m. and 3 p.m. At night, these larvae show no phototactic response, approximately half being found in each half of the box.

3rd instar larvae, which have c^+ in their genotype, (samples 4BE1 - 4BE8 inclusive), act in the same way as 1st and 2nd instar larvae, while those which are homozygous for c, (samples 4BE9 - 4BE16 inclusive) act in the same way as 4th and 5th instar larvae.

Discussion.

These results, then, indicate that a change from positive to negative phototaxis occurs during larval development, and that this change occurs at the start of the 4th larval instar if c^+ is present in the genotype, or at the start of the 3rd larval instar if c^+ is absent. The discrepancy in the frequency estimates for the alleles of gene c was due to a correlation between phototactic response and alleles c^+ and c, which led to the sample of larvae obtained by daytime sweep-netting not being random.

iii Fifth behaviour experiments.

To verify this, a set of tests were carried out using similar methods to those employed in the 3rd behaviour experiments. Two samples of 625 third instar larvae were used. These samples were named the "fifth behaviour experiment samples" (5BE1 and 5BE2). Each sample was split into five identical groups of 125 larvae, each made up of 25 larvae of the five genotypes, $c^+c^+b'b'aa(d^+ \text{ or } e^+)$, $ccb'b'aa(d^+ \text{ or } e^+)$, $ccb'b^oaa(d^+ \text{ or } e^+)$, $ccb^ob^oaa(d^+ \text{ or } e^+)$ and $ccb'b^oaa\ dde'e^o$. Two plots (plots 5 and 6) were prepared in the same way as the plots in the 3rd behavioural experiments. One group of larvae was put out on each of the plot

strips which again were marked A - E inclusive, and larvae were collected from these strips using the same techniques that were used for strips of the same letter in the 3rd behavioural experiments. The samples were put out at 12 p.m. and samples from plot 5 were re-collected after about 24 hours, whilst those from plot 6 were re-collected after approximately 36 hours.

Results and data analysis.

The results are given in Table 4Cii.

Analysis of the results from plot 5 shows that in the case of strips A, B and C, the number of c^+c^+ larvae in the samples was higher than that of any of the other four genotypes, this difference being greatest in the results from strips C and B, when the difference was significant, (for strip C, $X^2_4 = 16.636$, $0.01 > p > 0.001$; and for strip B, $X^2_4 = 12.574$, $0.02 > p > 0.01$), and least in those from strip A when the difference was not significant, ($X^2_4 = 2$, $0.8 > p > 0.7$). Larvae of the four genotypes containing cc were homogeneous in their action in all three of these strips, (for strip A, $X^2_3 = 0.370$, $0.95 > p > 0.9$; for strip B, $X^2_3 = 0.143$, $0.99 > p > .98$; and for strip C, $X^2_3 = 0.4$, $0.95 > p > 0.90$).

The number of larvae of the five genotypes were approximately equal within the samples from both strip D and strip E, any differences being insignificant, (for strip D, $X^2_4 = 0.049$, $p > 0.99$; and for strip E, $X^2_4 = 0.345$, $0.99 > p > 0.98$).

The analysis of the results from plot 6 shows that the action of all genotypes within a sample from any single strip are homogeneous, (for strip A, $X^2_4 = 0.283$, $p > 0.99$; for strip B,

Table 4Cii.

Fifth behaviour experiment. The number of third instar larvae of each genotype recollected from each strip in Plots 5 and 6.

Sample number	Genotypes in sample Alleles of genes C	B	A	D	E	Phenotypes	Number of larvae recollected by day from each strip in Plot 5 strip A	strip B	strip C	strip D	strip E	Total
5BE1	++	''	--	+ or +		3IG4Gr5Gr	19	16	12	25	20	92
	--	''	--	+ or +		3IG4Gr5Gr	13	6	3	24	21	67
	--	'o	--	+ or +		3OL4OL5OL	14	5	3	25	19	66
	--	oo	--	+ or +		3Br4Br5Br	12	5	2	25	18	62
	--	'o	--	--	'o	3PY4PY5PY	15	5	2	24	21	67
						Total	73	37	22	123	99	354
							Number of larvae recollected by day from each strip in Plot 6.					
							strip A	strip B	strip C	strip D	strip E	Total
5BE2	++	''	--	+ or +		3IG4Gr5Gr	18	13	10	25	20	85
	--	''	--	+ or +		3IG4Gr5Gr	17	18	13	23	15	87
	--	'o	--	+ or +		3OL4OL5OL	19	17	10	24	19	89
	--	oo	--	+ or +		3Br4Br5Br	18	15	13	24	15	85
	--	'o	--	--	'o	3PY4PY5PY	20	14	9	25	21	89
						Total	92	77	55	121	90	435

X^2_4 = 1.117, $0.9 > p > 0.8$; for strip C, X^2_4 = 1.273, $0.9 > p > 0.8$; for strip D, X^2_4 = 0.116, $p > 0.99$; and for strip E, X^2_4 = 1.778, $0.8 > p > 0.7$).

These tests, then, confirm that in the 3rd instar by day, larvae which have c^+ in their genotype may be found at higher levels in the herbage strata than those which are homozygous for c. At night, all the larvae used in the tests appear to act similarly with respect to level in the herbage strata, irrespective of genotypes.

D Conclusion

In conclusion, it seems that larval behaviour, with respect to those types of response being investigated, is not affected by the five major genes which control colour, except in the 3rd instar. All 1st and 2nd instar larvae are positively phototactic, whilst all 4th and 5th instar larvae are negatively phototactic.

As was shown in chapter 3C, the colour of larvae which are homozygous recessive for c, is controlled through the phenotypic expression of the alleles of genes B, D and E being advanced to commence at the start of the third instar by the action of cc, whilst the colour of those larvae with c^+ in their genotype is controlled by foodplant. The results of the 4th and 5th sets of behaviour experiments show that the recessive allele of gene C has a second role, in that it causes 3rd instar larvae which are homozygous for this allele to take on the phototactic response found normally in the 4th and 5th instars, that is to say, a negative response. 3rd instar larvae, which are not homozygous recessive with respect to gene C are positively phototactic.

The change to positive phototaxis in the 3rd instar may be caused directly by the recessive allele c. Alternatively, c may only be indirectly involved in the change, if it is caused by the alleles of genes, B, D and E, when these are expressed phenotypically in the 3rd instar.

Some of the possible reasons for the switch from a positive to a negative phototaxis between the early and late larval instars, are connected with the predation experiments, particularly those using avian predators, (see chapters 7 and 8), and so these reasons will be discussed in the General Discussion (chapter 9).

CHAPTER 5. THE STATUS OF THE LARVAL COLOUR POLYMORPHISM.

Having discovered that the larvae of *Phlogophora meticulosa* are genetically polymorphic with respect to colour in the 3rd, 4th and 5th instars (chapter 3), the next obvious step was to attempt to discover the way in which the polymorphism is maintained. The maintenance of the polymorphism poses some interesting evolutionary problems, particularly as the number of phenotypes is quite considerable and the genes involved are unlinked. However, before this problem could be closely investigated, it was necessary to determine whether the polymorphism was balanced, transient, or a combination of the two. This latter possibility arises because the variation in the larval colour of *P. meticulosa* is controlled by a number of unlinked genes, and so the polymorphic system is of a complex nature, in effect being made up of four genetic polymorphisms. Genes A, B and C each control one of these, whilst the fourth is based on genes D and E. In fact, it is probable that the allelomorphs of these two latter genes developed separately, but due to the interactions of these alleles, then, while confining the discussion to colour variation, their effect may be considered as producing a single polymorphism.

A transient polymorphism is produced by the temporary diversity which arises when a previously disadvantageous gene spreads through a population and displaces its allelomorph (Ford, 1940a). Such a case is that of the *carbonaria* form of

Biston betularia L. which has displaced the type form, to a greater or lesser extent, in many parts of Britain over the last 130 years (Kettlewell, 1958).

A balanced polymorphism is one in which the morphs are maintained at a fairly stable frequency by selective agencies which favour diversity and oppose uniformity (Ford, 1940a).

Initially, it was decided that the most accurate way to determine the status of the colour polymorphisms would be to calculate the frequencies of the various allelomorphs involved in the control of larval colour over a period of time.

Two methods of obtaining data from which to estimate allelic frequencies were employed. The first of these involved rearing larvae from females captured at light in the Ringwood area, and recording the phenotypes of these larvae during the 3rd, 4th and 5th instars.

The other method used, involved recording the colour types and instars of larvae taken by sweep-netting from low herbage at a number of sites in the Ringwood area. As these two methods employed rather different techniques, and posed very different problems, they will be dealt with separately.

A. Allelic frequency analysis using data from the progeny of light-trapped females.

Methods

To make allelic frequency estimates based on the progeny of wild females, light-trapping was carried out

around the Ringwood area during the six main emergence periods of P. meticulosa that occurred between September, 1975 and October, 1977.

All the trapped females were taken and treated in the same way as those used to obtain broods during the investigation into the control of larval colour variation (see basic breeding methods, chapter 2B).

The main emergences between September, 1975 and October, 1977 were in Sept./Oct. 1975; May, 1976; July, 1976; Sept./Oct. 1976; May/June, 1977; and Sept./Oct. 1977. The estimates for Sept./Oct. 1975 were made using the data from the larvae reared for the colour variation analysis (chapter 3A).

All larvae, except those from the Sept./Oct. 1977 main emergence were reared using the basic rearing methods described in chapter 2B. Larvae from eggs laid in Sept./Oct. 1977 were reared in a similar way, but were "forced" by being kept at a temperature maintained above 12°C. This was done simply so that the results would be available by the end of January, 1978. The evidence from the experiments to determine the effect of temperature on larval variation indicated that this treatment would not affect the allelic frequency estimates (see chapter 3Bi).

The samples obtained from light-trapped females were named the "first allelic frequency samples" (AF1).

Method of data analysis for genes A, B and C.

The data from the AF1 samples are tabulated in Table 5Ai.

Table 5Ai.

Experiments to determine the status of larval colour polymorphism in P. meticulosa. Colour analysis data from progeny of females taken during the six main emergences between September, 1975 and October, 1977.

Instar	Colour type	Period when females were taken					
		Sept/Oct. 1975	May, 1976	July, 1976	Sept/Oct. 1976	May/June 1977	Sept/Oct. 1977
Third	3IG	2883	7392	5410	11545	5384	8507
	OL	328	1003	662	1256	729	1001
	Br	76	279	168	271	205	234
	PY	13	34	24	52	23	39
	YG	5	12	9	22	9	15
	YB	3	9	6	10	7	9
	3IG,OL,Br	3287	8674	6240	13072	6318	9742
	PY,YG,YB	21	55	39	84	39	63
	Total	3308	8729	6279	13156	6357	9805
Fourth	Gr	1441	3265	2561	5984	2401	4229
	OL	1415	3975	2730	5482	2861	4183
	Br	330	1179	707	1213	820	988
	PY	47	119	87	187	87	138
	YG	16	39	28	65	27	47
	YB	11	32	22	43	24	34
	Gr,OL,Br	3186	8419	5998	12679	6082	9400
	PY,YG,YB	74	190	137	293	138	219
	Total	3260	8609	6135	12972	6220	9619

Table 5Ai. (Continued)

Experiments to determine the status of larval colour polymorphism in P. meticulosa. Colour analysis data from progeny of females taken during the six main emergences between September, 1975 and October, 1977.

Instar	Colour type	Period when females were taken					
		Sept/Oct. 1975	May, 1976	July, 1976	Sept/Oct. 1976	May/June 1977	Sept/Oct. 1977
Fifth	Gr	645	1146	973	2538	871	1789
	OL	581	1406	1071	2346	1047	1762
	Br	1943	5750	3820	7499	4019	5641
	PY	41	107	76	165	76	121
	YG	15	39	28	63	26	45
	YB	17	44	32	62	35	49
	Gr,OL,Br	3169	8302	5864	12383	5937	9192
	PY,YG,YB	73	190	136	290	137	215
	Total	3242	8492	6000	12673	6074	9407

The method of calculating allelic frequencies from this data is rather complicated. The complications are not due to any difficulty in the basic formulae used to estimate allelic frequencies, but rather, complications arose when trying to assess the genotypes of many larvae due to the complicated system of epistatic effects within the complex of alleles being studied.

To explain the method of estimating the allelic frequencies of the 11 alleles, I will use the data from the progeny of females taken in May, 1976. Broods from 41 females were reared, and a total of 8492 larvae reached the 5th instar.

The frequencies of the allelomorphs of gene B were estimated first. As the two alleles of gene B, b' and b^{o}, show no dominance, three main colour type classes controlled by these alleles can be identified in the 4th instar, these being Gr, OL and Br. If the class frequencies of these are symbolised by f, g and h respectively, then the proportion, s, of one of the alleles, say b', can be estimated by:

$$s = \frac{g + 2f}{2n_4},$$

where n_4 is the total number of 4th instar larvae in which the genotype with respect to gene B is identifiable. This equals the total number of 4th instar larvae minus the sum of 4th instar yellow type larvae. (In yellow type larvae the genotypic constitution of genes A and B, usually can not be ascertained with any degree of certainty. For this reason, in

the calculations to estimate the gene frequencies of A, B and C, the yellow larvae are excluded from the sample totals. This assumes that larvae which are yellow, i.e. contain neither d^+ nor e^+, are distributed at random throughout the population, showing no correlation to the genotypic make-up with respect to the allelomorphs of A, B and C.) Then:

$$s = \frac{3975 + 2.(3265)}{2(8419)}$$

$$= 0.6239.$$

If the proportion of the other allele, b^o, is r, then:

$$r = 1 - s$$

$$= 0.3761.$$

The standard error (S.E.) of these estimates may be calculated by:

$$SE = \sqrt{\frac{r(1 - r)}{2n}}$$

$$= 0.3885.$$ where n = 2 x number of broods for the sample.

As 1 - s = r, the standard error for s is obviously equivalent to that of r.

The allelic frequencies for b' and b^o for all main emergences are given in Table 5Aii.

As the heterozygote $b'b^o$ can be differentiated from both the homozygotes, b'b' and b^ob^o, phenotypically, expected class frequencies may be obtained from the estimated value of s by the ratio:

$$b'b' : b'b^o : b^ob^o = n_4(s^2) : 2n_4s(1 - s) : n_4(1 - s)^2,$$

and may be compared with observation by means of a general chi-squared test. The number of degrees of freedom for

Table 5Aii.

Experiments to determine the status of larval colour polymorphism in P. meticulosa. Allelic frequencies of genes A, B and C, estimated from the progeny of females taken during the six main emergences between September, 1975 and October, 1977. (Included are the estimated numbers of $b^{o}b^{o}$aa Fifth instar larvae and ccb'b' Third instar larvae used in estimating the allelic frequencies of genes A and C respectively).

Gene	Allele	Period when females were taken: Sept/Oct. 1975	May, 1976	July, 1976	Sept/Oct. 1976	May/June, 1977	Sept/Oct. 1977
B	b'	0.6744	0.6239	0.6546	0.6881	0.6300	0.6724
	b^{o}	0.3256	0.3761	0.3454	0.3119	0.3700	0.3276
	S.E.	0.5657	0.3885	0.3664	0.2508	0.3910	0.3085
A	a^{+}	0.3430	0.4021	0.3714	0.3396	0.3902	0.343
	a	0.6570	0.5979	0.6286	0.6604	0.6098	0.657
	S.E.	0.7342	0.4557	0.4250	0.2873	0.4425	0.3461
C	c^{+}	0.5262	0.5087	0.5183	0.5296	0.5074	0.52
	c	0.4738	0.4913	0.4817	0.4704	0.4926	0.48
	S.E.	0.7492	0.5003	0.4796	0.3421	0.4995	0.4070
Estimated number of $b^{o}b^{o}$aa Fifth instar larvae		142	416	273	517	290	417
Estimated number of ccb'b' Third instar larvae		334	812	618	1365	609	1010

these chi-squared tests will be one, (not two as might be thought, since one is forfeited by the calculation of s from the data). The chi-squared tests gave the following values:

for Sept./Oct. 1975 data, $X_1^2 = 0.4027$, $0.7 > p > 0.5$;

for May, 1976 data, $X_1^2 = 0.3094$, $0.7 > p > 0.5$;

for July, 1976 data, $X_1^2 = 0.2511$, $0.7 > p > 0.5$;

for Sept./Oct. 1976 data, $X_1^2 = 0.6891$, $0.5 > p > 0.3$;

for May/June, 1977 data, $X_1^2 = 0.4908$, $0.5 > p > 0.3$;

for Sept./Oct. 1977 data, $X_1^2 = 0.9536$, $0.5 > p > 0.3$.

When considering gene A, a different approach must be made as this gene has two alleles a^+ and $\underline{a}$, in which a^+ is fully dominant to $\underline{a}$. Again, excluding the yellow larvae, three main colour types are recognisable in the 5th instar, namely Gr, OL and Br. The Gr and OL larvae are known to be homozygous recessive for gene A in all cases, whilst the majority of Br larvae will contain the dominant allele a^+. However, some of the 5th instar Br larvae will have the genotype b^ob^oaa, being Br due to b^o being homozygous. To calculate the allelic frequencies of a^+ and $\underline{a}$, the number of b^ob^oaa larvae, k, must be estimated. This may be done using the formula:

$$k = \frac{h(i + j)}{f+g},$$

where f, g and h are the number of 4th instar Gr, OL and Br larvae respectively, and i and j equal the number of 5th instar Gr and OL larvae respectively.

Therefore:

$$k = 416.$$

(This assumes that there is no correlation between the

distribution of the alleles of A and B).

If the number of larvae with the allele a^+ in these genotypes is l, then:

$$l = n_5 - (i + j + k),$$

where n_5 is the total number of 5th instar larvae excluding the yellow colour-types. Thus:

$$l = 5334.$$

If the allelic frequencies of a^+ and a are t and u respectively, then:

$$u = \sqrt{\frac{(i + j + k)}{n_5}}$$

$$= 0.5979,$$

with the $$SE = \sqrt{\frac{(i - u^2)}{4n}}$$

$$= 0.4557,$$

and as $$t = 1 - u,$$

then $$t = 0.4021,$$

with the same standard error.

Turning to gene C, we have a similar situation to that when dealing with gene A. Gene C has a fully dominant allele c^+ and a recessive allele c. Excluding the yellow type larvae, three colour types may be identified in the 3rd instar, these being 3IG, OL and Br. Larvae which are OL or Br are known to be homozygous recessive for gene C, whilst the majority of 3IG larvae will be either homozygous dominant or heterozygous for C. However, some of the 3IG larvae will have the genotype ccb'b', the colour of these being indistinguishable from that of larvae with c^+ in their genotype. Therefore, to calculate the allelic frequencies of c^+ and c, the number of ccb'b'

larvae, m, must be estimated. This may be done using the formula:

$$m = \frac{f(p + q)}{(g + h)},$$

where p and q are the numbers of 3rd instar OL and Br larvae respectively. Therefore:

$$m = 812.$$

(This assumes that there is no correlation between the distribution of the alleles of B and C).

If the number of larvae with the allele c^+ in their genotype is o", then:

$$o'' = n_3 - (m + p + q),$$

where n_3 is the total number of 3rd instar larvae excluding the yellow colour types. Thus:

$$o'' = 6580.$$

If the allelic frequencies of c^+ and c are v and w respectively, then:

$$w = \sqrt{\frac{m + p + q}{n_3}}$$

$$= 0.4913,$$

with the $$SE = \sqrt{\frac{(1 - w^2)}{4n}}$$

$$= 0.5003.$$

As $$v = 1 - w,$$

then $$v = 0.5087,$$

with the same standard error.

The frequencies of the allelomorphs of genes A, B and C, estimated from the data collected from the progeny of light-trapped females during the six main emergences being

assessed, together with the estimated number of 5th instar $b^{o}b^{o}aa$ larvae, and the estimated number of 3rd instar ccb'b' larvae, are given in Table 5Aii, the frequencies being expressed graphically in Fig. 5i.

Method of data analysis for genes D and E.

When attempting to estimate the frequencies of the allelomorphs of genes D and E, different problems arise because of the epistatic interactions between these alleles (see chapter 3C). The respective dominant alleles of these genes both cause the colour of larvae to be controlled by the genes A, B and C. This frequently makes it impossible to ascertain which of the alleles of genes D and E are present in the genotype of a non-yellow larva. Even when dealing with yellow larvae, recognition of the exact genotypic configuration with respect to gene E is not always possible, unless the alleles of genes A and B present in the genotype are, either known, or may be calculated from the ratio of non-yellow phenotypes in the rest of the brood. Thus, when trying to calculate the frequencies of the alleles of genes D and E from the AF1 data, difficulties arise involving the designation of phenotypes to one genotype or another.

This problem proved to be insurmountable and the exact frequencies of the alleles d^{+}, d, e^{+}, e' and e^{o} have not been calculated. On the other hand, useful information on the class frequencies of the yellow producing genotypes was obtained from the AF1 data. To illustrate this, again the results from the May, 1976 broods will be used.

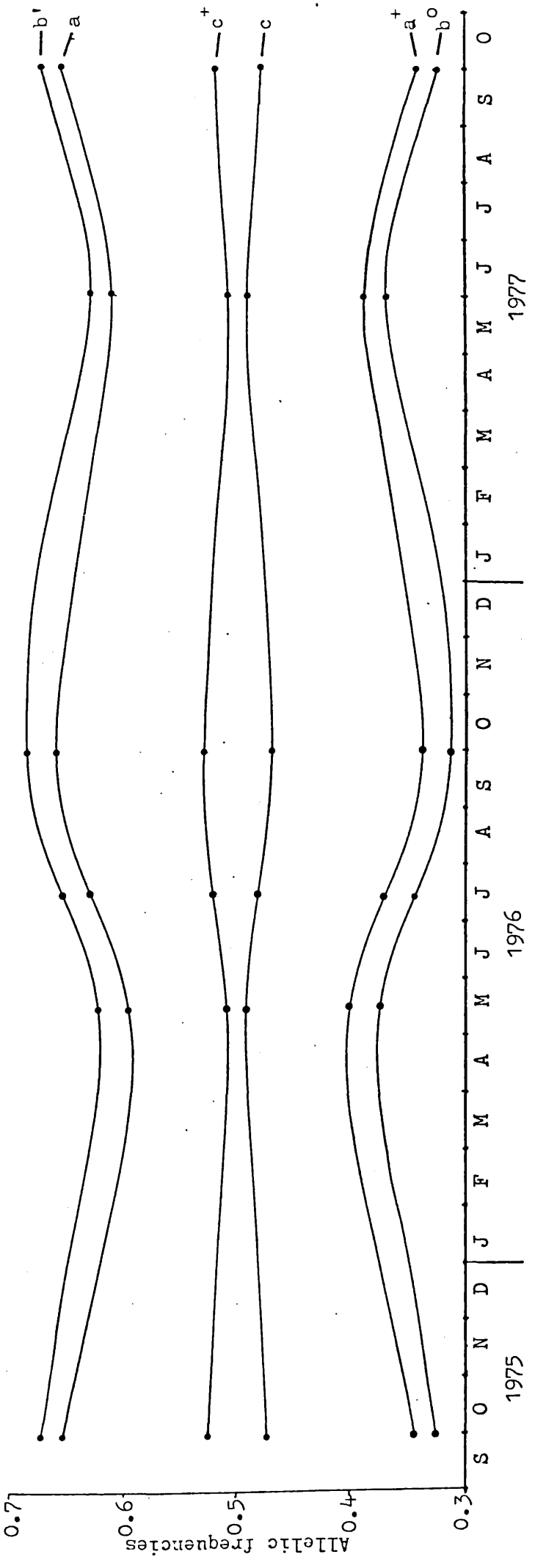


Fig. 5i. The frequencies of the allelomorphs of the genes A, B and C between September, 1975 and October, 1977 estimated from the AF1 data.

The class frequencies of the three yellow phenotypes in the 3rd, 4th and 5th instars are given in Table 5Aiii, the frequencies being given as a percentage of the total number of larvae in the respective instar.

An estimate of the percentage of larvae which are dde'e' may be made by considering the number of larvae which change from being PY in the 4th instar to YB in the 5th instar. All these larvae must have the genotype $b'b'a^{+}a^{x}dde'e'$. In the May, 1976 data this figure equals 12. From the frequency estimates for alleles a^{+}, a and b' the expected proportion of $b'b'a^{+}a^{x}$ larvae may be estimated as:

$$= s^2(t^2 + 2tu)$$

$$= 25.01\% \text{ of all 5th instar larvae.}$$

Therefore, as the number of larvae which are $b'b'a^{+}a^{x}dde'e'$ is 12, then the total number of dde'e' larvae will be:

$$12 \times \frac{100}{25.01} = 48,$$

which is 0.5652% of all 5th instar larvae.

An estimate of the percentage of larvae which are $dde^{o}e^{o}$ may be made by considering the number of larvae which are YG in both the 3rd and the 4th instars. In the May, 1976 broods this figure equals 12. All these larvae will be either $ccb'b'dde^{o}e^{o}$ or $ccb'b^{o}dde^{o}e^{o}$, (the alleles of gene A being immaterial). (Larvae which are $ccb^{o}b^{o}dde^{o}e^{o}$ will be PY in the 3rd instar due to the presence of e^{o} which inhibits the phenotypic expression of b^{o}).

From the frequency estimates of b', b^{o} and c, the expected proportion of ccb'b' larvae can be estimated as

Table 5Aiii

Experiments to determine the status of larval colour polymorphism in P. meticulosa. Proportions of the three yellow larval colour type producing genotypes estimated from the progeny of females. (Proportions given as a percentage of total number of larvae in the relevant instar).

Genotype	Period when females were taken					
	Sept/Oct. 1975	May, 1976	July, 1976	Sept/Oct. 1976	May/June, 1977	Sept/Oct. 1977
dde'e'	0.709	0.565	0.65	0.56	0.724	0.617
$dde^{o}e^{o}$	0.753	0.633	0.703	0.836	0.676	0.742
$dde'e^{o}$	1.046	1.088	1.073	1.105	1.052	1.086
Total	2.508	2.286	2.426	2.501	2.452	2.445

being s^2w^2, and that of ccb'b^o as $2rsw^2$. Thus, the expected proportion of ccb'b' larvae = 9.396% of all larvae, and the expected proportion of ccb'b^o = 11.328% of all larvae.

Thus, the total number of ccdde^{o}e^o larvae will equal the percentage of all 3rd instar larvae which are homozygous for c, divided by the percentage of larvae which are either ccb'b' or ccb'b^o, all multiplied by the percentage of all larvae which are YG in both the 3rd and 4th instars.

$$\text{This equals } \left[\frac{w^2}{(s^2w^2 + 2rsw^2)}\right] \times \left[\frac{12 \times 100}{8729}\right]$$

$$= 0.1601\% \text{ of all 3rd instar larvae.}$$

Therefore, the percentage of larvae which are dde^{o}e^o

$$= 0.1601 \times \frac{100}{w^2 \times 100}$$

$$= 0.6632\%.$$

One method of obtaining an estimate of ccb'b^o larvae involved the fact that all larvae lacking both the dominant alleles d^+ and e^+ are yellow. Thus, assuming the proportions of dde^{o}e^o and dde'e' larvae do not vary significantly in the 3rd, 4th and 5th instars due to differential fitness, then the percentage of dde'e^o larvae will equal the number of 4th or 5th instar yellow type larvae as a percentage of all larvae in the respective instar, minus the sum of the percentages of dde^{o}e^o and dde'e' larvae. (If the percentage of dde'e^o larvae is to be calculated from 3rd instar data, the total number of yellow larvae must be divided by w^2, as larvae with c^+ in their genotype will not be yellow, even if d^+ and e^+ are both absent).

Thus the percentage of dde'e^o larvae, calculated from

4th instar totals, equals 0.9776%, or from 5th instar totals, equals 1.0090%.

This method obviously incorporates any errors inherent in the methods of estimating the percentages of dde'e' and $dde^{o}e^{o}$ larvae. An alternative method of calculating the percentage of $dde'e^{o}$ larvae which will minimise these errors, involves considering the genotypes of all larvae which are PY in both the 4th and 5th instars.

From the May, 1976 data, 107 larvae fall into this category. This figure includes all $dde'e^{o}$ larvae plus the larvae which are b'b'aadde'e', (the allelomorphs of gene C being immaterial), and $b^{o}b^{o}dde^{o}e^{o}$, (the allelomorphs of genes C and A being immaterial).

From the estimates already made, the percentage of larvae which are b'b'aadde'e' equals 0.0786, and the percentage of larvae which are $b^{o}b^{o}dde^{o}e^{o}$ equals 0.0938%. Therefore, as the number of 5th instar PY larvae equals 1.2600 of all 5th instar larvae, but includes not only the $dde'e^{o}$ larvae, but also the two groups mentioned above, the percentage of all larvae which are $dde'e^{o}$

$$= 1.2600 - (0.0786 + 0.0938)$$

$$= 1.0876.$$

(This estimate must be made using the 5th instar proportion of PY larvae, otherwise the proportion of the $b'b'a^{+}a^{x}dde^{o}e^{o}$ larvae which are PY in the 4th instar, and YB in the 5th instar, must be taken into account if considering the 4th instar proportion of PY larvae).

To make these estimates, it was assumed that all the genes involved are in equilibrium, and that the actual frequency of each genotype is exactly equivalent to the expected frequency of that genotype calculated from the frequency estimates of the alleles of the genes involved. This is obviously not so in all cases, as can be seen most simply by comparing the actual proportion of 5th instar yellow-type larvae with the sum of the estimated proportions of dde'e', $dde^{o}e^{o}$ and $dde'e^{o}$ larvae (estimated using the second of the two methods outlined). These proportions should be equivalent if the assumptions made are true. The former proportion equals 2.2374% whilst the latter equals 2.3160%. Whilst this shows that the assumptions made are not completely valid, they must be nearly so, to give results which are so similar, and they are justifiable if only because they provide the most accurate means by which genotype frequency estimates may be made.

It is a simple matter to calculate the proportion of larvae with either d^{+} and/or e^{+} present in their genotype, as all non-yellow larvae must fall into this group. Thus, this proportion equals the sum of Gr, OL and Br 5th instar larvae, divided by the total number of larvae. Using the May, 1976 data this equals 97.76%.

Once the frequency of the three yellow producing genotypes had been found, an adaptation of the Hardy-Weinberg equilibrium was applied to the results, in an attempt to estimate the allelic frequencies of d^{+}, d, e^{+}, e' and e^{o}. If

the allelic frequencies of these five alleles are 'u, 'v, 'w, 'y and 'z respectively, then four equations may be produced, these being:

i) $(6'u^2 + 12'u.'v + 3'v^2)(3'w^2 + 6'w.'y + 6'w.'z + 3'y^2 + 6'z^2) = 97.76.$

ii) $'v^2.'y^2 = 0.5652.$

iii) $2'v^2.'y.'z = 1.0876.$

iv) $'v^2.'z^2 = 0.6632.$

Unfortunately, these equations have an infinite number of solutions as there are more variables than equations. This is because the phenotypic expressions of d^+ and e^+ are not distinguishable.

The estimated proportion of the three yellow-type producing genotypes, for each of the six main emergences under consideration, are given in Table 5Aiv.

When considering changes in the frequencies of the alleles estimated using data from different main emergences, it must be remembered that the estimates of the allelic frequencies of a^+, $\underline{a}$, c^+ and c, and the genotypic frequencies of dde'e', dde'e^o and dde^{o}e^o, where, to some extent, based on the results of 4th instar larvae. Thus, any departure from the Hardy-Weinberg equilibrium in the ratio between the Gr, Br and OL phenotypes observed in the 4th instar results would be transmitted, to some extent, to the 3rd and 5th instar results. Furthermore, as the 4th instar Gr, Br and OL phenotypes are controlled by gene B, then, any changes in the frequencies of the alleles of this gene, between the start of the 3rd instar

Table 5Aiv.

Experiments to determine the status of larval colour polymorphism in P. meticulosa. Proportions of the colour types observed in the data from the progeny of females taken during the six main emergences between September, 1975 and October, 1977. (Proportions given as a percentage of the total number of larvae in the relevant instar).

Instar	Colour type	Period when females were taken					
		Sept/Oct. 1975	May, 1976	July, 1976	Sept/Oct. 1976	May/June 1977	Sept/Oct. 1977
Third	3IG	87.15	84.68	86.16	87.75	84.69	86.76
	OL	9.92	11.49	10.54	9.55	11.47	10.21
	Br	2.30	3.20	2.68	2.06	3.22	2.39
	PY	0.39	0.39	0.38	0.40	0.36	0.40
	YG	0.15	0.14	0.14	0.17	0.14	0.15
	YB	0.09	0.10	0.10	0.08	0.11	0.09
	PY,YG,YB	0.63	0.63	0.62	0.65	0.61	0.64
Fourth	Gr	44.20	37.93	41.74	46.13	38.60	43.97
	OL	43.40	46.17	44.50	42.26	46.00	43.49
	Br	10.12	13.69	11.52	9.35	13.18	10.27
	PY	1.44	1.38	1.42	1.44	1.40	1.43
	YG	0.49	0.45	0.46	0.50	0.43	0.49
	YB	0.34	0.37	0.36	0.33	0.39	0.35
	PY,YG,YB	2.27	2.20	2.24	2.27	2.22	2.27

Table 5Aiv. (Continued)

Experiments to determine the status of larval colour polymorphism in P. meticulosa. Proportions of the colour types observed in the data from the progeny of females taken during the six main emergences between September, 1975 and October, 1977. (Proportions given as a percentage of the total number of larvae in the relevant instar).

Instar	Colour type	Period when females were taken					
		Sept/Oct. 1975	May, 1976	July, 1976	Sept/Oct. 1976	May/June 1977	Sept/Oct. 1977
Fifth	Gr	19.90	13.50	16.22	20.03	14.34	19.02
	OL	17.92	16.56	17.85	18.51	17.24	18.73
	Br	59.93	67.71	63.67	59.17	66.17	59.97
	PY	1.26	1.26	1.27	1.30	1.25	1.29
	YG	0.46	0.46	0.47	0.50	0.43	0.48
	YB	0.52	0.52	0.53	0.49	0.58	0.52
	PY,YG,YB	2.24	2.24	2.27	2.29	2.26	2.29

and the end of the 4th instar, would be manifest, to some extent, in the allelic frequency estimates of c^+ and c. Similarly, any changes in the frequencies of b' and b^o, between the start of the 4th instar and the end of the 5th instar, would affect the allelic frequency estimates of a^+ and a. Because of this, and because frequencies of the alleles of genes D and E could not be calculated, the proportion of each colour type in each instar was calculated. These are given in Table 5Aiii and are expressed graphically in Figs. 5ii, 5iii and 5iv.

It should also be realised that, due to the rarity of the yellow colour-types, and the level of the standard errors relating to the frequencies of the alleles of genes, A, B and C, the data with respect to the frequencies of the yellow colour-types must be viewed as being potentially unreliable, although the consistency of the phenotypic frequency values for these colour-types from both the AF1 and AF2 data implies that the values are realistic.

Results

Initially, if the results of the allelic frequencies of gene B are considered, it looks as though the frequency of b' falls during the winter months and rises during the summer months, so that all three Sept./Oct. values are similar to one another, and the two May (May/June) values are similar to one another, but are considerably lower than the Sept./Oct. values. The July, 1976 value is intermediate between those of May and Sept./Oct. 1976.

To look more closely at these results, the 4th instar data of Gr, OL and Br colour types, from pairs of consecutive main emergences, were compared using chi-squared. These tests gave highly significant probability values in all cases.

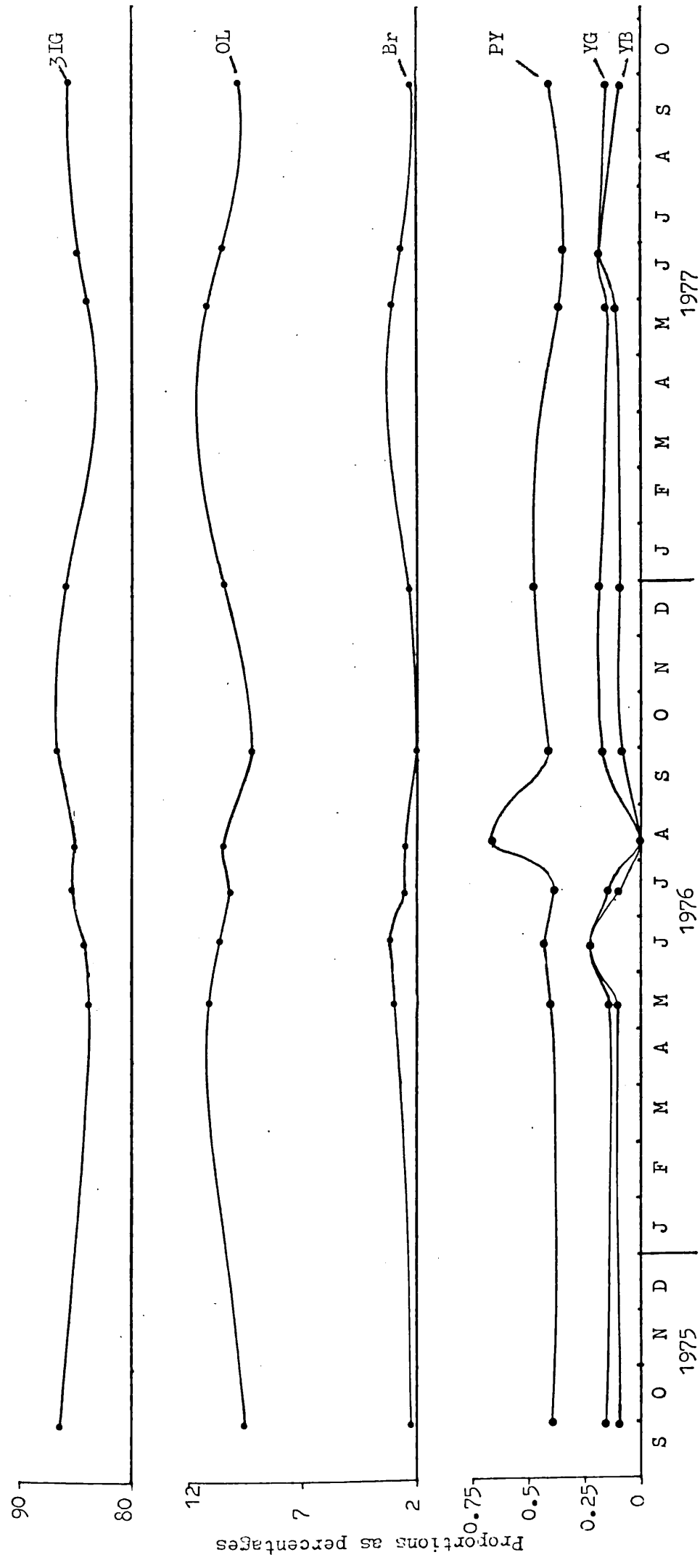


Fig 5ii. The proportions of each larval colour type in the 3rd instar between September, 1975 and October, 1977. Calculated from the AF1 and AF2 data.

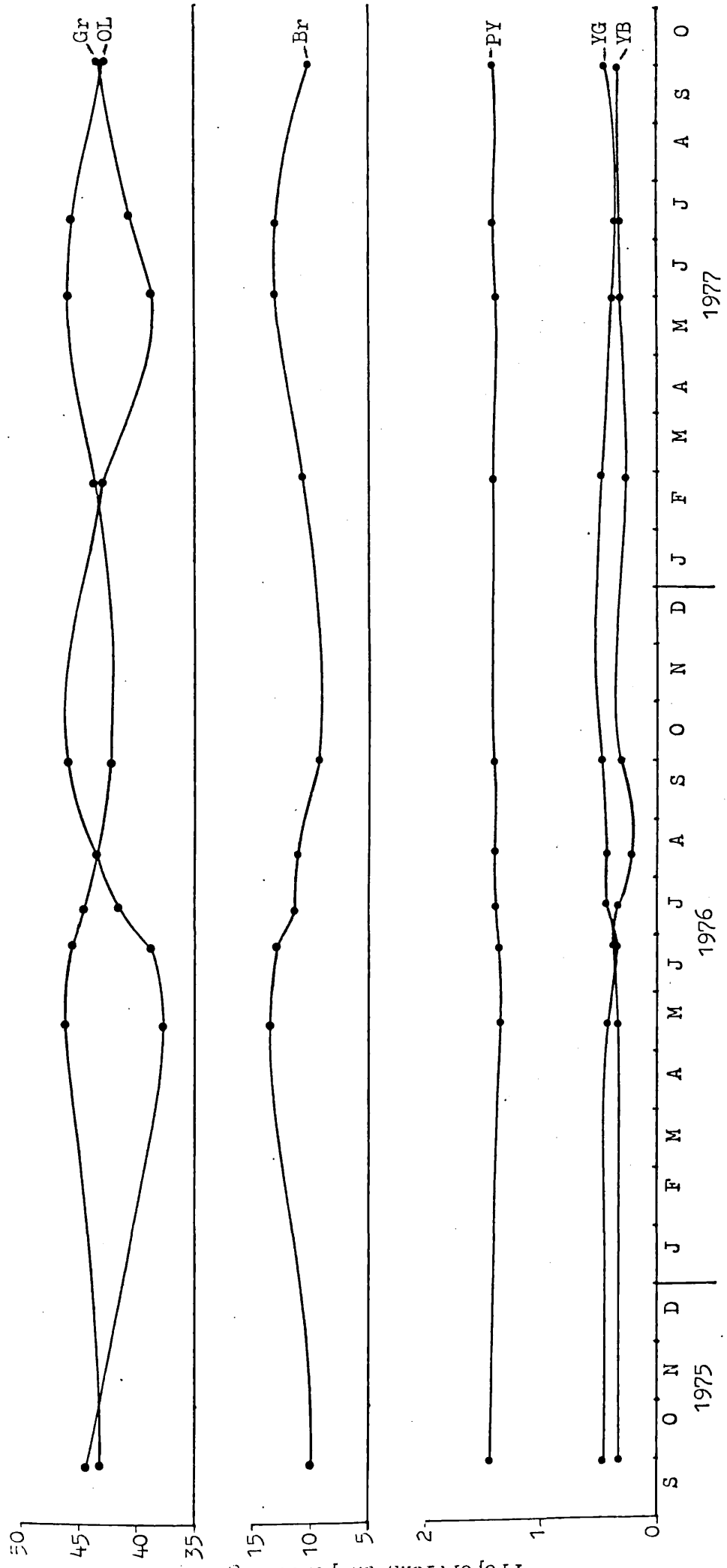


Fig. 5iii. The proportions of each larval colour type in the 4th instar between September, 1975 and October, 1977. Calculated from the AF1 and AF2 data.

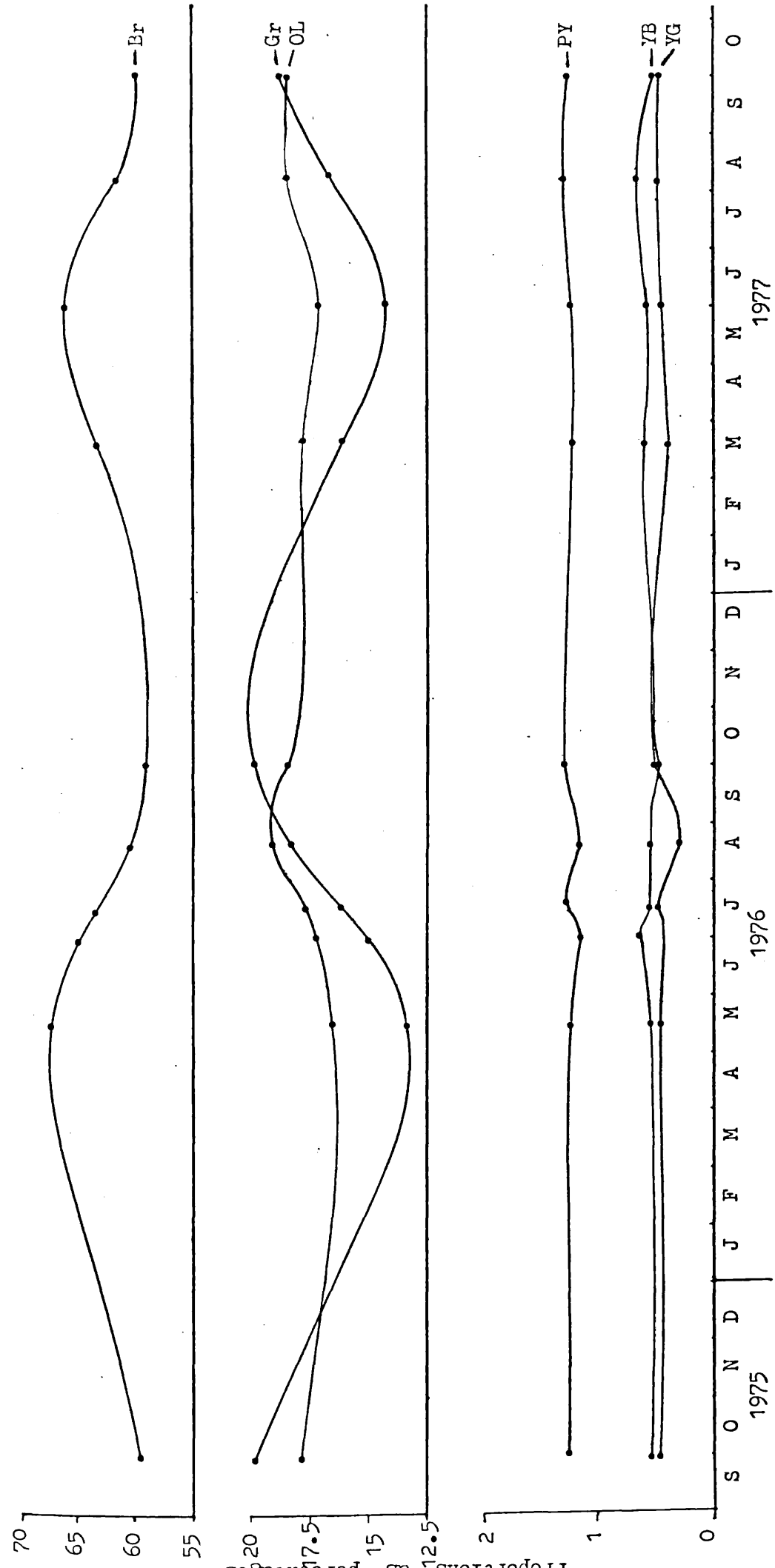


Fig. 5iv. The proportions of each larval colour type in the 5th instar between September, 1975 and October, 1977. Calculated from the AF1 and AF2 data.

(Comparing data from the main emergences:

Sept./Oct. 1975 and May, 1976, $X_2^2 = 51.23$, $p < 0.001$;

May, 1976 and July, 1976, $X_2^2 = 28.7$, $p < 0.001$;

July, 1976 and Sept./Oct.1976, $X_2^2 = 42.5$, $p < 0.001$;

Sept./Oct. 1976 and May/Jn.'77, $X_2^2 = 126.29$, $p < 0.001$;

May/Jn. 1977 and Sept./Oct.'77, $X_2^2 = 59.4$, $p < 0.001$).

On the other hand, if data from the three Sept./Oct. main emergences are compared, the probability value whilst still significant is not highly so, ($X_4^2 = 13.08$, $0.02 > p > 0.01$). If this data is further analysed by comparing the data from these three emergences in pairs, we find that the 1976 data is significantly different from that of both 1975 and 1977, whilst that of 1975 shows a high degree of homogeneity with that of 1977.

(Comparing data from the main emergences:

Sept./Oct. 1975 and Sept./Oct. 1976, $X_2^2 = 8.178$, $0.02 > p > 0.01$;

Sept./Oct. 1976 and Sept./Oct. 1977, $X_2^2 = 12.464$, $0.01 > p > 0.001$;

Sept./Oct. 1975 and Sept./Oct. 1977, $X_2^2 = 0.087$, $0.98 > p > 0.95$)

Turning to the data from the May, 1976 and May/June, 1977 main emergences, these proved to be homogeneous ($X_2^2 = 1.158$, $0.7 > p > 0.5$).

It seems then, that the allelic frequency of b' fell significantly during the winter of 1975/1976 only to rise again between May and July, 1976, and continue to rise between July and Sept./Oct. 1976, so that the allelic frequency of b' in Sept./Oct. 1976 was significantly higher than in Sept./Oct. 1975. The frequency dropped again during the winter of 1976/1977 to a level which was higher than the May, 1976 level, but

not significantly so. The frequency rose again during the summer of 1977 to reach a level which was very similar to that of Sept./Oct. 1975.

If the results from this relatively short period are taken to give a representative indication of the state of the polymorphism based on gene B, then we may deduce that this polymorphism is balanced and is controlled by selective agencies which favour the allele b' compared with b^o during the months between May and Sept./Oct., and favour the allele b^o compared with b' between Sept./Oct. and May. This would presumably be due to green larvae being selectively favoured over brown and possibly olive larvae during the summer months, whilst brown larvae are favoured during the winter.

The unusually high frequency of b' in Sept./Oct, 1976, (compared with its level in Sept./Oct. 1975 and 1977), may be due to P. meticulosa having three main emergences during 1976, whilst it had only two during 1975 and 1977. Thus, as selection favours b' during the summer months, the occurrence of an extra main emergence in July, 1976 may have increased the selection in favour of green larvae during the summer of 1976.

Turning to the estimated frequencies of a^+ and a, the frequency of a shows exactly the same directional changes as have been stated for b'. To discover whether these changes are significant, the expected number of larvae with a^+, and the expected number of larvae homozygous for a must be calculated. Then, comparisons may be made between different

main emergences. Comparisons of the number of each of the phenotypes, Gr, Br and OL, in the 5th instar, will not give a realistic indication of the significance of allelic frequency changes in a^+ and a. This is because the number of each phenotype in the 5th instar depends on the frequency of b' and b^o as well as the frequency of the alleles a^+ and a, so that changes in the allelic frequencies of b' and b^o will affect the relative proportions of the phenotypes Gr, Br and OL.

The number of larvae with a^+ and the number of larvae homozygous for a, (calculated from the allelic frequency estimates for a^+ and a), for each main emergence were compared with similar estimates from the following main emergence. These comparisons show that the changes in the allelic frequencies of a^+ and a between consecutive main emergences are highly significant.

(Comparing data from the main emergences:

Sept./Oct. 1975 and May, 1976 $X_1^2 = 53.616$, $p < 0.001$;

May, 1976 and July, 1976, $X_1^2 = 56.834$, $p < 0.001$;

July, 1976 and Sept./Oct. 1976, $X_1^2 = 20.703$, $p < 0.001$;

Sept/Oct. 1976 and May/June, 1977, $X_1^2 = 69.306$, $p < 0.001$;

May/June, 1977 and Sept/Oct. 1977, $X_1^2 = 45.275$, $p < 0.001$).

On the other hand, the three Sept./Oct. main emergences are homogeneous in this respect, ($X_2^2 = 0.662$, $0.8 > p > 0.7$), as are the two May main emergences, ($X_1^2 = 3.121$, $0.1 > p > 0.05$).

Thus, it seems that the frequency of a^+ rises significantly during the summer. It must be said that the allelic frequency

estimates for a^+ and a may be slightly inaccurate due to the fact that the calculation of the estimated number of b^ob^oaa larvae involved the 4th instar proportions of Gr, OL and Br. However, as less than 5% of larvae were estimated as being b^ob^oaa, then any bias caused by the estimates of b^ob^oaa larvae must be small, and these certainly could not have caused inaccuracies of the magnitude of the changes observed in the allelic frequencies of a^+ and a between main emergences.

Looking next at the alleles c^+ and c, the changes in the frequency estimates of these two alleles between main emergences show the same directional trends as those for b' and b^o respectively; however, the changes in c^+ and c are relatively small compared to those of b' and b^o.

The estimates of the allelic frequencies of c^+ and c may be inaccurate because the estimation of the number of ccb'b' larvae involved the 4th instar proportions of Gr, Br and OL. The proportion of ccb'b' larvae was estimated to be over 10% of the total number of larvae, and as the changes in the frequency estimates of alleles c^+ and c were small, a significant part of these changes may be due to the involvement of the phenotypic proportions of 4th instar Gr, Br and OL, in the calculation of the allelic frequencies of c^+ and c.

The number of larvae with c^+ and the number of larvae with c, (calculated from the allelic frequency estimates for c^+ and c), were compared with similar estimates from the following main emergences using chi-squared tests. These

comparisons showed that none of the changes in allelic frequencies of c^+ and c between consecutive instars were significant, except for that between Sept./Oct. 1976 and May/June, 1977.

(Comparing data from the main emergences:

Sept/Oct. 1975 and May, 1976, $X_1^2 = 3.716$, $0.1 > p > 0.05$;

May, 1976 and July, 1976, $X_1^2 = 1.759$, $0.2 > p > 0.1$;

July, 1976 and Sept/Oct. 1976, $X_1^2 = 2.754$, $0.1 > p > 0.05$;

Sept/Oct. 1976 and May/June, 1977, $X_1^2 = 11.037$, $p < 0.001$;

May/June, 1977 and Sept/Oct. 1977, $X_1^2 = 2.092$, $0.2 > p > 0.1$).

The three Sept/Oct. main emergences are homogeneous in this respect, ($X_2^2 = 2.694$, $0.3 > p > 0.2$), as are the two May main emergences, ($X_2^2 = 0.023$, $0.9 > p > 0.8$).

Thus, it seems that the polymorphism based on the alleles of gene C is balanced from year to year, and the seasonal variations in the frequencies of c^+ and c are less pronounced than those of the alleles of genes B and A, being, in most cases, non-significant.

Finally, looking at the proportion of the three types of yellow larvae, it appears that overall the proportion of yellow larvae remained very stable between September, 1975 and October, 1977, varying from 0.61% to 0.65% of the larvae reared in the 3rd instar, from 2.20% to 2.27% in the 4th instar, and from 2.24% to 2.29% in the 5th instar. The slight variations which do occur do not appear to be correlated with season in any way.

If the three yellow colour-types are considered

individually, then it is notable that changes in the proportion of YG larvae in all instars tend to be associated with similar changes in the proportion of green larvae at least as far as the direction of the changes are concerned. Similarly, in most cases, changes in the proportion of YB larvae are correlated with changes in the proportion of Br larvae. Both these features are to be expected as the YG phenotype is partly dependant on the presence in the genotype of the allele b' which controls green pigment production. The occurrence of the YB phenotype in the 3rd and 4th instars is partly dependant on the presence of b^{o}, and in the 5th instar YB will only occur if either b^{o} or a^{+} are present in the genotype.

B. Allelic frequency and phenotypic percentage analysis using data from larvae obtained by sweep-netting.

Method

The second method used to obtain samples for allelic frequency estimates involved sweep-netting larvae from low herbage at a number of sites in the Ringwood area. As no single site seemed likely to provide enough larvae for a realistic estimate of the allelic frequencies to be made, it had to be assumed that the frequency of the allelomorphs under consideration would not vary at a particular time over an area of about six square miles, around Ringwood, in which a number of suitable sites lay. Samples, which were named the "second allelic frequency samples" (AF2), were taken from these sites at appropriate periods during 1976 and 1977, so

that the frequencies of alleles could be estimated from the larvae of the three main emergences in 1976 and the May/June main emergence in 1977.

When data was being collected between June and August, 1976 the results of the behavioural experiments on 3rd instar larvae were not known, indeed it was partly the results from this data which led to the fourth and fifth behaviour experiments being undertaken.

In these behaviour experiments, it was shown that during the day, larvae which were homozygous recessive for gene C move from the leaves on which they feed at night, to the very low layers of ground herbage as they are negatively phototactic, whilst those which contain the dominant allele c^+ generally remain on the leaves of the food-plants throughout the daylight hours.

As the AF2 samples were collected by sweep-netting, then, in data obtained from larvae in the day, the behaviour of c^+c^+ or c^+c larvae as compared with that of cc larvae, would be expected to produce a preponderance of the c^+c^+ and c^+c larvae. The results of the fifth behaviour experiment, show that, even when the sweep-net is kept as close to the ground as possible during sweeps, only 54% of the total number of cc larvae on an area of ground are taken in the day, as compared to 74% of c^+c^+ larvae, (Table 4Cii, Strip A results, sample 5BE1). On the other hand, Table 4Cii shows that at night there is no significant difference between the numbers of cc and c^+c^+ larvae taken when sweeping either close to

the ground, (Strip A results, sample 5BE2), at a height of at least 100 mm from the ground, (Strip B results, sample 5BE2), or at a height of at least 250 mm above ground level, (Strip C results, sample 5BE2).

Fortunately, some of the data to be used for estimating the allelic frequencies of c^+ and c in larvae from the May, 1976 and July, 1976 main emergences was obtained by sweep-netting at night, and a record was kept of the time and date when data was collected. Therefore, when looking at the proportions of colour types in the 3rd instar, only the data collected by sampling at least two hours after sunset was used.

The second and third behaviour experiments indicated that there is no correlation between the alleles of genes A, B, D and E, and any behavioural correlation which might bias the data from 4th and 5th instar AF2 samples in favour of one phenotype or another. Indeed, this lack of correlation is indicated by a comparison of the proportions of the 4th and 5th instar larval phenotypes in the AF2 sample taken by day in June, 1976 (May, 1976 main emergence), with those in the AF2 sample taken at night in June, 1976. These proportions are very similar for a particular instar.

Results and analysis of data.

The results of recording the phenotypes of larvae obtained by sweep-netting are given in Table 5Bi.

When dealing with the data collected by sweep-netting,

Table 5Bi.

Experiments to determine the status of larval colour polymorphism in P. Meticulosa. Colour analysis data from larvae obtained by sweep-netting at specific periods between June, 1976 and August, 1977.

Instar	Colour type	Collected in June and July, 1976. (Larvae from May, 1976 main emergence) Night collected larvae	Day collected larvae	Total of all larvae	Collected in July and August, 1976. (Larvae from July, 1976 main emergence) Night collected larvae	Day collected larvae	Total of all larvae	Collected in Dec. 1976 or Jan. Feb, March or April, 1977. (Larvae from the Sept/Oct. 1976 main emergence) Night collected larvae	Collected in June, July or August, 1977. (Larvae from the May, 1977 main emergence) Night collected larvae
Third	3IG	394	518	912	262	478	740	928	515
	OL	52	42	94	33	32	65	109	65
	Br	15	10	25	8	7	15	26	18
	PY	2	1	3	2	1	3	5	2
	YG	1	1	2	0	0	0	2	1
	YB	1	0	1	0	0	0	1	1
	3IG+OL+Br	461	570	1031	303	517	820	1063	598
	PY+YG+YB	4	2	6	2	1	3	8	4
	Total	465	572	1037	305	518	823	1071	602
Fourth	Gr	198	256	454	185	172	357	427	327
	OL	233	281	514	185	177	362	433	356
	Br	66	84	150	48	43	91	108	91
	PY	7	9	16	6	6	12	14	11
	YG	2	2	4	2	1	3	5	3
	YB	2	3	5	1	1	2	3	3
	Gr+OL+Br	497	621	1118	418	392	810	968	774
	PY+YG+YB	11	14	25	9	8	17	22	17
	Total	508	635	1143	427	400	827	990	791
Fifth	Gr	76	69	145	63	82	145	135	103
	OL	88	85	173	65	77	142	149	114
	Br	329	300	629	207	253	460	529	378
	PY	6	6	12	4	5	9	10	8
	YG	2	3	5	1	1	2	3	3
	YB	3	3	6	2	2	4	5	4
	Gr+OL+Br	493	454	947	335	412	747	810	595
	PY+YG+YB	11	12	23	7	8	15	18	15
	Total	504	466	970	342	420	762	828	610

further difficulties arise. The results of the analysis of data from the AF1 samples show that, when considering the allelomorphs of genes A and B, there is a significant difference in the phenotypic frequencies from one brood to the next. The method of calculating allelic frequencies from this data, assumed that the frequencies of the alleles under consideration remained constant throughout the 3rd, 4th and 5th instars, and conformed to the Hardy-Weinberg equilibrium. This assumption seems to be reasonable when dealing with samples originating from ova laid by females taken at one particular season, the ensuing larvae being reared under controlled conditions. However, a similar assumption may not be made when dealing with data collected from wild larvae of various instars, as the frequencies of the allelomorphs in question may change from one instar to another due to differential mortality of the phenotypes in the wild.

Obviously, the frequencies of the alleles b' and b^{o} in the 4th instar could be calculated from the AF2 data, using the method outlined for the AF1 data. On the other hand, as the estimation of the frequencies of the alleles c^{+}, c, a^{+} and a depends to some extent on the frequency of the Gr, Br and OL phenotypes in the 4th instar, the allelic frequencies of c^{+}, c, a^{+} and a would be unrealistic if the frequencies of b' and b^{o} did not remain constant throughout the 3rd, 4th and 5th instars.

Estimations of the allelic frequencies of b' and b^{o} for the 4th instar are given in Table 5Bii. These show that

Table 5Bii.

Experiments to determine the status of larval colour polymorphism in P. meticulosa. Allelic frequencies of b' and b^{o}, estimated from data of samples obtained by sweep-netting larvae at specific periods between June, 1976 and August, 1977.

Allele	Period when larval samples were obtained			
	June & July, 1976 (May, 1976 main emergence)	July & August, 1976 (July, 1976 main emergence)	Dec, 1976, Jan-April, 1977 (incl) (Sept/Oct. 1976 main emergence)	June - Aug. 1977 (May, 1977 main emergence
b'	0.633	0.664	0.665	0.652
b^{o}	0.367	0.336	0.335	0.348
S.E.	0.0153	0.0166	0.0107	0.0121

the frequencies estimated from the AF2 data were intermediate between the frequency of the respective allele calculated using the AF1 data from females of the preceding main emergence and that of the subsequent main emergence. From this fact, it may be deduced that the change in the allelic frequencies of b' and b^{o}, between consecutive main emergences, is due to the two alleles having different survival values. There is, then, a change between the allelic frequency estimates of b' and b^{o} based on the 4th instar AF1 data for any particular main emergence, and similar estimates based on the AF2 data for the same main emergence. A further change is evident between these latter allelic frequency estimates and those from AF1 data of the ensuing main emergence. Because of these two changes, it may be deduced that the factors inducing the difference in the survival values of b' and b^{o}, between consecutive main emergences, act both before and after the average stage at which 4th instar larvae were sampled.

Therefore, as it is very probable that the allelic frequencies of b' and b^{o} do not remain constant throughout the 3rd, 4th and 5th instars, it was felt that there was little purpose in estimating the frequencies of c^{+}, c, a^{+} and $\underline{a}$ from the AF2 data. Instead, the proportion of each colour type was calculated for the 3rd, 4th and 5th instars. These proportions are given in Table 5Biii, and are expressed graphically in Figs. 5ii, 5iii and 5iv, with the relevant proportions from the AF1 data.

Table 5Biii.

Experiments to determine the status of larval colour polymorphism in P. meticulosa. Proportions of the colour types observed in the data from larvae obtained by sweep-netting at specific periods between June, 1976 and August, 1977. (Proportions given as a percentage of the total number of larvae in the relevant instar).

Instar	Colour type	Collected in June and July, 1976 (Larvae from May, 1976 main emergence) Night collected larvae *	Day collected larvae	Total of all larvae	Collected in July and August, 1976 (Larvae from July, 1976 main emergence) Night collected larvae *	Day collected larvae	Total of all larvae	Collected in Dec. 1976 or Jan. Feb, March or April, 1977. (Larvae from Sept/Oct. 1976 main emergence) Night collected larvae	Collected in June, July or August, 1977. (Larvae from the May, 1977 main emergence) Night collected larvae
Third	3IG	84.73	90.56	87.95	85.90	92.28	89.91	86.65	85.55
	OL	11.18	7.34	9.06	10.82	6.18	7.90	10.18	10.80
	Br	3.23	1.75	2.41	2.62	1.35	1.82	2.43	2.99
	PY	0.43	0.17	0.29	0.66	0.19	0.36	0.47	0.33
	YG	0.22	0.17	0.19	0	0	0	0.19	0.17
	YB	0.22	0	0.10	0	0	0	0.09	0.17
	PY,YG,YB	0.87	0.34	0.58	0.66	0.19	0.36	0.75	0.67
Fourth	Gr	38.98	40.31	39.72	43.33	43.0	43.17	43.13	40.59
	OL	45.87	44.25	44.97	43.33	44.25	43.77	43.74	45.58
	Br	12.99	13.23	13.12	11.24	10.75	11.00	10.91	11.65
	PY	1.38	1.42	1.40	1.41	1.5	1.45	1.41	1.41
	YG	0.39	0.31	0.35	0.47	0.25	0.36	0.51	0.38
	YB	0.39	0.47	0.44	0.23	0.25	0.24	0.30	0.38
	PY,YG,YB	2.16	2.20	2.19	2.11	2.00	2.05	2.22	2.17
Fifth	Gr	15.08	14.81	14.95	18.48	19.52	19.03	16.30	16.89
	OL	17.46	18.18	17.84	19.06	18.33	18.64	18.00	18.69
	Br	65.28	64.38	64.85	60.53	60.24	60.37	63.53	61.97
	PY	1.19	1.29	1.24	1.17	1.19	1.18	1.21	1.31
	YG	0.40	0.64	0.52	0.29	0.24	0.26	0.36	0.49
	YB	0.60	0.64	0.62	0.59	0.48	0.52	0.60	0.66
	PY,YG,YB	2.19	2.57	2.38	2.05	1.91	1.96	2.17	2.46

*Note, only the night collected samples were used to look at the changes in the proportions of third instar phenotypes

When the proportion of the Gr, Br and OL colour-types in the 5th instar are considered in relation to those obtained from the AF1 data, it is evident that the phenotypic proportions from the AF2 data are intermediate between the proportions of phenotypes from the AF1 data of the preceding main emergence and that of the following main emergence. The only exception to this is the proportion of 5th instar OL larvae in the sample taken in August/September, 1976, (i.e. larvae from the July, 1976 main emergence), which is higher than that from the light-trapped female data of either the preceding or the subsequent main emergences. Thus, the variation in the phenotypic proportions of the different main emergences would seem to be due to selective forces, which act on the insects both before and after the average stage in the 5th instar at which larvae were sampled.

Similarly, the proportion of Gr, Br and OL phenotypes in the 3rd instar, from the samples obtained by sweep-netting at night, were usually intermediate between the proportions for the respective phenotypes from the preceding and subsequent main emergence data obtained from light-trapped females. The only exceptions are the values of Gr and OL from the August/September samples, (July main emergence), the former of which is slightly lower, and the latter slightly higher than the respective proportions from both the preceding and subsequent sets of AF1 data.

If the data from sweep-netting by day is compared with that from sweep-netting at night for the May and July, 1976

main emergences, then, in the 3rd instar, the number of Br, OL and yellow type larvae, is proportionally lower in the day-collected samples than the night-collected samples. When chi-squared tests were used to compare the data, these showed that the differences between the day and night collected samples were significant, (for the May, 1976 main emergence, $X^2_3 = 9.024$, $0.05 > p > 0.02$; for the July, 1976 main emergence, $X^2_3 = 8.640$, $0.05 > p > 0.02$). The reason for this involves differences in the behaviour of larvae which are homozygous for c and those which contain c^+, (see chapter 4B).

Finally, looking at the sum of the proportions of the three types of yellow larvae from the AF2 samples, it is evident that, although these vary more than those from the AF1 samples, - a feature which may be due to the smaller size of the AF2 samples, - they are fairly similar. This would accord with the suggestion that the proportion of yellow type larvae does not vary with season.

C. Discussion

As previously mentioned, there are in effect four polymorphic systems involved in the colour variation found in larvae of P. meticulosa.

Analysis of the data from the AF1 samples, showed that the polymorphic system controlled by gene B is balanced; however, there is seasonal variation in the frequencies of the two alleles, such that allele b' is present at its highest frequency in adults which emerge in September and

October, and at its lowest frequency in adults of the May main emergences. Furthermore, the AF2 sample data indicate that the changes in the allelic frequencies of b' and b^o are due to selective factors which act both before, and after, the average stage at which 4th instar larvae were sampled. As the alleles of gene B may be expressed phenotypically in both the 3rd and 5th instars as well as the 4th instar, then it seems likely that the factors affecting the frequency of b' and b^o will act on larvae in all three of these instars.

The polymorphic system controlled by gene A also seems to be balanced when considered from one year to the next, but again the frequencies of the two alleles show seasonal variations. The AF1 data shows that allele a^+ is at its highest frequency in adults of the May main emergences, and at its lowest in those of the Sept./Oct. main emergences. As the alleles of gene A are only expressed phenotypically in the 5th instar, then, if the selective factors affecting the frequency of its alleles are acting differently on larval colour, these factors are only likely to act in this instar. The data from the AF2 samples indicate that the factors causing changes in these allelic frequencies, act throughout the 5th instar. It is likely that the changes in the frequency of alleles b' and b^o affect the proportions of the various colour types in the 5th instar; however, this effect is almost certainly small when compared with the overall changes which were observed.

The data from the AF1 samples also indicate that the polymorphic system controlled by gene C is balanced. Here again seasonal variations occur. However, these variations are small in comparison with those of the alleles of genes B and A. It must also be noted that as the alleles of b' and b^{o} may be expressed in the 3rd instar, the changes in the frequency of these alleles will affect the proportions of the 3rd instar phenotypes, and as the changes in these proportions are relatively small, this effect could be appreciable.

Data from both the AF1 and AF2 samples indicate that the polymorphic system controlled by genes D and E is balanced; however, the numbers involved are rather small, and this should be taken into account when considering the results.

Finally, looking at the larval colour system as a whole, rather than in individual sections, it seems that the proportion of Gr larvae in the later instars falls between September and May, and rises over the summer months between May and September, the converse being true for Br larvae. The proportion of OL larvae rises and falls in the same way as that of Br larvae in the 3rd and 4th instars, and in the same way as Gr larvae in the 5th instar. This would be expected from the changes in the proportions of Gr and Br larvae, because the proportion of a particular heterozygote in a population will rise when the frequency of the rarer allele rises, and fall when the frequency of the commoner allele rises. (The apparent frequency of b' in the 5th instar is

lower than its real value as b' is hypostatic to a^+ in this instar).

It appears then, that during the winter and early spring months Br larvae have a selective advantage over Gr larvae, and during the summer and early autumn months Gr larvae are at a selective advantage to Br larvae.

CHAPTER 6. THE MAINTENANCE OF THE LARVAL COLOUR POLYMORPHISM.

A. Introduction

From the data collected to investigate whether the polymorphic colour system in P. meticulosa was balanced or transient, it has been concluded that while the proportions of 3rd, 4th and 5th instar phenotypes vary from one main emergence to the next, the polymorphism seems to be balanced when considered from year to year. The length of time over which the evidence, that led to these conclusions, was collected, is far too short to justify making a categorical statement to this effect. However, I have examined collections of set larvae dating back as far as 1850, and these indicate that the Gr, OL, Br and PY colour types have existed for over 100 years. YG and YB larvae seem also to have existed, but because blown and set larvae fade with age, it is difficult to distinguish the YG and YB colour types from the Gr and Br colour types in old larvae. The fact that the colour types appear to have existed for over 100 years, implies that the variation is reasonably stable. Therefore, on the assumption that the polymorphism is balanced, an investigation to determine the way in which this balance is preserved was undertaken. Therefore, I think that it is appropriate to mention here a number of general conclusions concerning polymorphism.

Discontinuous variation when genetically controlled must be maintained by a switch mechanism of one kind or another. This may be either in the form of a major gene, a super-gene, or a chromosome reconstruction such as an inversion (Ford, 1964).

When dealing with a genetic polymorphism in which the forms are distinguishable by morphological characters, such as colour or markings, there is no reason to suppose that these characters are necessarily of any direct adaptive significance. However, in most cases, these characters will undoubtedly be associated with other changes of a less obvious manifestation which are so. Indeed, there are good reasons for believing that, in the vast majority of cases, genetic switch mechanisms have a multiple effect. Direct evidence for this view has been supplied by work on Drosophila melanogasterMeigen, in which every one of the hundreds of switch mechanisms studied seem to act, not only on the morphology of the insect, but also on its viability, length of life or fertility (Ford, 1964). Thus, it must be realised that when looking into the selective forces controlling a balanced polymorphism, one must consider not only whether a character, like colour pattern, influences the survival value of the organism, but also, and often more importantly, whether the gene controlling it does so.

It seems then, that major genes generally have multiple effects. The view is often taken that many of these effects are of negligible importance for survival. This can not be the case when they control a polymorphism. It has been shown that, if one allelomorph of a gene is to be of neutral survival value compared with another, the balance of advantage and disadvantage involved must be remarkably exact (Fisher, 1930b). It was also demonstrated that a mutant

gene of neutral survival value will spread very slowly in a population, so that, if derived from a single mutant, the number of individuals which possess it can not greatly exceed the number of generations since it arose (Fisher, 1930a). Moreover, mutations take place so rarely, that its recurrence is not likely to hasten the process substantially. Therefore, genes of neutral survival value will be very rare, more so in fact because, except in very small populations, before their frequency can increase appreciably, the fine balance, needed for their neutrality, will have been upset by alterations in the overall genetic make-up of the organism, and changes in the environment.

When a gene is actually disadvantageous it will tend to be eliminated by natural selection and its spread will be arrested at an early stage. Therefore, it will be maintained at a level determined, on the one hand, by its mutation rate, and, on the other, by the counter selection to which it is exposed. It is clear then, that if a unifactorial character occurs at a frequency which is significantly higher than that expected from the mutation rate, it must have been favoured by selection at some time.

Yet, mutant genes which have a greater survival value than their normal alleles, will, if this advantage persists, spread through a population until they displace the normal alleles which will be reduced to rare mutants. While this process is taking place, the population will be in a state of transient polymorphism (Ford, 1940a).

Such situations must be relatively uncommon as advantageous genes will in most cases already have been incorporated into the organism's genetic constitution. Only when a mutant gene becomes advantageous, due to a change in the environment or gene complex of the population, will it be possible to study its spread through the community.

The majority of genetic polymorphisms, then, are of a different type; that is to say, they are balanced, being maintained at a level determined by the strength of the opposing selective forces to which they are subject. This ensures diversity, which may often be virtually permanent, as is shown by the existence of two sexes in many species.

The mechanisms which, at least theoretically, seem capable of maintaining a genetic polymorphism in a balanced state, have been examined by a number of authors (e.g. Dempster, 1955; Sheppard, 1958; Williamson, 1958; Maynard Smith, 1962; Ford, 1965). However, the amount of experimental work to identify relevant selective forces is limited. One outstanding exception concerns polymorphic mimicry in Lepidoptera (e.g. Carpenter and Ford, 1933; Clarke and Sheppard, 1959a, b, 1960a, b, c, d, 1962; Ford, 1953; Sheppard, 1959, 1961a, b), where the polymorphism is maintained largely by the relative frequencies of the mimics and models.

There are, however, a large number of species with colour polymorphism where mimicry is obviously not involved. Giving a complete or even representative list of these species

is clearly beyond the scope of this study and no comprehensive review exists. Examples, however, are to be found in many different phyla throughout the animal kingdom and Table 6Ai gives a number of examples which it is hoped will give some idea of the range of species involved. Dealing specifically with Lepidoptera, Robinson (1971) outlines many suitable examples.

What, then, are the selective agencies which have the potential to maintain non-mimetic polymorphism?

In a few cases experimental evidence has produced probable answers. Sheppard (1952) has shown that females of the three genotypes, dominula, medionigra and bimacula, which are each phenotypically distinct in the moth Callimorpha dominula L., all prefer to mate with males of another, rather than their own, form. This obviously opposes uniformity, although it is not the only agency which does so in this species. Clarke (1962a), attempting to discover the way in which colour and marking variation is maintained, particularly in Cepaea spp., concluded that the colour and banding polymorphisms of these species are probably kept in a balanced state by selective predation in the absence of mimicry.

Ford (1964) takes the view that the most general basis of genetic polymorphism is a balance of opposed advantage and disadvantage, such that the heterozygote is favoured compared with either homozygote. The basis of the theory involves, firstly, that when a rare gene becomes an asset owing to changed conditions, the resulting advantage

Table 6Ai.

Examples of species with colour polymorphisms which do not involve mimicry.

Metridium senile (Coelenterata, Anthozoa), FOX AND PANTIN (1961)

Pomatoceros triqueter (Annelida, Polychaeta), FØYN AND GJØEN (1954)

Cepaea spp. (Mollusca, Gastropoda), VARIOUS AUTHORS

Limicolaria spp. (Mollusca, Gastropoda), CROWLEY AND PAIN (1970)

Armadillium nasutum (Crustacea, Isopoda), ADAMKEWICZ (1969)

Paratettix tetanus (Arthropoda, Orthoptera), FISHER (1939)

Biston betularia (Arthropoda, Lepidoptera), VARIOUS AUTHORS

Ophiocamina nigra (Echinodermata, Ophiuroidea), FONTAINE (1962)

Xiphophorus maculatus (Chordata, Pisces), GORDON AND GORDON (1957)

Eleutherodactylus spp. (Chordata, Amphibia), GOIN (1950)

Natrix sipedon (Chordata, Reptilia), CAMIN AND EHRLICH (1958)

Poephila gouldiae (Chordata, Aves), SOUTHERN (1945)

Malaconotus spp. (Chordata, Aves), HALL, MOREAU AND GALBRAITH (1966)

will be an average of the advantages and disadvantages conferred by the different aspects of its action. Secondly, selection will tend to make the favourable effects of the gene dominant and the unfavourable ones recessive. This dominance modification will cause the heterozygote to have nothing but advantages, and be superior to the homozygotes which will have both advantages and disadvantages (Sheppard, 1953). This situation ensures that a balanced polymorphism will be maintained, the frequencies of the morphs depending on the relative strength of the selection and counter-selection involved. (The work of Caspari (1950) on the moth Ephestia keuhniella Zeller illustrates the point well).

One of the obvious cases in which there is definite evidence that heterozygous advantage can maintain a balanced polymorphism is that of sickle-cell anaemia in man (Allison, 1954, 1956).

A further example is provided by the work of Gustafsson and his colleagues which gave instances of mutants, arising in experimental stocks of both animal and plant material, that produce heterozygotes superior in viability to the wild type, though their homozygotes are markedly weak or lethal (Gustafsson, 1946; Gustafsson, Nybom and Von Wettstein, 1950). Sheppard (1953) points out that heterozygous advantage, due to dominance modification, can also result from a chromosome inversion. Inversions will generally carry many genes but will behave as a single genetic unit. Some of the genes on an inversion will be dominant and

others recessive in action. Thus, the inversion may at once produce a stable polymorphism due to its genetic content; on the other hand, if not of a type to do so at first, polymorphism may evolve through dominance-modification along the lines already indicated. Evidence supporting this theory has been provided by work on Drosophila pseudoobscura and D. persimilis (Dobzhansky, 1947, 1958; Dobzhansky and Levene, 1948; Pavan, Dobzhansky and Da Cunha, 1957; Spiess, 1958).

However, whether heterozygous advantage is as ubiquitous as has been suggested in some reviews of polymorphism, has yet to be satisfactorily demonstrated. When dealing with colour polymorphisms, the lack of definite evidence may often be due to the problems of distinguishing between homozygotes and heterozygotes in the wild.

This, and the following two chapters, explain some of the experiments and tests which were designed to investigate some of the mechanisms by which the larval polymorphisms of P. meticulosa may be maintained. For these purposes the mechanisms will be split into two groups; those which do not involve larval predators and will be dealt with in this chapter, and those which involve predators or other organisms such as parasites, or viral and bacterial diseases which cause death. This latter group of mechanisms will again be split into two sections, the first, to be dealt with in chapter 7, including larval mortality due to organisms which do not seem to use colour vision to find prey. The second,

which is dealt with in chapter 8, being concerned with predation by birds which have colour vision and appear to look for their prey.

B. Non-random mating

Introductory remarks.

The amount of work carried out on non-random mating mechanisms in animals is very limited. Examples of genes having both morphological and recognisable physiological effects are well known, but very few genes are known to influence the "choice" of mate. The majority of reports on non-random mating between individuals, which differ by a single major-gene, suggest that like genotypes usually tend to mate together.

On the other hand, one of the most documented examples of non-random mating involves the tendency for unlike genotypes of C. dominula to pair (Sheppard, 1952). If the non-random mating in C. dominula were based upon the differences in the coloration and markings of the three adult forms, then the situation would not be potentially applicable to the variation in P. meticulosa larval colour, as the system controlling this variation does not seem to manifest itself phenotypically in the morphology of the imago. However, some of Sheppard's data indicated, although not conclusively, that the mating behaviour is not influenced by visual stimuli, and Sheppard speculates that scent may be important as females use it to "assemble" the males. In butterflies at least, males are known which

employ "scent organs" to induce the female to mate. As mentioned in chapter 2B, males of P. meticulosa certainly have scent-glands and brush-like organs which seem to be used to distribute male pheromones when flying in proximity to a female.

It is, then, interesting to speculate on the effect of non-random mating. A tendency to pair with a mate of a different genotype will cause a polymorphism, unless other selective factors over-ride this effect. On the other hand, a tendency to pair with a mate of similar genotype, will, when taken to its extreme, where there is no inter-genotype mating, cause the irradication of heterozygotes, and, then, if one homozygote has a selective advantage over the other, a unimorphic population should result. In cases where both homozygotes are able to survive, the two morphs will be liable to speciation.

Sheppard's tests on C. dominula seem, from the data published, to have been designed simply to determine whether there is a tendency for pairing between similar or different phenotypes. However, there is no reason to think that non-random mating tendencies should simply be confined to these two alternatives. A biallelic gene has three genotypic configurations, two homozygotes and the heterozygote. Therefore, it is at least theoretically possible that moths of one of the genotypes may not only show a tendency to avoid mating with moths of a similar genotype, but may also show a preference between the other two genotypes. Furthermore,

not all genotypes need necessarily show similar types of tendencies. If, for example, the two homozygotes show a tendency to mate with the other homozygote, whilst the heterozygotes favoured pairing with moths of the same genotype, the system would promote an excess of heterozygotes relative to the value expected in a random mating system. Moreover, if this were the only character controlled by the gene, the frequencies of the two homozygotes would eventually become equivalent and the system would produce a balanced polymorphism in which the proportion of heterozygotes was greater than half the population, and could reach 66.6% of the population if the mating preferences were absolute.

In chapter 5, it was seen that the number of Olive 4th instar larvae in the Ringwood area always slightly exceeded the value expected by calculation from the allelic frequency estimates of b' and b^{o}. This excess may of course be due to chance, to physiological factors which lead to heterozygous advantage, or to any one of a number of other selective agencies; however, a system similar to that just described, could account for the excess, if some other selective agency prevented the proportions of the two homozygotes equalising by favouring green 4th instar larvae compared with brown.

Other theoretical systems of preferential mating could undoubtedly be devised to produce stable polymorphisms; however, it is hoped that it has been sufficient to describe only one here to show that, in some cases at least, detailed

tests, comparing all three genotypes of a biallelic gene, may be needed to discover whether preferential mating is influencing the maintenance of a polymorphism.

Methods

A number of cages, similar to those described in chapter 2 for pairing, were hung from trees in my garden at Ringwood, and the moths used in a particular test were placed inside these during the morning. The moths used came from the main stocks which were bred primarily as stock for predation experiments. Moths were used as soon after emergence as was possible. The tests were arranged in two series of four sets. Three of these sets concentrated attention on one particular gene, the fourth being concerned with the two genes, D and E, which control the occurrence of yellow larvae. To do this, in each test moths were used which, as far as was ascertainable, were of uniform genotype except for the alleles of the gene under investigation. In the first series of tests a female of a particular genotype was placed in a cage with three, or, in the case of the fourth set, four males, which varied in genotypic make-up with respect to the gene under consideration. The second series of tests were carried out in the same way, but with one male being caged with three or four females. In the three sets of tests designed to investigate the influence of genes A, B and C on mating, each test was repeated 20 times. In the fourth set only ten tests were carried out with each set of genotype combinations because of the rarity of the

yellow larval forms.

As the allelomorphs of the five genes involved are not as yet distinguishable morphologically in the imago, some method of making moths of the same sex recognisable had to be devised. This was done by putting two or three minute spots of black cellulose paint on the moths. In the first three sets of tests two spots of paint were put on the upperside of the frontal margin of the forewings, so that for one genotype both spots were on the right wing, for the second genotype both were on the left wing, and for the third, one spot was painted on each wing. In the fourth set of tests, three spots were used, so that either all three spots were placed on one wing, or they were split up, two on one wing and one on the other. To ensure that the paint spots did not affect results, the spot configuration used was varied between the genotypes in different tests. The moths were only left in the cages for one night, so only pairings which occurred on the first night were recorded.

Results and analysis of data.

The results are given in Tables 6Bi and 6Bii. Table 6Bi deals with tests using one female and three or four males whilst Table 6Bii deals with the reverse situation.

Chi-squared analysis of the results show that there is no correlation between a particular gene and a tendency to mate with a moth of either the same or of a different genotype. Furthermore, if all the trials of any particular test are compared, whether considering series 1 or series 2, in no

Table 6Bi.

Experiments to determine whether mate choice is correlated to the genes controlling larval colour. Results from tests using one female and three or four males.

Set Number	Test Number	Number of Tests	The gene being tested	Female	Male 1	Male 2	Male 3	Male 4 (where applicable)	Genotypic configuration of other genes	The number of matings between the females and: Male 1	Male 2	Male 3	Male 4 (where applicable)	Total
1	1	20	A	aa	aa	a^+a	a^+a^+		$b'b^occd^+$ or e^+	5	6	8		19
	2	20	A	a^+a^+	a^+a^+	a^+a	aa		$b'b^occd^+$ or e^+	7	6	5		18
	3	20	A	a^+a	a^+a	aa	a^+a^+		$b'b^occd^+$ or e^+	8	6	5		19
2	1	20	B	b'b'	b'b'	$b'b^o$	b^ob^o		$aaccd^+$ or e^+	6	5	7		18
	2	20	B	b^ob^o	b^ob^o	$b'b^o$	b'b'		$aaccd^+$ or e^+	4	6	8		18
	3	20	B	$b'b^o$	$b'b^o$	b'b'	b^ob^o		$aaccd^+$ or e^+	6	9	5		20
3	1	20	C	cc	cc	c^+c	c^+c^+		$aab'b^od^+$ or e^+	5	6	6		17
	2	20	C	c^+c^+	c^+c^+	c^+c	cc		$aab'b^od^+$ or e^+	7	6	6		19
	3	20	C	c^+c	c^+c	cc	c^+c^+		$aab'b^od^+$ or e^+	5	5	8		18
4	1	10	D and E	d^+ or e^+	d^+ or e^+	$dde'e^o$	dde'e'	dde^oe^o	$aab'b^occ$	3	2	3	1	9
	2	10	D and E	$dde'e^o$	$dde'e^o$	dde'e'	dde^oe^o	d^+ or e^+	$aab'b^occ$	2	1	2	3	8
	3	10	D and E	dde'e'	dde'e'	dde^oe^o	d^+ or e^+	$dde'e^o$	$aab'b^occ$	2	4	2	2	10
	4	10	D and E	dde^oe^o	dde^oe^o	d^+ or e^+	dde'e'	$dde'e^o$	$aab'b^occ$	3	2	2	2	9

Table 6Bii.

Experiments to determine whether mate choice is correlated to the genes controlling larval colour. Results from tests using one male and three or four females.

Set Number	Test Number	Number of Tests	The gene being tested	Male	Female 1	Female 2	Female 3	Female 4 (where applicable	Genotypic configuration of other genes	The number of matings between the Males and: Female 1	Female 2	Female 3	Female 4 (where applicable)	Total
1	1	20	A	aa	aa	a^+a	a^+a^+		$b'b^ocd^+$ or e^+	5	7	6		18
	2	20	A	a^+a^+	a^+a^+	a^+a	aa		$b'b^ocd^+$ or e^+	7	8	5		20
	3	20	A	a^+a	a^+a	aa	a^+a^+		$b'b^ocd^+$ or e^+	5	5	8		18
2	1	20	B	$b'b'$	$b'b'$	$b'b^o$	b^ob^o		$aaccd^+$ or e^+	7	5	5		17
	2	20	B	b^ob^o	b^ob^o	$b'b^o$	$b'b'$		$aaccd^+$ or e^+	8	6	6		20
	3	20	B	$b'b^o$	$b'b^o$	$b'b'$	b^ob^o		$aaccd^+$ or e^+	5	6	8		19
3	1	20	C	cc	cc	c^+c	c^+c^+		$aab'b^od^+$ or e^+	6	6	7		19
	2	20	C	c^+c^+	c^+c^+	c^+c	cc		$aab'b^od^+$ or e^+	4	6	7		17
	3	20	C	c^+c	c^+c	cc	c^+c^+		$aab'b^od^+$ or e^+	6	6	6		18
4	1	10	D and E	d^+ or e^+	d^+ or e^+	$dde'e^o$	$dde'e'$	dde^oe^o	$aab'b^occ$	2	3	2	2	9
	2	10	D and E	$dde'e^o$	$dde'e^o$	$dde'e'$	dde^oe^o	d^+ or e^+	$aab'b^occ$	1	3	2	1	7
	3	10	D and E	$dde'e'$	$dde'e'$	dde^oe^o	d^+ or e^+	$dde'e^o$	$aab'b^occ$	3	2	3	2	10
	4	10	D and E	dde^oe^o	dde^oe^o	d^+ or e^+	$dde'e^o$	$dde'e'$	$aab'b^occ$	3	1	2	3	9

instance is there a significant tendency towards mating with one genotype in preference to another. The results of these chi-squared tests are given in Appendix Table 6Bi.

Conclusions.

Although the data from the tests are not as substantial as might be wished, particularly when considering genes D and E, the evidence indicates that there are no mating preferences between the various genotypes.

C. The investigation of heterozygous advantage with respect to gene B.

(i) Introduction

Heterozygous advantage, as mentioned earlier, may maintain a genetic polymorphism by a balance of opposed advantage and disadvantage, such that the heterozygote is favoured compared with either homozygote. The maintenance of many balanced polymorphisms has been attributed to this phenomenon, in some cases with supportative evidence and, in other cases, simply because evidence for any other explanation is lacking.

Sheppard (1953) notes that heterozygous advantage may result from dominance modification, (see page 248). Ford (1964) notes that the establishment of heterozygous advantage constitutes the normal course of events to be expected in polymorphism, and suggests, that although this situation has not been looked for very often, it is frequently realized. Furthermore, the genetic structure of organisms with their

large number of rare mutants, whose alleles have the status of 'normal' major-genes, demonstrates that many of these must have passed through a phase of transient polymorphism which has not become a balanced one. On the other hand, some balanced polymorphisms are known in which no evidence of heterozygous advantage has been uncovered, even though it has been sought. Ford quotes C. dominula as an example of this and speculates that, as the medionigra gene is only found naturally in one locality, and the heterozygote is visibly distinct from both homozygotes, it appears that this polymorphic situation arose fairly recently. Moreover, as the rarer of the two allelomorphs occurs at a relatively low frequency, then the heterozygous advantage may not yet have had sufficient opportunity to become established.

Muller (1950, 1958) opposes the view that polymorphism is a widespread phenomenon, and that it is generally associated with maintenance by heterozygous advantage. He suggests that when a polymorphism, controlled by two alleles, super-genes, or inversions, is maintained in stable equilibrium by heterozygous advantage, the population would be better adjusted to the environment if it were monomorphic for another allele which was equal or superior in fitness to the heterozygote of the polymorphic system. He goes on to suggest that natural selection must lead to a monomorphic state, and he says that balanced polymorphisms .....

> "..... are to be regarded as temporary makeshifts that arise in the stress of comparatively rapid evolutionary flux and that they are due to be

> rectified ultimately, where a long-term natural selection repairs its short-term imperfections and miscarriages".

At best this statement over-generalises the situation; however, the evidence seems to indicate that it is in fact a fallacy. Dobzhansky and Pavlovsky (1960) point out that the statement is based upon the misconception that the survival value of a heterozygote can always be equalled, if not exceeded, by another allele arising by mutation. They give evidence taken from both laboratory tests which were continued over a long period of time and from situations found in nature, to show that polymorphisms are not simply 'temporary makeshifts', but that they may be extremely stable. Thus, in the laboratory experiments, heterozygous advantage was maintained over many generations. Furthermore, in Drosophila willistoni, some inversion heterozygotes occur over vast territories, and as this species is not associated with man it is suggested that a genetic variant must have existed for a long time to have become incorporated in the gene pools of such widespread populations.

Further indication of the fallacy of Muller's statement may be gained by looking at the fossil evidence of colour and banding in the snail Cepaea nemoralis, which shows that the polymorphism has been in existence since the Pleistocene period (Diver, 1929), or by considering some of the human blood-groups and taste test distinctions, for which the Great Apes also are polymorphic (Ford, 1964).

Thus, it seems to be generally accepted that

heterozygous advantage can maintain a balanced polymorphism, although there is strong opposition from some quarters to the suggestion that it is of widespread importance.

In P. meticulosa the chief difficulty in investigating whether heterozygous advantage is important in maintaining the polymorphisms, is the occurrence of fully dominant alleles of four of the five genes controlling larval colour variation. Only the heterozygote of gene B can be distinguished phenotypically from both its homozygotes with any degree of certainty, and then only in the 4th instar when either d^+ or e^+ is present in the genotype to prevent the expression of alleles d, e' and e^o. For this reason, initially, the investigation into the existence of heterozygous advantage in P. meticulosa was confined to gene B.

(ii) Methods.

Three methods were used to seek heterozygous advantage in the polymorphism controlled by gene B. The first of these involved simply looking at the number of progeny of each genotype produced in broods where both parents were thought, on the basis of the chapter 3C results, to be $b'b^o$. This method does not involve a circular argument, relating to the fact that the genotypes were established by looking at 4th instar phenotypic ratios, as it may seem at first. The system put forward, (that the alleles of gene B control Gr, OL and Br 4th instar larvae, see chapter 3C pp 147-148), may be reached without considering the 4OL x 4OL broods, because the b^o and b' system is the only one which will cater

for the results of, 4Gr x 4Gr broods producing all 4Gr progeny, and 4Br x 4Br broods producing all 4Br progeny, and 4Gr x 4Br broods producing all 4OL progeny, (see Table 3Ci, broods CC2 - CC8 inclusive). Furthermore, whilst 4OL x 4OL broods endorse the existence and details of the gene B system, in that 4OL x 4OL broods give progeny which do not deviate from a 1:2:1 ratio of 4Gr:4OL:4Br, it is possible that, if a number of 4OL x 4OL broods are considered, there may be a general tendency for the number of 4OL progeny to exceed the sum of 4Gr and 4Br progeny. Therefore, this method is used to try to detect heterozygous advantage which is not detectable by considering individual broods, but which may be rendered apparent by the consideration of a number of broods.

The second method was to employ an adaptation of Ford's work on differential viability between melanic and non-melanic forms of the moth Alcis repandata L. which involved submitting broods of larvae to adverse environmental conditions (Ford, 1940b).

The third method involved calculating the expected number of heterozygotes in a natural population from allelic frequency estimates derived from the same population, and comparing this figure with the observed number of heterozygotes.

Method 1.

This simply involved submitting the observed results of 22 of the control-cross broods, in which both parents were $b'b^{o}$, to chi-squared tests, taking the expected frequency of

the results to be based on a 1:2:1 ratio of Gr:OL:Br 4th instar forms in each case. The drawback of this method is that the larvae being scored were reared in an environment where there was an abundant food supply, and where temperature, humidity and larval density were controlled to a greater or lesser extent. Furthermore, the larvae were not submitted to natural conditions with respect to rainfall or wind, and were protected from predators, parasites and to some extent disease. Therefore, this method may not give results which are directly comparable with a natural situation.

Method 2.

Because of the drawbacks inherent in method 1, a second set of data was collected from larvae of broods, resulting from $b'b^{o}$ parents, which were reared under adverse conditions of food supply or larval density.

Ford (1937, 1940b) provided evidence that larvae of the melanic form of A. rependata survived semi-starvation better than larvae of the non-melanic form.

A similar method was used to test for increased heterozygote vigour in P. meticulosa. Ten broods of larvae were reared. These broods were named the "heterosis experimental broods", and were numbered HEB1 - 10 inclusive. Each brood was reared from parents known to be heterozygous for gene B, homozygous recessive for genes A and C, and thought to be homozygous dominant for gene D and/or gene E, although, due to the epistatic and hypostatic interactions of these two genes, no more definite statement may be made. From

these parental genotypes, all the broods should conform to a 1:2:1 ratio of Gr:OL:Br colour-types in the 3rd, 4th and 5th instars.

The eggs of each brood were split at random into two groups, so that one group comprised one third of the larvae and the other group two-thirds. The smaller groups from each brood were reared as controls using the basic rearing methods described in chapter 2B. The other group from five of the broods (HEB1 - 5 inclusive) were submitted to semi-starvation. The method employed was to alternately provide larvae with sorrel for 24 hours, and then remove all food for 24 hours. Otherwise the larvae were treated in the same way as the control groups.

The larger groups of larvae from broods HEB6 - 10 inclusive were submitted to overcrowding treatment, all larvae in each group being reared in a single box measuring 250 mm x 200 mm x 80 mm. The number of larvae in a group varied from 72 to 237. Otherwise the larvae were treated in the same way as the control groups.

Method 3.

The final method employed uses the idea that heterozygous advantage is shown if there is an excess of heterozygotes over the expected number when mating is random. The results of the tests to investigate mating behaviour indicated that mate selection is at random with respect to the allelomorphs of gene B (see chapter 6B).

The results in chapter 5 showed that the allelic

frequencies of b' and b^{o} do change substantially between one main-emergence and the next in the wild population studied. The estimates of the allelic frequencies of b' and b^{o} were based on the 4th instar phenotypes of larvae resulting from females taken during the main emergences between September, 1975 and October, 1977. These larvae were reared in captivity. It was assumed that the conditions under which the larvae were reared did not favour one phenotype more than another, and therefore, that the allelic frequency estimates produced for the 4th instar larvae were approximately representative of the frequencies of b' and b^{o} in the population from which the parents originated, at the time when the females were taken. If this is so, then when the observed number of 4th instar larvae of each of the phenotypes Gr, Br and OL, are compared with the expected numbers of these phenotypes calculated from the allelic frequency estimates of the relevant main emergences, these values should be virtually equivalent.

Such comparisons were carried out for each of the six main emergences between September, 1975 and October, 1977 using chi-squared tests.

(iii) Results and analysis of data.

Method 1 results.

The results of the 22 control cross broods in which both parents were $b'b^{o}$ are given in Table 6Ci.

If the numbers of homozygotes are compared with the number of heterozygotes in these 22 broods, 18 show an excess

Table 6Ci.

Investigation to determine whether the maintenance of the polymorphism controlled by gene B involves heterozygous advantage. The results of a number of control cross broods in which both parents were heterozygous for gene B. Fourth instar results.

Brood Number	Origin of the male parent	Phenotype of the male parent	Genotype of the male parent Alleles of gene: C	B	A	Origin of the female parent	Phenotype of the female parent	Genotype of the female parent Alleles of gene: C	B	A	Number of eggs laid	Number of fourth instar larvae of colour types: Gr	OL	Br	Total number of fourth instar larvae	Total number of green and brown larvae (homozygote)	Whether the excess is of homozygotes or heterozygotes	Chi-squared values from a comparison of the numbers of homozygotes and heterozygotes, taking a 1:1 expected ratio	Probability values. (Degrees of freedom equal 1 throughout
CC1	IB1	3Gr 4OL 5OL	+-	'o	--	IB1	3Gr 4OL 5OL	+-	'o	--	76	14	31	16	61	30	Heterozygotes	0.016	0.9 - 0.8
CC9	CC8	3Gr 4OL 5OL	+-	'o	--	CC8	3Gr 4OL 5OL	+-	'o	--	138	32	68	31	131	63	Heterozygotes	0.191	0.7 - 0.5
CC11	IB3	3Gr 4OL 5Br	(++)	'o	+-	IB3	3Gr 4OL 5Br	(+x)	'o	+-	264	60	126	53	239	113	Heterozygotes	0.707	0.5 - 0.3
CC38	IB6	3Gr 4OL 5OL	++	'o	--	IB6	3Gr 4OL 5OL	++	'o	--	39	8	20	7	35	15	Heterozygotes	0.714	0.5 - 0.3
CC39	IB6	3Gr 4OL 5OL	++	'o	--	IB6	3Gr 4OL 5OL	++	'o	--	64	13	28	12	53	25	Heterozygotes	0.170	0.7 - 0.5
CC40	IB6	3Gr 4OL 5OL	++	'o	--	IB6	3Gr 4OL 5OL	++	'o	--	407	89	197	95	379	182	Heterozygotes	0.594	0.5 - 0.3
CC41	IB6	3Gr 4OL 5OL	++	'o	--	IB6	3Gr 4OL 5OL	++	'o	--	293	63	139	61	263	124	Heterozygotes	0.856	0.5 - 0.3
CC50	IB14	3OL 4OL 5OL	--	'o	--	IB14	3OL 4OL 5OL	--	'o	--	431	93	210	90	393	183	Heterozygotes	1.884	0.2 - 0.1
CC51	IB14	3OL 4OL 5OL	--	'o	--	IB14	3OL 4OL 5OL	--	'o	--	62	15	27	12	54	27	-	0	1
CC52	IB14	3OL 4OL 5OL	--	'o	--	IB14	3OL 4OL 5OL	--	'o	--	194	40	84	43	167	83	Heterozygotes	0.006	0.95 - 0.90
CC53	IB14	3OL 4OL 5OL	--	'o	--	IB14	3OL 4OL 5OL	--	'o	--	28	4	11	7	22	11	-	0	1
CC54	IB19	3Gr 4OL 5Br	(++)	'o	(++)	IB19	3Gr 4OL 5Br	(+x)	'o	(+x)	235	52	107	55	214	107	-	0	1
CC55	IB19	3Gr 4OL 5Br	(++)	'o	(++)	IB19	3Gr 4OL 5Br	(+x)	'o	(+x)	314	65	136	69	270	134	Heterozygotes	0.015	0.95 - 0.90
CC56	IB19	3Gr 4OL 5Br	(++)	'o	(++)	IB19	3Gr 4OL 5Br	(+x)	'o	(+x)	309	59	142	64	165	123	Heterozygotes	1.362	0.3 - 0.2
CC57	IB21	3Gr 4OL 5Br	+-	'o	(++)	IB21	3Gr 4OL 5Br	+-	'o	(+x)	401	87	182	89	358	176	Heterozygotes	0.101	0.8 - 0.7
CC58	IB21	3Gr 4OL 5Br	+-	'o	(++)	IB21	3Gr 4OL 5Br	+-	'o	(+x)	69	14	32	15	61	29	Heterozygotes	0.148	0.7
CC59	IB23	3OL 4OL 5Br	--	'o	(++)	IB23	3OL 4OL 5Br	--	'o	(+x)	256	59	114	52	225	111	Heterozygotes	0.04	0.9 - 0.8
CC60	IB23	3OL 4OL 5Br	--	'o	(++)	IB23	3OL 4OL 5Br	--	'o	(+x)	161	36	72	37	145	73	Homozygotes	0.067	0.8 - 0.7
CC61	IB23	3OL 4OL 5Br	--	'o	(++)	IB23	3OL 4OL 5Br	--	'o	(+x)	222	42	94	48	184	90	Heterozygotes	0.087	0.8 - 0.7
CC62	IB23	3OL 4OL 5Br	--	'o	(++)	IB23	3OL 4OL 5Br	--	'o	(+x)	147	33	61	27	121	60	Heterozygotes	0.008	0.95 - 0.90
CC83	CC1	3OL 4OL 5OL	--	'o	--	CC1	3OL 4OL 5OL	--	'o	--	304	68	144	66	178	134	Heterozygotes	0.360	0.7 - 0.5
CC85	CC11	3Gr 4OL 5Br	(++)	'o	+-	CC11	3Gr 4OL 5Br	(+x)	'o	+-	107	22	49	23	94	45	Heterozygotes	0.429	0.7 - 0.5
Totals											4521	968	2074	972	4014	1940	Heterozygotes	4.473	0.05 - 0.02

of heterozygotes, three have equal numbers of homozygotes and heterozygotes and in one brood there were more homozygotes than heterozygotes. However, these excesses were shown to be non-significant when the number of heterozygotes was compared with the number of homozygotes by chi-squared tests taking the expected ratio between the two as 1:1, the lowest probability value being between 0.2 and 0.1. The chi-squared values are given in Table 6Ci.

On the other hand, as so many of the broods showed an excess of heterozygotes, there is an indication that the heterozygotes may be slightly more viable.

In fact, if the phenotypic results of all these broods are summed, then the resulting totals of homozygotes and heterozygotes should still conform to a 1:1 ratio. When the summed totals of Gr and Br larvae were compared with the number of OL larvae using chi-squared, the value obtained was significant though not highly so, ($X^2_1 = 4.473$, $0.05 > p > 0.02$).

Therefore, it appears that the heterozygotes ($b'b^o$) have a slightly greater chance of surviving until the 4th instar than either of the homozygotes, $b'b'$ and b^ob^o, under artificial rearing conditions.

Although gene B is only known to affect the morphology of P. meticulosa in the later larval instars, there is no reason to suppose that any physiological effects it has are confined to this larval stage. Indeed genes are known which affect more than one stage in the life-cycle of insects as is

illustrated by the occurrence of a recessive gene found in Colias philodice Godt., which changes the egg, larval, pupal and adult eye colour from the typical yellowish-green to blue (Gerould, 1921, 1926). Therefore, the slight selection against homozygotes indicated by the excess of heterozygous 4th instar larvae may continue to act after the 4th instar. For this reason it became imperative to compare the number of larvae of each of the three colour types to actually reach the imago stage. Fortunately, records of the number of imagines subsequently emerging from each larval phenotype were kept, and these are given in Table 6Cii.

Analysing this data in the same way as the 4th instar larval data gave very similar results, heterozygotes giving rise to imagines more often than homozygotes in 18 broods whilst the reverse was true in only one brood. The excesses were again not significant except when the results of all the broods were summed, and even then the probability was above 0.02, ($X^2_1 = 4.859$, $0.05 > p > 0.02$). Moreover, if phenotypes of larvae which died between the 4th instar and the adult stage are considered, these are very close to a 1:1 ratio of homozygotes to heterozygotes, the actual figures being 132 homozygotes and 131 heterozygotes. Therefore, it appears that mortality after the 4th instar is not related to the genotype with respect to gene B. It is also worth noting that the proportions of the two homozygotes do not differ significantly from a 1:1 ratio, whether the results of 4th instar larvae are examined, ($X^2_1 = 0.008$, $0.95 > p > 0.9$),

Table 6Cii.

Investigation to determine whether the maintenance of the polymorphism controlled by gene B involves heterozygous advantage. The results of control cross broods in which both parents were heterozygous for gene B. (For origins, genotypes and phenotypes of the parents of these broods, see Table 6Ci

Brood Number	The Number of larvae to reach the imaginal state b'b' (larvae which were green in the 4th instar)	b'b° (larvae which were olive in the 4th instar)	b°b° (larvae which were brown in the 4th instar)	Total number of imagines	Number of imagines from green and brown 4th instar larvae	Whether the excess is of homozygotes or heterozygotes	Chi-squared values from a comparison of the numbers of homozygotes and heterozygotes, taking a 1:1 expected ratio	Probability values (Degrees of freedom equal 1 throughout)
CC1	12	28	15	55	27	Heterozygotes	0.018	0.9 - 0.8
CC9	28	64	30	122	58	Heterozygotes	0.295	0.7 - 0.5
CC11	56	121	52	229	108	Heterozygotes	0.738	0.5 - 0.3
CC38	8	18	6	32	14	Heterozygotes	0.5	0.5 - 0.3
CC39	11	25	12	48	23	Heterozygotes	0.083	0.8 - 0.7
CC40	82	184	91	359	173	Heterozygotes	0.471	0.5 - 0.3
CC41	57	130	58	245	115	Heterozygotes	0.918	0.5 - 0.3
CC50	88	196	85	369	173	Heterozygotes	1.434	0.3 - 0.2
CC51	11	25	12	48	23	Heterozygotes	0.083	0.8 - 0.7
CC52	39	77	38	154	77	-	0	1
CC53	4	9	7	20	11	Homozygotes	0.2	0.9 - 0.8
CC54	49	102	49	200	98	Heterozygotes	0.08	0.8 - 0.7
CC55	60	127	64	251	124	Heterozygotes	0.036	0.9 - 0.8
CC56	53	137	60	250	113	Heterozygotes	2.304	0.2 - 0.1
CC57	80	167	84	331	164	Heterozygotes	0.027	0.9 - 0.8
CC58	13	26	13	52	26	-	0	1
CC59	52	109	50	211	102	Heterozygotes	0.232	0.7 - 0.5
CC60	34	68	34	136	68	-	0	1
CC61	41	90	43	174	84	Heterozygotes	0.207	0.7 - 0.5
CC62	30	56	25	111	55	Heterozygotes	0.009	0.95 - 0.90
CC83	65	138	64	267	129	Heterozygotes	0.303	0.7 - 0.5
CC85	21	46	22	89	43	Heterozygotes	0.101	0.8 - 0.7
Totals	894	1943	914	3751	1808	Heterozygotes	4.859	0.05 - 0.02

or the number of these phenotypes to reach the imago stage are considered, (X^2_1 = 0.221, $0.7 > p > 0.5$), so the situation does not involve the heterozygote being more viable than just one of the homozygotes.

To sum up then, the results from these 22 control crosses indicate that some degree of increased vigour may be associated with the heterozygotes, but if so, this is slight, and the evidence is far from conclusive.

Method 2 results.

Turning then to the results produced by rearing larvae under conditions of either semi-starvation or high larval density. The results of broods HEB1 - 10 inclusive are given in Table 6Ciii.

Chi-squared values were calculated for the results of both the control samples and the samples submitted to supposedly adverse conditions for each brood. These values were calculated from the data relating to the number of each phenotype that attained the imaginal state. Unfortunately, two of the samples submitted to high larval density were wiped out by disease epidemics. The fact that two of the samples submitted to conditions of high larval density were completely wiped out by disease is not surprising, for as many breeders of Lepidoptera will know, larvae kept at a high density seem to be particularly susceptible to this type of epidemic, (see chapter 7 p.289). The chi-squared values quoted for these two samples, (HEB7 and HEB8) are based on the 4th instar data, and the numbers of each colour-

Table 6Ciii.

Investigation to determine whether the maintenance of the polymorphism controlled by gene B involves heterozygous advantage. The results of experiments to determine the effects of semi-starvation and larval density on genotype viability - The HEB broods. Results of the fourth instar colour types, and the number of larvae of each colour type that subsequently gave rise to imagines. All parents were Olive in the third, fourth and fifth instars, and had the genotype ccb'b^{o}aa (σ^{+} or φ^{+}).

Type of conditions to which samples were submitted	Brood number	Origin of the male parent	Origin of the female parent	Number of eggs laid	Number of fourth instar larvae of colour types: Gr	OL	Br	Number of imagines resulting from 4th instar larvae of colour types: Gr	OL	Br	Total number of homozygotes (green or brown 4th instar larvae) to produce imagines	Percentage mortality	Total number of imagines	Chi-squared values from a comparison of the number of homozygotes and heterozygotes to produce imagines, taking a 1:1 expected ratio	Probability values (Degrees of freedom equal 1 throughout)
A. Reared using basic rearing methods described in chapter 2B. (employed as controls)	HEB1	MSO19	MSO18	38	11	26	13	10	24	11	21	22.4	45	0.2	0.7 - 0.5
	HEB2	MSO16	MSO20	121	24	53	27	22	50	26	48	19.0	98	0.041	0.9 - 0.8
	HEB3	MSO13	MSO12	40	7	17	7	6	17	6	12	27.5	29	0.862	0.5 - 0.3
	HEB4	MSO12	MSO13	97	21	43	19	20	40	16	36	21.6	76	0.211	0.7 - 0.5
	HEB5	MSO20	MSO18	81	17	35	15	14	33	14	28	24.7	61	0.410	0.7 - 0.5
	Totals							72	164	73	145		309	1.168	0.3 - 0.2
B. Reared under conditions of semi-starvation	HEB1	MSO19	MSO18	113	12	30	16	9	21	8	17	67.8	38	0.421	0.5 - 0.3
	HEB2	MSO16	MSO20	243	39	86	36	24	53	21	45	59.7	98	0.653	0.5 - 0.3
	HEB3	MSO13	MSO12	30	11	24	10	7	16	8	15	61.3	31	0.052	0.9 - 0.8
	HEB4	MSO12	MSO13	195	32	68	31	20	44	19	39	57.4	83	0.301	0.7 - 0.5
	HEB5	MSO20	MSO18	154	25	62	29	17	40	18	35	54.3	75	0.333	0.7 - 0.5
	Totals							77	174	74	151		325	1.628	0.3 - 0.2
C. Reared using basic rearing methods described in chapter 2B. (employed as controls)	HEB6	MSO13	MSO15	33	8	17	6	7	15	6	13	20	28	0.143	0.8 - 0.7
	HEB7	MSO16	MSO13	78	18	41	16	17	35	16	33	12.8	68	0.059	0.9 - 0.8
	HEB8	MSO14	MSO16	51	13	28	12	11	27	10	21	21.3	48	0.75	0.5 - 0.3
	HEB9	MSO18	MSO12	107	21	49	24	21	47	21	42	16.8	89	0.281	0.7 - 0.5
	HEB10	MSO18	MSO11	118	27	60	25	24	56	24	48	11.9	104	0.615	0.5 - 0.3
	Totals							80	180	77	157		337	1.570	0.3 - 0.2
D. Reared under conditions of high larval density	HEB6	MSO13	MSO15	72	13	27	13	12	23	12	24 **	34.7	47	0.021	0.9 - 0.8
	HEB7	MSO16	MSO13	156	21	43	17	0	0	0	28 *	100	81	0.309 *	0.7 - 0.5
	HEB8	MSO14	MSO16	123	17	39	19	0	0	0	36 *	100	75	0.12 *	0.8 - 0.7
	HEB9	MSO18	MSO12	214	42	91	40	40	83	37	77	25.2	160	0.225	0.7 - 0.5
	HEB10	MSO18	MSO11	237	46	93	43	40	86	38	78	30.8	164	0.390	0.7 - 0.5
	Totals							92	192	87	253 *		527	1.837 *	0.5 - 0.3

* Note that in samples from HEB7 and HEB8, which were reared under conditions of high larval density, no imagines emerged due to a viral disease epidemic. Thus, in chi-squared tests and totals, the results from the 4th instar stage of these broods were used.

** Note, in this brood, more homozygotes than heterozygotes reached the imaginal state whilst in all other samples the converse was true.

type in the 4th instar for these two broods are also used in the totals for samples submitted to high larval density.

Chi-squared values were also calculated from the total number of each phenotype to reach the imaginal state in each of the four sets of samples. All the chi-squared values are given in Table 6Ciii.

Dealing first with broods HEB1 - 5 inclusive, none of the chi-squared values from individual samples were significant, although in every case there were more heterozygotes than homozygotes. The excesses of heterozygotes from the semi-starved samples are very similar to those of the control samples. This indicates that the advantage of the heterozygote over the homozygotes is not increased under conditions of semi-starvation, even though the percentage mortality, in the samples submitted to this treatment, was between two and three times as great as in the control samples.

A chi-squared test to compare the total number of each phenotype that reached the adult stage in the semi-starved and the control samples, gave a non-significant result, ($X^2_2 = 0.069$, $0.98 > p > 0.95$). It is notable that the proportional excesses of heterozygotes from individual semi-starved samples is similar in magnitude to those from the control cross broods (figured in Table 6Ci). I have little doubt that if considerably more broods were submitted to conditions of semi-starvation, and the results of these broods were summed, the resulting totals would show a significant

deviation from the expected 1:1 ratio of heterozygotes to homozygotes.

Turning to the results from broods HEB6 - 10 inclusive; similar comments are applicable with the exception that one of the samples submitted to over-crowded conditions produced 24 imagines from homozygotes and only 23 from heterozygotes. The total number of each phenotype to reach the imaginal stage, (or in the case of HEB7 and HEB8, to reach the 4th instar), in the "over-crowded samples" was not significantly different from similar figures from the control samples of these broods, (X^2_2 = 0.171, 0.95 > p > 0.9).

Perhaps the most telling result from the analysis of the HEB sample data was produced by a test comparing the four sets of samples and the total number of imagines resulting from homozygotes and heterozygotes in the control cross broods in Table 6Ci. This gave a high probability value which indicates a high degree of homogeneity, (X^2_4 = 0.696, 0.98 > p > 0.95).

These results then, seem to support, though still not conclusively, the hypothesis that the heterozygote $b'b^o$ is likely to reach the imaginal stage slightly more than twice as often as either of the homozygotes, b'b' and b^ob^o. On the other hand, the submission of larvae to adverse conditions, in the form of either limited food supply or overcrowding, does not appear to enhance the effect of any difference in the viability of the homozygotes and the heterozygotes.

Method 3 results.

Moving to the results of the comparisons between the observed and expected numbers of the Gr, Br and OL phenotypes in the 4th instar of the AF1 data. These observed and expected numbers are given in Table 6Civ, together with the estimated allelic frequencies of b' and b^o for each main-emergence, and the chi-squared and probability values from the comparisons.

It may be seen from these results that in every case the observed number of 4th instar OL larvae exceeds the expected number and both the Gr and Br observed values are lower than expected; however, in no case are these differences significant when the Gr and Br values are classed together as homozygotes, (the probability values always being greater than 0.3 and less than 0.7).

Even when the totals of all the main emergences are added together and compared using a chi-squared test, the result is still not significant at the 5% level, ($X^2_1 = 2.395$, $0.2 > p > 0.1$), and in this case, the results of over 45,000 larvae are being considered!

(iv) Discussion.

The evidence from the three groups of tests to determine whether the polymorphism, based on gene B, in P. meticulosa, is maintained by heterozygous advantage, does not give a clear cut answer to the question.

In all the tests, the observed number of olive larvae exceeded the expected numbers, but when considering individual

Table 6Civ.

Investigation to determine whether the maintenance of the polymorphism controlled by gene B involves heterozygous advantage. Observed and expected results from the Gr, OL and Br colour types in the 4th instar from the AF1 data.

		Sept/Oct. '75	May '76	July '76	Sept/Oct. '76	May/June '77	Sept/Oct. '77
Number of Gr 4th instar larvae	(Observed	1441	3265	2561	5984	2401	4229
	(Expected	1449.0	3277.1	2570.0	6003.3	2413.9	4249.9
Number of Br 4th instar larvae	(Observed	330	1179	707	1213	820	988
	(Expected	337.7	1190.9	715.6	1233.4	832.6	1008.8
Number of OL 4th instar larvae	(Observed	1415	3975	2730	5482	2861	4183
	(Expected	1399.2	3951.0	2712.3	5442.3	2835.4	4141.2
Total number of Gr and Br 4th instar larvae	(Observed	1771	4444	3268	7197	3221	5217
	(Expected	1786.7	4468.0	3285.6	7236.7	3246.5	5258.7
Total number of larvae		3186	8419	5998	12679	6082	9400
Chi-squared value		0.318	0.275	0.201	0.507	0.430	0.787
Probability value (D.F. = 1)		0.7-0.5	0.7-0.5	0.7-0.5	0.5-0.3	0.7-0.5	0.5-0.3

broods, excesses were not significant, and in the AF1 data the excesses were very small indeed. However, the ubiquity of the trend for greater numbers of Olive larvae than expected leads to the conclusion that the heterozygote $b'b^{o}$ is at an advantage, albeit very slight, over the homozygotes. The results of the first set of tests indicate that this is due to advantage over both the homozygotes, $b'b'$ and $b^{o}b^{o}$, and the factors producing the excess of heterozygotes act before or during the 4th instar. The results of the second set of tests indicate that the heterozygous advantage is not increased by submitting larvae to unfavourable conditions such as high larval density or limited food supply.

The results of the allelic frequency estimates of b' and b^{o} have shown that these estimates vary considerably from spring to autumn (see chapter 5). These variations can not be due to heterozygote advantage, which should lead to a balanced polymorphism in which the frequencies of the alleles concerned remain stable, if the strength of the selective factors involved remains stable as is indicated by the AF1 data comparisons.

Therefore, having said that the heterozygote $b'b^{o}$ is at an advantage over the homozygotes, it should be added that whilst this advantage may contribute to the overall maintenance of the polymorphism, the fluctuations in the frequencies of b' and b^{o} imply that other selective factors of far greater magnitude are involved.

(v) Gamete compatability and oval viability considered with respect to heterozygous advantage in gene B.

The first set of tests on heterozygous advantage indicate that the excess of the heterozygote, $b'b^{o}$, over the two homozygotes, b'b' and $b^{o}b^{o}$, is due to factors which act at some point between mating and the end of the 4th larval instar. As the genotypes b'b', $b'b^{o}$ and $b^{o}b^{o}$ only become recognisable in the 3rd or 4th instars, then unless some other character controlled by gene B is found which will enable the genotypes to be distinguished, the exact period over which these factors act will not be ascertainable.

It is interesting to note that the submission of broods to unfavourable conditions did not enhance the heterozygote excess. One theoretical explanation of this is that gametes containing different allelomorphs of B are more compatible than those containing similar alleles. If female gametes with b' were fertilised by male gametes with b^{o} more often than those with b' in situations where both b' and b^{o} male gametes were present, and similarly, if female gametes with b^{o} were fertilised more often by b' than b^{o} male gametes, a disproportionately high number of heterozygotes would result. The genotypes b'b', $b'b^{o}$ and $b^{o}b^{o}$, are not phenotypically distinct in the oval stage and so this hypothesis can not be tested directly. However, if zygote formation between gametes, containing the same allele of gene B, occurs less often than between gametes which have different alleles, then crosses between different homozygotes

might be expected to produce a greater number of fertile eggs, or at least a smaller proportion of infertile eggs, than broods where both parents were of the same genotype with respect to gene B. It should be noted that were this shown to be the case it would either support the partial incompatability hypothesis, or suggest that the heterozygous zygote was more viable in the very early stages of development, (i.e. until the larva hatched from the egg), than the homozygotes. On the other hand, if the proportion of infertile eggs was similar in all cases, the incompatability hypothesis can not be rejected, because the preference for fertilization to occur between non-like gametes may only produce a significant effect under conditions of competition, i.e. where both b' and b^{o} male gametes are present in the proximity of the female gametes.

Method

To investigate the hypothesis, the eggs laid by 120 females were counted as were the number of eggs which hatched from each of these broods. 40 of the broods (MSG1 - 40 inclusive), were the result of parents which were both homozygous for b'. A further 40 (MSB1 - 40 inclusive), were the result of parents, both of which were homozygous for b^{o}. The final 40 broods, (MSO1 - 40 inclusive) were the result of b'b' x $b^{o}b^{o}$ crosses.

Results

The results of these 120 broods are given in Table 6Cv.

Table 6Cv.

Investigation into gamete compatability and oval viability considered with respect to heterozygous advantage in gene B.

	Results from the MSG1 - 40 broods	Results from the MSO1 - 40 broods	Results from the MSB1 - 40 broods	Combined results from all 120 broods
Number of eggs laid	8379	8845	8801	26025
Number of eggs to hatch	8027	8397	8394	24818
Percentage of eggs laid to hatch	95.799	94.935	95.376	95.362
Percentage of eggs laid not to hatch	4.201	5.065	4.624	4.638
Mean number of eggs laid per brood	209.475	221.125	220.025	216.875
Mean number of eggs to hatch per brood	200.675	209.925	209.85	206.817

It may be seen from the results that the b'b' x $b^{o}b^{o}$ crosses produced slightly more eggs than the other types of cross. However, as the greatest difference in the totals was only 466, (i.e. between the b'b' x $b^{o}b^{o}$ broods and the b'b' x b'b' broods), and the number of eggs in individual broods varied from 24 to 504, this difference is not considered to be significant. Furthermore, the proportion of eggs which did not hatch was slightly greater in the b'b' x $b^{o}b^{o}$ broods, although not significantly so, ($X_2^2 = 0.172$, $0.95 > p > 0.9$).

Thus, these tests give no evidence to support the theory that heterozygote advantage with respect to gene B in P. meticulosa may be due to greater compatability between gametes which contain different allelomorphs of this gene when compared with gametes having similar alleles. However, as has already been noted, the results of these tests can not disprove the hypothesis. On the other hand, there is evidence that zygotes with $b'b^{o}$ are no more viable in the early stages of development than those containing $b^{o}b^{o}$ or b'b'.

D. The investigation of heterozygous advantage with respect to gene A.

The next problem considered was whether heterozygous advantage was maintaining the polymorphism based on the alleles of gene A.

As one of the alleles of gene A is completely dominant to the other allele, the homozygote dominant $a^{+}a^{+}$ and the

heterozygote a^+a are indistinguishable as all are Br in the 5th instar. Further, if the recessive allele a is homozygous, and is present with b^ob^o, these larvae again are brown in the 5th instar. Thus, to make a direct comparison of the ratios of heterozygotes to both homozygotes is not possible. However, in broods where the genotypes of the parents meet certain specifications, the number of larvae which are heterozygous or homozygous with respect to A may be ascertained. These specifications are that at least one of the parents must be homozygous for b', and that one of the parents must be homozygous for a whilst the other is heterozygous, i.e. a^+a. Under these conditions, all Br 5th instar progeny will be heterozygous for gene A whilst all Gr and OL 5th instar larvae will be homozygous for a.

Method

To determine whether heterozygous advantage is important in the maintenance of the polymorphism controlled by gene A, the results of ten such broods, reared using the basic rearing methods, were studied. Here again we are not seeking heterozygote advantage of a magnitude to cause significant deviations from the expected 1:1 ratios of heterozygotes (a^+a) to homozygotes (aa), but rather we are looking for a general trend in which the heterozygote is at a slight advantage such that this is only manifest when a number of broods are considered.

Results and analysis of results

The results of these ten broods are given in Table 6Di.

Table 6Di.

Investigation to determine whether the maintenance of the polymorphism controlled by gene A involves heterozygous advantage. 5th instar results of various IB and CC broods, the genotypes of the parents meeting the stipulations, outlined in chapter 6D.

Brood number	Genotype of male parents, allele of gene: B	A	Genotype of female parent, allele of gene: B	A	Phenotype of male parent	Phenotype of female parent	Number of eggs laid	Number of 5th instar larvae: non-brown (aa)	Br (a^+a)	Total	Whether the excess is of heterozygotes or homozygotes	Chi-squared values from a comparison of the number of aa and a^+a larvae taking a 1:1 expected ratio	Probability values (Degrees of freedom equal 1 throughout)
IB5	''	(+-)	''	(--)	4Gr 5Br	4Gr 5Gr	317	137	146	283	heterozygote	0.286	0.7 - 0.5
IB29	''	(+-)	''	(--)	4Gr 5Br	4Gr 5Gr	204	92	85	177	homozygote	0.277	0.7 - 0.5
CC4	''	--	''	+-	4Gr 5Gr	4Gr 5Br	78	34	37	71	heterozygote	0.127	0.8 - 0.7
CC8	''	--	oo	+-	4Gr 5Gr	4Br 5Br	174	74	69	143	homozygote	0.175	0.7 - 0.5
CC12	''	--	'o	+-	4Gr 5Gr	4OL 5Br	184	83	79	162	homozygote	0.099	0.8 - 0.7
CC13	''	--	''	+-	4Gr 5Gr	4Gr 5Br	312	142	151	293	heterozygote	0.276	0.7 - 0.5
CC14	''	--	oo	+-	4Gr 5Gr	4Br 5Br	179	71	76	147	heterozygote	0.170	0.7 - 0.5
CC16	''	--	''	+-	4Gr 5Gr	4Gr 5Br	284	122	137	259	heterozygote	0.869	0.5 - 0.3
CC21	''	--	''	+-	4Gr 5Gr	4Gr 5Br	301	149	136	285	homozygote	0.593	0.5 - 0.3
CC30	''	+-	''	--	4Gr 5Br	4Gr 5Gr	131	51	58	109	heterozygote	0.450	0.7 - 0.5
Totals								955	974	1929	heterozygote	0.187	0.7 - 0.5

Comparisons were made between the number of aa larvae and the number of a^+a larvae, using chi-squared tests, taking the expected ratios between the two genotypes to be 1:1. The chi-squared and probability values from these tests are also given in Table 6Di.

The results show that in six of the ten broods, more heterozygotes were produced, whilst in the other four broods there was an excess of homozygotes. Furthermore, none of the results differed significantly from the expected 1:1 ratio, and a comparison between the total number of each of the two genotypes in the ten broods also produced a non-significant result.

Conclusion

These results indicate that the heterozygote a^+a is not at an advantage over the homozygote aa.

E. The investigation of heterozygous advantage with respect to gene C.

Finally, we turn to a consideration of the possibility of heterozygote advantage maintaining the polymorphism controlled by gene C. Here we have a similar situation to that just mentioned in connection with gene A, in that one of the alleles of gene C is completely dominant to the other, with the result that the heterozygote c^+c is not phenotypically distinguishable from the homozygous dominant c^+c^+, as both these genotypes produce green 3rd instar larvae. Again, the

situation is complicated by the fact that some larvae which are homozygous recessive, i.e. cc, are not recognisably different from those with c^+ in the genotype, this situation arising when larvae are ccb'b' and, thus, again green. However, again this difficulty can be overcome by considering broods where the genotypes of the parents comply with certain conditions, these being that at least one parent is homozygous for b^o, and that one parent is homozygous recessive for gene C whilst the other is heterozygous. These conditions ensure that all 3rd instar green progeny are heterozygous for gene C whilst any olive or brown larvae are homozygous recessive for this gene, and that a 1:1 ratio of c^+c larvae to cc larvae should result.

Method

Ten broods were reared using the basic rearing methods. These were named the "heterozygote advantage gene C broods", (HGC1 - 10 inclusive). For each of these broods one parent was homozygous for b' whilst the other was homozygous for b^o, with the result that all the progeny were $b'b^o$, which produces olive when the alleles of gene B are expressed phenotypically.

Results and analysis of results

The results of the HGC broods are given in Table 6Ei.

Comparisons were made between the number of cc larvae and the number of c^+c larvae using chi-squared tests and taking the expected ratios between the two genotypes to be

Table 6Ei.

Investigation to determine whether the maintenance of the polymorphism controlled by gene C involves heterozygous advantage. 3rd instar results of the HGC broods.

Brood number	Genotype of male parent, allele of gene: C	B	Genotype of female parent, allele of gene: C	B	Phenotype of male parent	Phenotype of female parent	Number of eggs laid	Number of 3rd instar larvae of colours: Gr (with c^+c)	OL (with cc)	Total	Whether the excess is of heterozygotes or homozygotes	Chi-squared values from a comparison of the numbers of Gr and OL larvae taking a 1:1 expected ratio	Probability values (Degrees of freedom equal 1 throughout
HGC1	--	''	+-	oo	3Gr 4Gr	3Gr 4Br	197	103	34	187	Heterozygote	1.930	0.2 - 0.1
HGC2	--	''	+-	oo	3Gr 4Gr	3Gr 4Br	284	134	112	246	Heterozygote	1.967	0.2 - 0.1
HGC3	--	''	+-	oo	3Gr 4Gr	3Gr 4Br	71	37	25	62	Heterozygote	2.323	0.2 - 0.1
HGC4	+-	''	--	oo	3Gr 4Gr	3Br 4Br	49	19	17	36	Heterozygote	0.111	0.8 - 0.7
HGC5	+-	''	--	oo	3Gr 4Gr	3Br 4Br	465	232	186	418	Heterozygote	5.062	0.05 - 0.02
HGC6	+-	oo	--	''	3Gr 4Br	3Gr 4Gr	196	76	86	162	Homozygote	0.617	0.5 - 0.3
HGC7	+-	oo	--	''	3Gr 4Br	3Gr 4Gr	270	128	109	237	Heterozygote	1.523	0.3 - 0.2
HGC8	+-	oo	--	''	3Gr 4Br	3Gr 4Gr	160	81	60	141	Heterozygote	3.128	0.1 - 0.05
HGC9	--	oo	+-	''	3Br 4Br	3Gr 4Gr	241	118	83	201	Heterozygote	6.094	0.02 - 0.01
HGC10	--	oo	+-	''	3Br 4Br	3Gr 4Gr	381	187	154	341	Heterozygote	3.194	0.1 - 0.05
Totals								1115	916	2031	Heterozygote	19.498	< 0.001

1:1 for all the broods. The chi-squared and resulting probability values from these tests are also given in Table 6Ei.

The results show that, in nine of the ten HGC broods, the heterozygotes outnumbered the homozygotes, and, in two of the broods, the excesses of heterozygotes were significant at the 5% level. In the one brood where there were more homozygotes than heterozygotes, (HGC6), the excess was not significant. When the total numbers of each of the two genotypes in the ten broods were compared, the result produced was highly significant, ($X^2_1 = 19.498$, $p < 0.001$).

Discussion

The results of these tests indicate that the heterozygote c^+c is at a definite advantage over the homozygote cc. It is difficult to ascertain whether c^+c is also at an advantage over the dominant homozygote c^+c^+, because these two genotypes are indistinguishable phenotypically and because, as the results of the HGC broods show, the advantage of c^+c over cc shows considerable inter-brood variation. Possibly the degree of departure from the 3:1 ratio expected in $c^+c \times c^+c$ crosses could be compared with the departure from a 1:1 ratio expected in $c^+c \times cc$ crosses, but due to the variation in the degree of heterozygous advantage in different broods, the experiments would have to be very extensive to give a definite indication one way or the other.

Evidence indicating whether c^+c is at an advantage to c^+c^+ is needed before the question of whether the

polymorphism based on gene C is maintained by heterozygote advantage can be given a satisfactory answer. However, it may be noted that, unlike the situation with respect to the alleles of genes B and A, the frequencies of the alleles c^+ and c are relatively constant throughout the year, and the slight fluctuations which do occur may be due at least in part to the method by which the frequencies of c^+ and c were calculated (see chapter 5). Thus, in this case there is no indication, as there are in the cases of genes B and A, that there must be other selective forces of considerable magnitude acting on the allelic frequencies which cause seasonal fluctuations in the frequencies.

CHAPTER 7. EXPERIMENTS ON SELECTION BY ORGANISMS WHICH KILL LARVAE: (a) ORGANISMS WHICH DO NOT USE COLOUR-VISION TO FIND LARVAE

A. Introduction

When considering the mechanisms involving predators or other organisms which may maintain the larval colour polymorphism in P. meticulosa, it is tempting to search for a correlation between larval colour and predation by organisms which have colour vision. However, the possibility that the polymorphism may be controlled by predators, other organisms such as parasites, or viral and bacterial diseases, which do not use colour vision to find larvae, must not be overlooked. This chapter deals with tests to investigate some selection mechanisms of this type.

It has already been noted that morphological characters, by which the forms of a polymorphic species are often distinguished, may not necessarily be of any adaptive significance, as in the vast majority of cases genetic switch mecharisms seem to have a multiple effect. Therefore, it is conceivable that the genes involved in the larval colour polymorphism in P. meticulosa may affect such factors as scent, defence behaviour, immunity to certain diseases, or at least reduced susceptibility to some diseases, and acceptibility to predators with respect to taste, all of which may give one particular genotype a selective advantage over others. This advantage, whilst being correlated with colour, will not be due to it. The fact that many predators search for food without using colour vision does not necessarily mean that they will not selectively affect the

colours of the prey directly. They may do this by selecting for the tone of the prey. Ford (1964) notes that C. nemoralis may be preyed upon by rodents which, although lacking colour vision, may select for tone. Cain (1953) also notes that rabbits are colour blind (Walls 1942), and suspects that they exert visual selection by tone on Cepaea.

The effects of a gene need not be confined to one stage in the life-cycle of an insect (see chapter 6 page 267), and thus, selection at more than one stage in the life-cycle may affect gene frequency. On the other hand, a comparison of AF1 sample data with that from the AF2 samples (chapter 5), indicates that the selective factors causing the fluctuations in the frequencies of the alleles of gene A and gene B act during the larval stage, although they may also act at other stages.

Four causes of larval mortality, due to organisms which do not seem to use colour vision to find larvae were considered. These were:

i) Death caused by viral disease.
ii) Death caused by insect (hymenopteran and dipteran) parasitism.
iii) Death due to beetle predation.
iv) Death due to mammal predation.

B. Experimental studies of selection by viral disease.

One of the most common causes of death in laboratory stocks of Lepidoptera larvae is that due to viral disease, and Ford (1955) states that, without a doubt, virus disease constitutes the most serious menace to many species of Lepidoptera. Any

breeder of butterflies and moths will be familiar with the symptoms of viral infection which usually develop in the later larval instars. The infected larvae become limp and pass liquid from both the mouth and the anus. The condition seems to be fatal in all cases once the symptoms become apparent, and death usually follows within 72 hours, (personal observation). The excreta are highly infective, and, consequently, rearing larvae in crowded conditions, or failure to remove frass from rearing boxes, tends to spread an epidemic, as is indicated by some of the results of the HEB and ELB samples (see chapters 6C and 3Bc; and Tables 6Ciii and 3Bc2/i), the former samples being reared in very crowded conditions, and the latter being reared without removing frass from the rearing boxes.

The tests carried out were designed to determine whether viral disease affected larvae of different genotypes to similar or different extents.

Method

The method used was to purposely expose uninfected larvae to conditions under which they might come into contact with infected larvae, the duration of the tests being such that up to 50% became infected.

Three pairs of samples of 150 and one pair of samples of 200 larvae, of known genotypes, were made up from eggs of a number of broods. The samples were named the "viral infection samples" (VIS1 - 8 inclusive). The genotypes of larvae in each of the samples are given in Table 7Bi. The samples were reared

Table 7Bi.

Experimental studies of selection by viral disease. Numbers of larvae in samples VIS1 - 8 inclusive which became infected and died after being exposed to larvae infected with a viral disease. Included are the genotypes of the larvae in each sample, and the contingency chi-squared and probability values from tests to compare the number of larvae of each genotype, within a particular sample, which became infected.

Sample Number	Gene being investigated	Genotype. Alleles of gene: C	B	A	D	E	Phenotype of larvae	Instar in which larvae were exposed to virus infection	Number of larvae to become infected	Chi-squared and probability values comparing the number of larvae of each genotype which became infected. Chi-squared value	Degrees of freedom	Probability value
VIS1	C	++	oo	--	+ or +		3Gr 4Br 5Br	2nd	15	1.735	2	0.5 - 0.3
		+-	oo	--	+ or +		3Gr 4Br 5Br		19			
		--	oo	--	+ or +		3Br 4Br 5Br		13			
									Total 47			
VIS3	B	++	''	--	+ or +		3Gr 4Gr 5Gr	2nd	16	1.630	2	0.5 - 0.3
		++	'o	--	+ or +		3Gr 4OL 5OL		16			
		++	oo	--	+ or +		3Gr 4Br 5Br		11			
									Total 43			
VIS5	A	+x	''	++	+ or +		3Gr 4Gr 5Br	2nd	12	2.381	2	0.5 - 0.3
		+x	''	+-	+ or +		3Gr 4Gr 5Br		12			
		+x	''	--	+ or +		3Gr 4Gr 5Gr		18			
									Total 42			
VIS7	D and E	--	'o	--	+ or +		3OL 4OL 5OL	2nd	20	1.900	3	0.7 - 0.5
		--	'o	--	--	'o	3PY 4PY 5PY		14			
		--	'o	--	--	oo	3YG 4YG 5YG		17			
		--	'o	--	--	''	3YB 4YB 5YB		15			
									Total 66			
VIS2	C	++	oo	--	+ or +		3Gr 4Br 5Br	3rd	15	7.637	2	0.05 - 0.02
		+-	oo	--	+ or +		3Gr 4Br 5Br		14			
		--	oo	--	+ or +		3Br 4Br 5Br		26			
									Total 55			
VIS4	B	++	''	--	+ or +		3Gr 4Gr 5Gr	4th	27	0.673	2	0.8 - 0.7
		++	'o	--	+ or +		3Gr 4OL 5OL		26			
		++	oo	--	+ or +		3Gr 4Br 5Br		23			
									Total 76			
VIS6	A	+x	''	++	+ or +		3Gr 4Gr 5Br	5th	27	1.920	2	0.5 - 0.3
		+x	''	+-	+ or +		3Gr 4Gr 5Br		21			
		+x	''	--	+ or +		3Gr 4Gr 5Gr		27			
									Total 75			
VIS8	D and E	--	'o	--	+ or +		3OL 4OL 5OL	3rd	26	2.083	3	0.7 - 0.5
		--	'o	--	--	'o	3PY 4PY 5PY		22			
		--	'o	--	--	oo	3YG 4YG 5YG		29			
		--	'o	--	--	''	3YB 4YB 5YB		27			
									Total 104			

using the basic rearing methods until they were to be exposed to the infected larvae. All the larvae from a particular sample were put into a 0.6 m x 0.5 m x 0.175 m perspex box with muslin covered aeration holes. If, at this point, any of the genotypes in a sample were not phenotypically distinguishable, white cellulose paint marks were used to ensure the genotype of each larva was recognisable. In samples where paint marks were used, all larvae were marked, the different genotypes being rendered distinguishable by placing marks for a particular genotype on specific segments. Similar marking techniques were used in the experimental studies of selection by carabids and hedgehogs (chapters 7D and 7E).

One of each of the pairs of samples was exposed to infected larvae whilst in the 2nd instar, the other being exposed during one of the instars in which the colour effects of the alleles of the gene being considered were expressed phenotypically.

The uninfected larvae were exposed to infected larvae by introducing one infected 5th instar larva, of each of the genotypes in a particular sample, into the box which contained that sample. Thus, samples VIS1 - 6 inclusive were each exposed to 3 infected larvae, and samples VIS7 - 8 were exposed to 4 infected larvae. The infected larvae used, had all developed the symptoms of viral infection less than 24 hours before their use, and, whenever possible, in larvae used for a particular test, the symptoms of viral disease had become apparent almost simultaneously.

The infected larvae, which were rendered recognisable by marking them with red paint, were removed from the boxes after 24 hours[1] and the samples were then completely split so that all larvae were reared individually after exposure. Throughout, larvae were fed on sorrel which had been carefully washed. Great care was taken at all times to prevent larvae in the samples from accidentally coming into contact with infected larvae either directly or indirectly.

Results and analysis of data

The numbers of larvae of each genotype which died in each of the eight samples are given in Table 7Bi.

Comparing these figures for each sample using contingency chi-squared tests gave non-significant results in most cases, (these chi-squared values being given in Table 7Bi); however, in the case of sample VIS2, the result was significant, the probability value being between 0.05 and 0.02. Furthermore, a chi-squared test comparing the total number of larvae which died from each sample gave a highly significant result ($X^2_7 = 30.289$, $p < 0.001$). By inspection, it seems that the number of larvae infected in those samples exposed to the diseased larvae whilst in the 2nd instar was lower than in those exposed in later instars. Chi-squared tests showed that there was no significant difference in the number of larvae to become infected in each sample of the former group, (i.e. VIS1,

[1] Preliminary tests had shown that a period of between 20 and 35 hours was most suitable to produce a 50% incidence of infection using 4 infected 5th instar larvae with a sample of 200 uninfected 5th instar larvae.

3, 5 and 7) (X^2_3 = 0.923, $0.9 > p > 0.8$), nor in the number to become infected in each sample of the latter group, (i.e. VIS2, 4, 6 and 8) (X^2_3 = 5.037, $0.2 > p > 0.1$). On the other hand, if the total number of larvae to be infected in each of these two sets of samples are compared, the difference is highly significant (X^2_1 = 24.693, $p < 0.001$).

Two explanations for these results seem plausible. Firstly, larvae in the 2nd instar may be less susceptible to infection than those in the later instars because of some physiological mechanism. Secondly, 2nd instar larvae may be infected less often than those in later instars because they behave in a different way. In chapter 4 it was shown that larvae in which colour is foodplant controlled are positively phototactic, whilst those in which colour is not affected by foodplant are negatively phototactic. As all the larvae were reared in normal light conditions, larvae which were negatively phototactic would thus be likely to move more during a 24 hour period than those which were positively phototactic, as they would migrate downwards in the boxes at dawn to "hide" below the leaves of the foodplant, and return to the higher foodplant layers at or after dusk, while the positively phototactic larvae remain in the upper layers of the food. As all the infected larvae introduced into the boxes were in their 5th instar, these would be negatively phototactic. This would mean that infected larvae would tend to spend a greater part of the tests in the proximity of, and be more likely to infect, the 3rd instar cc larvae, or larvae in the 4th or 5th instars, than those in the 2nd instar, or 3rd instar larvae with c^+c^x in their

genotype. The results of VIS2, which show that the proportion of cc larvae which were infected was greater than the proportion of either c^+c^+ or c^+c larvae to be infected, imply that this second theory is the more probable. In sample VIS2, one of the three genotypes, cc, produces larvae which are negatively phototactic, the other two producing positively phototactic 3rd instar larvae. Thus, it would be expected that cc larvae would be infected more than the latter two types, which should be equally liable to infection. Indeed, if the numbers of cc larvae to be infected and uninfected are compared, using a two by two contingency chi-squared test, with the sums of the c^+c^+ and c^+c larvae to be infected and uninfected, then a highly significant result is produced (X^2_1 = 7.600, $0.01 > p > 0.001$).

In conclusion, it seems that none of the genes which control larval colour affect the degree of susceptibility to viral disease except gene C. The advantage conferred upon larvae with c^+ over those homozygous for c may well be due to the difference in behaviour of larvae with or without the allele c^+. Moreover, if this is so, it is probable that viral disease confers a selective advantage upon allele c^+ compared with its allelomorph c in the wild with respect to viral disease, as the behaviour of larvae with respect to the alleles of gene C in the wild, seems to be similar to that noted in the laboratory. The selection due to viral disease should then produce a transient polymorphism, with the frequency of c^+ rising whilst that of c falls, if viral disease is the only selective factor which affects the genotypes c^+c^+, c^+c and

cc differently. However, as chapter 5 has indicated that the polymorphism based on gene C is balanced, it is likely that there is some other selective factor which balances the advantage conferred on c^+ by viral disease, if this is relevant to field conditions.

C. Experimental studies of selection by parasites (and viral disease).

Apart from virus diseases, probably the most serious menace to which many species of Lepidoptera are exposed, is that due to parasitic insects. These belong to two super-families, the Chalcidoidea and the Ichneumonidea. The second of these is again divided, between the true Ichneumons (family Ichneumonidae) and the closely related Braconids (family Braconidae).

Moss (1933), who made a study of the braconid Apanteles glomeratus L. in South Buckinghamshire, found that 84.2% of the 3026 caterpillars of the Large White butterfly (P. brassicae) that he studied, harboured this parasite. Dowdeswell (1961) found, from a series of elegantly designed experiments on natural selection in Maniola jurtina L., that selection pressures of the order of 70% operate, during the last seven weeks of pre-imaginal life, against larvae and pupae destined to give rise to females of two or more spots, and that the principal selective agent responsible for the reduction in spotting under natural conditions is the bracionid parasite Apanteles tetricus Reinhard.

It thus appears that the mortality rate in Lepidoptera due to insect parasites may be very high, and therefore, if

these insects parasitise the different genotypes of P. meticulosa to different extents, and the overall density of parasites varies with season, the seasonal variation in the allelic frequencies of genes A and B could be wholly or partly explained by differential parasitism.

The tests carried out were designed to discover whether the death rate of larvae due to hymonopteran and dipteran parasites varied with season and larval genotype. The data from these tests also provided further information on selection by viral disease.

Method

Six samples of larvae were obtained from wild populations near Cadnam in Hampshire[1] by sweep-netting at night. These samples were named the "parasite selection experiment samples" (PE1 - 6 inclusive). Three samples, (PE1, 2 and 3) were collected during March and April, 1977, the other three (PE4, 5 and 6) being collected during July and August, 1977. Larvae of samples PE1 and PE4 were in the 3rd instar, those of PE2 and PE5 were in the 4th instar, and larvae of samples PE3 and PE6 were in the 5th instar. The samples obtained were reared using the basic rearing methods, except that all were reared singly, and the containers used for rearing varied in size and shape.

The phenotypes of all the larvae were recorded in each instar, and from this data the genotype of each larva was deduced as far as possible. The number of larvae of each

[1] Cadnam is approximately 12 km north-west of Ringwood.

genotype which died,was recorded together with the apparent cause of death and the stage at which they died.

Results and analysis of data

The data thus collected is tabulated in Tables 7Ci and 7Cii. Three classes of mortality were recognised. The first of these was that due to virus disease, (for symptoms see chapter 7B). The second was that due to insect parasitism, diagnosed by the emergence of parasites. Both hymenopteran and dipteran species were found to parasitise the larvae of P. meticulosa, the former group doing so more frequently. However, as tests were designed to discover the overall effect of insect parasitism on the frequencies of the larval genotypes, the taxonomic groups of parasites are not differentiated in these tables.

The third class of mortality was named "others", and included all larvae or pupae whose death was thought to be due to other reasons such as fungal infection or mishandling.

Table 7Ciii gives the proportions of larvae of certain genotypes which died from viral or parasitic infection, larvae collected in different instars being treated separately. In each case,the figure given refers to the number of larvae of a particular type that died, due to viral disease, or parasitic attack, as a percentage of the total number of larvae known to be of that genotype. The proportions of yellow larvae which died are not given as the numbers of yellow larvae in the samples were far too small to give results of any great value.

The data collected yielded a large amount of data on a

Table 7Ci.

Experimental studies of selection by insect parasites (and viral disease). Numbers of larvae in samples PE1 - 3 inclusive which died due to either parasitic attack or viral disease or other causes. (Samples collected by sweep-netting during March and April, 1977).

Sample number	Instar in which larvae were collected	Phenotype of larvae in instar 3	4	5	Deduced genotype of larvae alleles of gene: C	B	A	D	E	Number of larvae collected	Number of larvae to reach the 3rd larval ecdysis	Number of larvae at the start of the 4th instar	Number of larval deaths during the 4th instar due to: viral disease	parasite attack	other causes	Number of larvae to reach the 4th larval ecdysis	Number of larvae at start of the 5th instar	Number of larval deaths after start of 5th instar due to: viral disease	parasite attack	other causes	Number of imagines to emerge
PE1	3rd	Gr	Gr	Gr	X	''	--	+	or +	194	161	83	3	0	1	79	35	7	6	1	21
				Br	X	''	+x	+	or +								44	10	7	0	27
			OL	OL	+x	'o	--	+	or +			61	1	0	0	60	26	4	4	0	18
				Br	+x	'o	+x	+	or +								34	6	6	0	22
			Br	Br	+x	oo	X	+	or +			16	1	0	0	15	15	3	2	1	9
			Y	Y	+x	X	X	--	--			1	0	0	0	1	1	0	0	0	1
		OL	OL	OL	--	'o	--	+	or +		23	23	3	0	0	20	7	2	0	0	5
				Br	--	'o	+x	+	or +								13	5	1	1	6
		Br	Br	Br	--	oo	X	+	or +		6	6	0	0	0	6	6	2	0	0	4
		Y	Y	Y	--	X	X	--	--		2	2	0	0	1	1	1	0	0	0	1
Totals											192	192	8	0	2	182	182	39	26	3	114
PE2	4th	-	Gr	Gr	X	''	--	+	or +	391		164	5	0	1	158	68	18	17	1	32
		-		Br	X	''	+x	+	or +								90	27	23	2	38
		-	OL	OL	X	'o	--	+	or +			176	3	0	2	171	71	19	21	1	30
				Br	X	'o	+x	+	or +								100	26	28	1	45
		-	Br	Br	X	oo	X	+	or +			43	1	0	1	41	41	12	10	0	19
		-	Y	Y	X	X	X	--	--			8	0	0	0	8	8	3	1	0	4
Totals												391	9	0	4	378	378	105	100	5	168
PE3	5th	-	-	Gr	X	''	--	+	or +	213							35	7	9	0	17
		-	-	OL	X	'o	--	+	or +								35	8	8	1	18
		-	-	Br	X	oo	or +x	+	or +								140	35	32	3	70
		-	-	Y	X	X	X	--	--								3	0	0	0	3
Totals																	213	50	49	4	103

Table 7Cii.

Experimental studies of selection by insect parasites (and viral disease). Numbers of larvae in samples PE4 - 6 inclusive which died to either parasitic attack or viral disease or other causes. (Samples collected by sweep-netting during July and August, 1977).

Sample number	Instar in which larvae were collected	Phenotype of larvae in instar: 3	4	5	Deduced genotype of larvae alleles of gene: ~ C	B	A	D	E	Number of larvae to reach the 4th larval ecdysis	Number of larvae at start of the 5th instar	Number of larval deaths after start of 5th instar due to: viral disease	parasite attack	other causes	Number of imagines to emerge	Number of larvae collected	Number of larvae to reach the 3rd larval ecdysis	Number of larvae at the start of the 4th instar	Number of larval deaths during the 4th instar due to: viral disease	parasite attack	other causes
PE4	3rd	Gr	Gr	Gr	X	''	--	+	or +	62	21	4	5	1	11	149	127	65	2	0	1
				Br	X	''	+x	+	or +		41	8	11	1	21						
			OL	OL	+x	'o	--	+	or +	42	18	5	5	0	8			45	2	0	1
				Br	+x	'o	+x	+	or +		24	4	3	2	15						
			Br	Br	+x	oo	X	+	or +	14	14	3	5	0	6			15	0	0	1
			Y	Y	+x	X	X	--	--	1	1	0	0	0	1			1	0	0	0
		OL	OL	OL	--	'o	--	+	or +	16	6	1	1	0	4		18	17	1	0	0
				Br	--	'o	+x	+	or +		10	4	1	0	5						
		Br	Br	Br	--	oo	X	+	or +	3	3	1	0	0	2		4	4	1	0	0
		Y	Y	Y	--	X	X	--	--	0	0	0	0	0	0		0	0	0	0	0
Totals										138	138	30	31	4	73		149	147	6	0	3
PE5	4th	-	Gr	Gr	X	''	--	+	or +	152	64	17	14	1	32	367		157	4	0	1
		-		Br	X	''	+x	+	or +		88	18	21	2	47						
		-	OL	OL	X	'o	--	+	or +	156	67	14	16	1	36			164	6	0	2
		-		Br	X	'o	+x	+	or +		89	23	18	1	45						
		-	Br	Br	X	oo	X	+	or +	37	37	8	6	0	23			38	0	0	1
		-	Y	Y	X	X	X	--	--	8	8	2	1	0	5			8	0	0	0
Totals										353	353	82	76	5	188			367	10	0	4
PE6	5th	-	-	Gr	X	''	--	+	or +		30	6	6	0	18	174					
		-	-	OL	X	'o	--	+	or +		34	8	6	1	19						
		-	-	Br	X	oo	or +x	+	or +		106	22	19	1	64						
		-	-	Y	X	X	X	--	--		4	1	1	0	2						
Totals											174	37	32	2	103						

Table 7Ciii.

Experimental studies of selection by insect parasites (and viral disease). Proportions of larvae of certain genotypes which died from viral disease or parasitic attack.

Cause of death and genotype	Samples on which proportion is based. Figures given as percentages PE1	PE2	PE3	PE4	PE5	PE6
Viral disease in known cc larvae	36.4			38.10		
Viral disease in known c^+c^x larvae	18.75			22.95		
Parasitic attack in known cc larvae	3.03			9.52		
Parasitic attack in known c^+c^x larvae	15.00			21.31		
Overall mortality in known cc larvae	42.4			47.62		
Overall mortality in known c^+c^x larvae	37.5			49.18		
Viral disease in known b'b' larvae	24.10	30.49	20.00	21.54	24.84	20.00
Viral disease in known $b'b^o$ larvae	24.42	27.27	22.86	27.42	26.22	23.53
Viral disease in known b^ob^o larvae	22.73	30.23		26.32	21.05	
Parasitic attack in known b'b' larvae	15.66	24.39	25.71	24.62	23.57	20.00
Parasitic attack in known $b'b^o$ larvae	12.79	27.84	22.86	16.13	20.73	17.65
Parasitic attack in known b^ob^o larvae	9.09	23.26		26.32	15.79	
Overall mortality in known b'b' larvae	42.17	55.70	51.43	50.77	49.68	40.00
Overall mortality in known $b'b^o$ larvae	39.29	56.14	51.43	48.39	50.61	44.12
Overall mortality in known b^ob^o larvae	38.10	53.66		57.89	39.47	

Table 7Ciii. (Continued)

Experimental studies of selection by insect parasites (and viral disease). Proportions of larvae of certain genotypes which died from viral diseases or parasitic attack.

Cause of death and genotype	Samples on which proportion is based. Figures given as percentages PE1	PE2	PE3	PE4	PE5	PE6
Viral disease in known aa larvae	19.12	26.62	21.43	22.22	23.66	21.88
Viral disease in known a^+a^x larvae	23.08	26.84		24.44	23.16	
Viral disease in known a^+a^x or b^ob^o larvae			25.00			20.75
Parasitic attack in known aa larvae	14.71	27.34	24.29	21.33	22.90	18.75
Parasitic attack in known a^+a^x larvae	15.39	26.84		20.00	22.03	
Parasitic attack in known a^+a^x or b^ob^o larvae			22.86			17.92
Overall mortality in known aa larvae	35.29	55.40	50.00	48.89	48.09	42.19
Overall mortality in known a^+a^x larvae	36.26	56.32		45.33	44.00	
Overall mortality in known a^+a^x or b^ob^o larvae			50.00			39.62
Overall mortality due to viral disease	24.23	29.16	23.47	24.49	25.07	21.26
Overall mortality due to parasitic disease	13.40	25.58	23.01	21.09	20.71	18.39
Overall mortality due to all causes	39.69	57.03	48.36	50.34	48.77	41.95

number of topics, from the ecology of parasitic Hymenoptera and Diptera to the frequencies of some of the alleles and genotypes controlling larval colour in P. meticulosa. However, only those topics relating directly to the maintenance of the larval colour polymorphism will be dealt with in detail. Suffice it to say, allelic and phenotypic frequency estimates based on the samples collected in the Cadnam area are in complete accordance with those from the AF2 data, and thus suggest that the proportions of each of the larval colour-types in the Cadnam populations are similar to those of populations in the Ringwood area.

Looking at the proportions of the various genotypes in the samples from the Cadnam area which suffered from viral disease, it may be seen that in the samples collected during March and April, the proportion of the infected larvae was greatest in those collected whilst in the 4th instar. The same is true for the samples taken during July and August, but to a lesser extent. The increase in infection between larvae collected in the 3rd and 4th instars seems to be due to the low number of 3rd instar larvae with c^+ in their genotype which were infected. The reduction in the proportion of infected larvae in samples PE3 and PE6, when compared with PE2 and PE5 respectively, is probably due to the fact that the majority of infected larvae died in the 5th instar, and therefore, a substantial number of infected larvae may have died in the wild early in the 5th instar. In general the proportion of larvae which were infected was lower in the spring samples than in the late summer samples.

Only a small part of the genotype of many of the larvae could be deduced from the phenotypic information obtained from the samples. This fact, coupled with the small size of the samples, makes the validity of the deductions drawn from the data open to question, particularly when dealing with larval colour-types, which are rare in some instars, such as Br in the 4th instar or Gr in the 5th instar.

That said, it seems that the alleles of genes A and B do not substantially affect the incidence of viral infection. On the other hand, as has already been shown in laboratory tests, (see chapter 7B), the alleles of gene C do appear to affect the incidence of viral infection. In sample PE1, about 36% of the known cc larvae were infected compared with approximately 19% of known c^+c^x larvae, the equivalent figures from PE4 being 38% and 23% respectively.

It has already been noted (chapter 7B) that the reason for these differences is thought to be related to the behaviour of the two types of larvae. However, it is notable that larvae with c^+ in their genotype seem to be favoured more in the field than they were in the laboratory tests, a feature which is probably due to the limitation placed on the movement of larvae reared in boxes in the laboratory.

Considering the proportion of larvae which died due to attack by insect parasites; the data from PE1 produced a value which was considerably lower than that from either PE2 or PE3, both of which gave similar results with the value from PE2 being slightly higher than that from PE3. The values from the

three samples collected during July and August were all around 20%.

When considering the level of parasitism in relation to particular genotypes of genes A and B, the differences in the proportion of larvae of different genotypes are small, but one or two trends may be noted. The proportions of $b'b^{o}$ larvae parasitised in PE1, 4 and 5 were lower than the levels in b'b' larvae; however, in PE2 the reverse was true. Similarly, the proportions of $b^{o}b^{o}$ larvae which were parasitised were lower than those of the $b^{o}b'$ larvae in samples PE1, 2 and 5, the reverse being true in PE4. If the proportions of parasitised $b^{o}b^{o}$ larvae are compared with those of parasitised b'b' larvae, again the $b^{o}b^{o}$ values are lower in PE1, 2 and 5 but higher in PE4. The proportion of $a^{+}a^{x}$ and aa larvae which were parasitised were in all cases very similar in a particular sample. In general though, the allelomorphs of genes A and B do not seem to affect the level of parasitism differently.

On the other hand, the levels of parasitism in $c^{+}c^{x}$ and cc larvae do vary considerably in some instars. In samples PE1 and 4, the $c^{+}c^{x}$ larvae were parasitised far more heavily than the cc larvae. These figures may be unrealistic, being biased by the small number of larvae known to be cc; however, it is notable that, in both cases, these differences are greater than the differences with respect to any other genotypes.

It is also interesting to note that whilst virus infection favours $c^{+}c^{x}$ larvae over cc larvae, insect parasites tend to attack $c^{+}c^{x}$ larvae more than cc larvae and these two factors seem to virtually balance one another out, so that if

the overall mortality in c^+c^x and cc larvae is considered, then in PE1, 37.5% of c^+c^x larvae died whilst 42.4% of cc larvae died. In PE4 these figures were even more similar, 49.18% of c^+c^x larvae dying and 47.619% of cc larvae dying. However, the fact that in sample PE1 the c^+c^x larvae were at an advantage over cc larvae, whilst in sample PE4 the reverse was the case, would cause changes directly opposed to the slight seasonal variations which occur in the allelic frequencies of c^+ and c. One possible reason for the greater incidence of parasite infection in c^+c^x larvae is that these larvae remain in the higher exposed layers of the herbage strata during the day when in the 3rd instar, whilst the cc larvae migrate down to the lower herbage layers. Thus, the c^+c^x larvae may be more exposed to those parasites which fly in the day time than the cc larvae.

D. Experimental studies of selection by Carabid beetles.

Carabid beetles, particularly the larger species, are known to prey upon Noctuid larvae (personal observation). Little work has been done upon the way in which these insects hunt for food; however, Mason (1972) indicates that the majority are nocturnal and that they find food by accidental encounters and recognise it by touch and possibly scent. Two of the largest species which are known to hunt and feed in grassland are Carabus nemoralis Müll. and Carabus violaceus L. (Alexander personal communication). Tests were carried out to find out whether these two species preferentially prey on any particular genotype.

Method

40 beetles of each of the two Carabid species mentioned previously were collected from the wild and were kept in round perspex boxes with a 105 mm diameter. On four consecutive evenings each of these beetles was offered three or four larvae of specific genotypes, so that by the end of four nights each beetle had been offered 13 larvae, (one of each of the genotypes given in Table 7Di). The larvae offered to each beetle on a specific night were monogenotypic for all genes except one (or in the case of genes D and E, two) so that each test was designed to discover whether the beetles favoured one or other of the genotypes at a particular locus (or loci). The orders in which samples were offered to Carabids were decided by random number tables. In each test the genotype of the first larvae to be eaten was recorded. All the beetles were used for the first tests within three days of being captured. All the tests were carried out at night, and moonlight or a very dim electric light was used by which to observe the beetles and larvae. Again, where the genotypes of larvae in a particular sample were not distinguishable phenotypically, all the larvae in that particular sample were marked using the method described in chapter 7B.

Results and analysis of data

In all the tests the beetles attacked one of the larvae within 25 minutes of the larval samples being placed in the boxes with the beetles.

The results of the tests are given in Table 7Di.

Table 7Di.

Experimental studies of selection by carabid beetles. The number of larvae of a specific genotype attacked and eaten first by Carabus nemoralis or Carabus violaceus when 3 or 4 larvae of different genotypes were offered to the beetles.

Test letter and number of trials	Gene to which tests specifically relate	The genotypes of the larvae offered to the beetles with respect to genes: C	B	A	D	E	Instar when larvae were offered	The phenotype of larvae when offered	Number of larvae of each genotype taken first by C. nemoralis	Number of larvae of each genotype taken first by C. violaceus	Total
V	C	(++	oo	--	+ or +		3rd	Gr	2	3	5
20 trials		(+-	oo	--	+ or +		3rd	Gr	4	3	7
		(--	oo	--	+ or +		3rd	Br	4	4	8
W	B	(++	''	--	+ or +		4th	Gr	4	2	6
20 trials		(++	'o	--	+ or +		4th	OL	3	3	6
		(++	oo	--	+ or +		4th	Br	3	5	8
Y	A	(+x	''	++	+ or +		5th	Br	3	3	6
20 trials		(+x	''	+-	+ or +		5th	Br	3	5	8
		(+x	''	--	+ or +		5th	Gr	4	2	6
Z	D and E	(--	'o	--	+ or +		5th	OL	2	2	4
20 trials		(--	'o	--	--	'o	5th	PY	5	2	7
		(--	'o	--	--	oo	5th	YG	1	4	5
		(--	'o	--	--	''	5th	YB	2	2	4

The tests relating to a particular gene are given a specific letter in the tables, those relating to genes C, B, A, and D and E together, being given the letters V, W, Y, and Z respectively.

The differences in the number of larvae in a particular genotype taken first by the two species of beetles were not significant, so the results for the two species were added together. Chi-squared tests were then carried out on the results of tests relevant to each particular gene, and all these tests gave non-significant results, (for V tests, X_2^2 = 0.7, $0.8 > p > 0.7$; for W tests, X_2^2 = 0.4, $0.9 > p > 0.8$; for Y tests, X_2^2 = 0.4, $0.9 > p > 0.8$; for Z tests, X_2^2 = 1.2, $0.7 > p > 0.5$).

Discussion

The evidence from these tests indicated that neither of the beetles used showed a significant preference for larvae of one genotype to those of another, although again the figures were too small to be taken as providing conclusive evidence.

These tests were carried out in very artificial conditions, and I think it is relevant to consider what might happen in a natural situation. Most beetles, which are large enough to prey upon P. meticulosa larvae in the later instars, are ground feeding species (Alexander personal communication). It has been stated that the majority of Carabid beetles find prey by accidental encounters, and it has been noted that these beetles generally eat the first edible object with which they come into contact, (personal observation, and Alexander personal

communication). Thus, in the wild, beetles may prey on 3rd instar larvae which are homozygous recessive for gene C more than those which have the allele c^+ in their genotype. This is because the former type are more likely to be found at ground level in the day, due to their negative phototactic response. However, as the beetles are usually nocturnal predators, the effect is likely to be very slight.

E. Experimental studies of selection by hedgehogs.

Predation by mammals undoubtedly contributes significantly to the overall death rate of many Noctuid species during the larval stage. A number of British mammals are known to eat larvae of Noctuids. These range from members of the Insectivora, such as the European Hedgehog, Erinaceus europaeus L. and the Common Shrew, Sorex araneus L., to larger carnivorous and scavenging mammals such as the Badger, Meles meles L. and the Red Fox, Vulpes vulpes L., Harris (personal communication). Yalden (1976) notes that for hedgehogs, the most important class of food is lepidopterous larvae, which comprised over 25% of the contents of the 177 hedgehog stomachs he examined. He also mentions that almost all of the larvae seemed to be Noctuids. Noctuid larvae have also been found in some quantities in the stomachs of foxes and badgers, (Harris, personal communication) and the stomach of one fox contained over 150 larvae. Most of these were closely comparable with Noctua pronuba, which feeds on low-growing plants from September to May (personal observation); however, three of the larvae were 5th instar P. meticulosa.

The tests carried out were designed to determine whether hedgehogs show selection for P. meticulosa larvae of any particular genotype when offered a choice between larvae of various genotypes.

It has been noted that hedgehogs have a pure-rod retina (Bridges and Quillian, 1973) and that they are thus colour blind; however, Herter (1965) asserts that he was able to teach hedgehogs to distinguish between yellow and blue even when these were of the same tone. In any case, hedgehogs are almost entirely nocturnal, although they may be seen occasionally at dusk and dawn. Corbet & Southern (1977) note that they have poor eyesight, but acute senses of smell and hearing. Hedgehogs are active from April to October, although, if conditions are suitable, they are not uncommonly found active outside this period (Reeve personal communication).

Methods

The methods used for the tests assumed that hedgehogs did not hunt by sight at all. Two hedgehogs were taken from the wild in late August. One of these was a young female, the other an adult male. The hedgehogs were kept in cages for two weeks before being used for tests. The cages were made of wood and wire, covered with muslin. They had white bases and dimensions of 1 m x 1 m x 0.5 m. The hedgehogs were kept separately. During this period, the hedgehogs were fed in the evening upon a mixed diet of worms, slugs and minced beef. For the tests 16 samples of larvae of specific genotypes, ranging in number from 30 to 60, were made up. These samples

were named the "hedgehog selection samples" (HS1 - 16 inclusive), and the exact make-up of each with respect to larval genotype is given in Table 7Ei. Samples HS1 - 8 inclusive were given to the female hedgehog, (hedgehog 1) and samples HS9 - 16 inclusive were given to the male (hedgehog 2), one sample being given every other evening over a period of 15 days. During this period, on evenings when the hedgehogs were not given a sample of larvae, they were again fed on the diet of slugs, worms and mince.

Each sample was given to a hedgehog by scattering the larvae over the bottom of the cages about half an hour after sunset. The hedgehogs were then kept under observation using the natural evening light, moonlight, or, where necessary, very dim artificial light until they had consumed half the larvae in the sample. The hedgehogs were then removed and the genotypes of the remaining larvae were recorded. Again, when two genotypes in a sample were indistinguishable phenotypically, they were marked by similar methods to those used in the viral experiments (see chapter 7B). The samples were given to each hedgehog in a random order.

Results and analysis of data.

The results of these tests are given in Table 7Ei.

Chi-squared tests comparing the number of larvae of each genotype to be eaten by each hedgehog gave non-significant results in all cases. The chi-squared and probability values of these tests are given in Table 7Ei. Further tests, comparing the total number of larvae of each genotype to be

Table 7Ei.

Experimental studies of selection by hedgehogs. The numbers of larvae of specific genotypes in samples HS1 - 16 inclusive, eaten by hedgehogs.

Sample numbers	Larval instars	Number of larvae per sample	Gene being tested	Alleles of gene being tested	Alleles of other genes C	B	A	D	E	Number of larvae taken by Hedgehog 1 from samples:			Number of larvae taken by Hedgehog 2 from samples:			Overall Total	Chi-squared and probability values comparing the predation of the two hedgehogs (degrees of freedom = 1) Chi-squared	Probability
										HS1	HS2	Total	HS9	HS10	Total			
HS1, 2, 9 & 10	3rd	(20	C	c^+c^+	X	oo	--	+ or +		13	10	23	7	13	20	43	0.209	0.7 - 0.5
		(20	C	c^+c	X	oo	--	+ or +		11	9	20	9	7	16	36	0.444	0.7 - 0.5
		(20	C	cc	X	oo	--	+ or +		6	11	17	14	10	24	41	1.195	0.3 - 0.2
										HS3	HS4	Total	HS11	HS12	Total			
HS3, 4, 11 & 12	4th	(16	B	b'b'	++	X	--	+ or +		9	6	15	10	8	18	33	0.273	0.7 - 0.5
		(16	B	$b'b^o$	++	X	--	+ or +		7	7	14	7	6	13	27	0.037	0.9 - 0.8
		(16	B	b^ob^o	++	X	--	+ or +		8	11	19	7	10	17	36	0.111	0.8 - 0.7
										HS5	HS6	Total	HS13	HS14*	Total			
HS5, 6, 13 & 14	5th	(10	A	a^+a^+	+x	''	X	+ or +		3	6	9	3	5	8	17	0.059	0.9 - 0.8
		(10	A	a^+a	+x	''	X	+ or +		6	4	10	8	3	11	21	0.048	0.9 - 0.8
		(10	A	aa	+x	''	X	+ or +		6	5	11	4	6	10	21	0.048	0.9 - 0.8
										HS7	HS8	Total	HS15	HS16	Total			
HS7, 8, 15 & 16	5th	(10	D and E	d^+d^x or e^+e^x	--	'o	--	X	X	4	5	9	6	3	9	18	0	1
		(10	D and E	$dde'e^o$	--	'o	--	X	X	4	4	8	4	5	9	17	0.059	0.9 - 0.8
		(10	D and E	$dde'e'$	--	'o	--	X	X	5	8	13	5	7	12	25	0.04	0.9 - 0.8
		(10	D and E	dde^oe^o	--	'o	--	X	X	7	3	10	5	5	10	20	0	1

* Error in procedure, test stopped when 14 larvae out of 30 had been eaten.

eaten in a series of samples for testing the reaction of genotypes of one particular gene, also gave non-significant results, (for HS1, 2, 9 and 10, $X^2_2 = 0.65$, $0.8 > p > 0.7$; for HS3, 4, 11 and 12, $X^2_2 = 1.313$, $0.7 > p > 0.5$; for HS5, 6, 13 and 14, $X^2_2 = 0.536$, $0.8 > p > 0.7$; for HS7, 8, 15 and 16, $X^2_3 = 1.9$, $0.7 > p > 0.5$).

The results show that the reactions of the two hedgehogs to larvae of a particular genotype did not vary to a significant extent. Furthermore, none of the genotypes being considered seem to provide larvae of that genotype with an advantage over larvae of any other genotype when predation by hedgehogs is being considered.

As in the tests on selection by Carabid beetles, these artificial tests are not representative of a natural situation, and it is possible that hedgehogs selectively favour c^+c^x larvae in the 3rd instar as they are more likely to encounter and eat larvae which are homozygous for c which may be found near to the ground at dusk and dawn; however, if this is the case the selection in favour of c^+ would again be very slight as hedgehogs are mainly nocturnal.

CHAPTER 8. EXPERIMENTS ON SELECTION BY PREDATORS WHICH HUNT BY SIGHT AND POSSESS COLOUR VISION.

A. Introduction

Having considered some of the selective factors which may indirectly affect the frequencies of the alleles controlling larval colour in P. meticulosa, the cause of the seasonal variations in the frequencies of the alleles of genes A and B are still unexplained, and the balance of the polymorphisms based on genes A, B, D and E have not been explained conclusively, although heterozygous advantage may be involved in some cases. Therefore, we turn now to a consideration of selection by predators which hunt by sight, and which possess colour vision.

Many birds prey upon Lepidoptera larvae in the wild. Thompson (1964) notes that there can be no doubt that the vast majority of diurnal birds possess colour vision, although it is thought that the majority of nocturnal ones do not. Experiments on domesticated birds such as chickens and pigeons have indicated that the range of colours visible to them is about the same as in man[1]. This means that birds which prey upon the larvae of P. meticulosa in the later instars, may prey upon the different phenotypes to different extents due to their colours, and thus confer a selective advantage upon some of the colour controlling alleles over others.

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[1] At one time it was thought that birds were "blue-blind", but later experiments have shown that this was due to misinterpretation of experimental data, and, although most species tested do show a decrease in sensitivity to blue, they are by no means blind to this colour. This relative insensitivity is thought to be due to the profusion of yellow and orange droplets in their retinae (Thompson, 1964).

There are two basic mechanisms which may lead to birds preying on one phenotype more than others. Firstly, as the larvae of P. meticulosa are thought to be basically cryptic, one phenotype may be less easily detected by predators because it matches its background better. Selection by palatable animals which do not match their background is now generally accepted, and circumstantial evidence indicating the advantage of cryptic coloration may be obtained by considering the huge number of species which use camouflage for protection. Examples of such species may be found in almost every animal phylum. Kettlewell's, now classical, studies on the peppered moth, Biston betularia, have proved beyond doubt that those forms which do not match their background very well are attacked more than those which do (Kettlewell, 1955b, c, 1956, 1958). Several authors have also noted that balanced polymorphic species may also be subject to strong visual selection (e.g. Sheppard, 1951; Clarke, 1960; Ford, 1964; Allen, 1972b.)

The second mechanism involves the fact that some birds which prey upon protectively coloured insects, tend to hunt for prey of a type that they have recently found, even to the exclusion of others that seem more obvious, thus making use of the experience they have just gained (de Ruiter, 1952). This type of behaviour involves the formation of searching images. The term "searching image" was first used by Von Uexkull (1934), who noted that toads (Bufo bufo L.) fed on earthworms, subsequently snapped at matchsticks when given a choice of other items. That searching images may be formed very quickly and may be extremely specific, was demonstrated by de Ruiter's work on the effectiveness

of crypsis in the larvae of the Geometer Ennomos alcanara L. (de Ruiter, 1952). Using caged hand-reared birds as predators, he showed that when twigs were added to the cages, the birds initially pecked at them. Once the birds had become habituated to the twigs, dead caterpillars were added. These were not attacked at first, but once one was found others were also sought and discovered. At first, in the search for larvae, the twigs were also pecked, but the birds soon became more efficient.

The idea of searching image formation was first used in an ecological context by Tinbergen (1960) working on titmice which fed on insect prey in pine forests. He discovered that, when a particular type of prey became abundant, there was often a lag of a few days before the titmice began to utilize this new sort of food to any great extent. Tinbergen considered that accidental encounters with the new prey would lead to an improvement in the birds ability to find it.

The tendency to find prey using searching images is the basis of what Clarke (1962b) termed "apostatic selection", which maintains that predators preferentially search for and eat the most common forms of a polymorphic species. Allen (1972b) notes that under such conditions the rarer forms, even if relatively conspicuous, should be protected from predation and hence be maintained in the population. However, it should be stressed that, if a rare phenotype is much more conspicuous than a common one, it may appear to the predators to be the commoner of the two (Ford, 1964), and in such conditions would be selected against (Allen, 1972b).

Over the last 15 years the theory of apostatic selection has received considerable attention, and there has been a steady build-up in evidence supporting this theory, (e.g. Owen, 1963; Allen and Clarke, 1968; Greenwood, 1969; Croze, 1970; den Boer, 1971; Smith, 1971; Allen, 1972a, b, 1974, 1976), and various authors have shown that this type of mechanism is capable of maintaining a polymorphism, (e.g. Wright, 1948; Clarke, 1962a; Li, 1962; Moment, 1962; Clarke and O'Donald, 1964).

Let us look now more specifically at the situation in P. meticulosa.

Cain et al., (1960), and Turner, (1961) have suggested that disruptive selection may act to eliminate the more conspicuous phenotypes of polymorphic prey species, such that the optima come to resemble particular elements of the background. Certainly a number of species of Lepidoptera do appear to exhibit such "cryptic polymorphism" and in many cases some morphs are green and others brown. The previous work in this study has shown that P. meticulosa is polymorphic in the later instars, having six main colour forms, which involve the three colours green, brown and yellow, either singly, or in mixtures of any two. The environment which these larvae inhabit in the Ringwood area is, in general, made up of numerous shades of green, brown and yellow.

In chapter 5, it was noted that the frequencies of the alleles controlling the colours of the larvae do not vary to any great extent from year to year at a particular season, but there is considerable seasonal variation in the frequencies of

the alleles of genes A and B.

If apostatic selection is considered in relation to P. meticulosa, a number of extreme problems arise. In the first place, the number of main colour-types present in a population at any one time varies, theoretically, from one, if all larvae are in the first or second instars, to six, if larvae of later instars are present; however, as mentioned in chapter 2, in reality all stages of the life cycle occur throughout the year as the insect does not have a totally rigid annual cycle.

Secondly, Croze (1967, 1970) and Allen (1972b), have both demonstrated that the closer the degree of resemblance of an unfamiliar prey to the familiar type, the more likely it is to be taken. The similarities of the larval colours of P. meticulosa are difficult to assess because of the variability within colour types. This means that, if a number of birds are trained to search for a particular larval colour type, then, if each of the birds is subsequently given a choice of two types of larvae, including that with which it is familiar, the bias produced in results due to similarity between the two types would be difficult to assess.

Thirdly, the ratio of the colour types in the population may vary very considerably over a short period of time, i.e. a week or two. For instance, in the third week of June, 1976, approximately 78% of larvae in a sample from a site in the Ringwood area were in the 4th instar, and of these almost 90% were either green or olive. In samples from the same site which were recorded between seven and ten days later, approximately

69% of the larvae were in the 5th instar and, of these over 60% were brown in colour. The effect of this sudden change in the colour of the majority could be similar to that noted by Tinbergen (1960) when working on the predator prey relationships between titmice and insects (p. 316), i.e. brown larvae of P. meticulosa would be preyed upon less heavily at the start of the 5th instar than would be expected if predation was random, as the bird predators would have formed searching images for the previously commoner green and olive forms. This feature will be dealt with in greater detail in chapter 9.

Fourthly, whilst experiments to determine whether apostatic selection may act upon the polymorphic larvae of P. meticulosa could be carried out using methods similar to those used by Clarke and Allen (1968) and Allen (1972b), either by substituting dead larvae for their lard and flour baits, or by using caged birds, the results would have very limited relevance to the maintenance of larval colour polymorphism in this species in the wild. Allen (1972b) notes that a testable prediction can be made as to the effect of apostatic selection on two closely related polymorphic prey. It is essential, for this prediction to be valid, that the two species should, (a) possess visibly similar polymorphisms, (b) live in the same habitat, and (c) share the same predators. Allen goes on to say that, if they satisfy these criteria, it is probable that predators would treat them both as the same species and this would mean that in effect one polymorphism would be maintained

by predators selecting apostatically. The system would operate in such a way that, if a particular form of one species is maintained at a high frequency by selective factors not directly related to the visible characteristics of the form, then the similar form of the second species would only be at an advantage when present at a low frequency. The polymorphism as a whole would then be maintained in equilibrium at a point determined by a balance of the visual and non-visual selective forces.

The only species to have been studied with regard to this type of prediction are C. nemoralis and C. hortensis, Clarke, (1962b, 1969), Carter, (1967), and the evidence outlined in these three papers has been a matter of some controversy between the two authors. However, this controversy is outside the scope of this thesis.

Allen (1972b) omits to mention one other condition which must be considered if a prediction on the effect of apostatic selection on two species is to be made. This is that, if any other species, whether monomorphic or polymorphic, which are at some stage closely similar to one or more of the morphs of the two species under consideration, exist in the same habitat and share the same predators as them, then these will contribute to the overall apparent frequencies of the forms with which they are similar. These other species must, therefore, be taken into account.

In their natural habitat in the Ringwood area and elsewhere, P. meticulosa larvae coexist with larvae of a considerable number of other species of Lepidoptera, many

of which are similar in colour to one or other of the larval forms of P. meticulosa. At one site near Ringwood, whilst sweep-netting for larvae, 17 other species of Lepidoptera larvae which were either green or brown in colour, were taken. In actual fact, the number of species which have larval forms similar to one or other of those of P. meticulosa, and which share both the same habitats and the same predators at some stage during the year, must be considerably higher than this, the majority of the species which fulfil these criteria probably being members of the family Noctuidae. Therefore, to produce a valid prediction as to the effect of apostatic selection on the larval forms of P. meticulosa, the frequencies of all the forms of these other species which are similar to any of the P. meticulosa larval colour-types would have to be calculated and taken into account. Work of this type is clearly outside the scope of this thesis and an immense amount of experimental work would have to be carried out before the situation involving P. meticulosa, (or any of the other species), could be discovered.

Because of these problems in investigating the effect of predator selection on larval colour polymorphism in P. meticulosa, my intention when embarking on these selection experiments was not to attempt to discover the way in which the polymorphism is maintained, but rather to carry out preliminary work which would give some indication of the ways that bird predation might affect the polymorphism. Furthermore, it was hoped that these preliminary experiments

would give indications as to the direction that further work should take.

Therefore, the experiments carried out were designed with two main aims in mind. Firstly, to determine whether apostatic selection by birds can affect the frequencies of larval colour types in Lepidoptera. Whilst these tests were carried out using P. meticulosa larvae as prey, any conclusions which may be drawn from the results should also apply to other species which share similar habits, habitats and predators to those of P. meticulosa and have similar polymorphic larvae. Secondly, the experiments were designed to determine whether there might be a correlation between the seasonal variation in the larval colour type frequencies and seasonal variations in the relative extents to which the different morphs may be preyed upon by birds.

B. General Materials and Methods

One of the most important considerations, when designing experiments to determine the effect of selection by predators using colour vision to search for prey, was to give results which would be comparable, at least to some extent with a natural situation.

For this reason, three basic experimental designs were developed.

Design 1. This design was used for tests to investigate various aspects of the reaction of birds to living larvae of P. meticulosa, presented at high density. Caged starlings

which had been captured by mist-netting, were used as predators. The tests were carried out in cages which measured 2m x 2m x 2m. These had a wooden frame covered on five sides with wire mesh. The cages had a white chip-board base, and were completely covered with black muslin. A number of wooden perches were placed in each cage, and shelter boxes which could be closed, were secured inside each cage at the top of one of the sides. A door in one of the sides gave access to the cages. The cages were kept outside, except in exceptionally bad weather when they were moved under a shelter.

Design 2. This design was used for tests to investigate the predation of "trained" or "untrained" caged starlings to various samples of living larvae, in what, for the larvae, usually represented a fairly natural environment as far as vegetation was concerned. The tests took place in cages which measured 7.07m square and 2m high. The cages were constructed of wood and wire in the same way as those used in design 1, with the exceptions that, (a) extra wooden struts were used for support, (b) the cages were bottomless, (c) the vertical struts were 2.25 metres long, the extra length extending down past the wire so that this length could be stuck into the ground. Again the cages were furnished with perches, which in this case were made of birch (Betula pubescens) and hornbeam (Carpinus betulus) branches and twigs of the sort which gardeners use when growing sweet peas.

Each cage was set up in the following manner. An area of reasonably flat ground of the same area as the cage was marked out. The plots chosen (except in tests 1 - 4 inclusive of

experiments 7 and 8) were all in an area which had been "grazed" by domesticated muscovy ducks in previous years, and thus, the vegetation in these areas was fairly rich in both the profusion of growth and the variety of plant species present. The vegetation consisted of grasses and low growing plants, and in all cases, common sorrel was the most plentiful plant apart from the grasses. The overall vegetation of the area in which the plots were situated was of grassland and low growing plants, with a few clumps of rhodedendron and scattered mature trees of various types. Once a plot had been selected, it was prepared in a similar manner to the plots used in the third and fifth behaviour experiments (see chapter 4 pages 182-183), with the exception that, during the four month preparation, the muslin covering the plots was anchored to the ground in the centre of the plot so that the muslin would slope down from the corrugated iron sides of the plots to the centre, and leaves and twigs falling on the plot would be caught in the resulting trough. The debris thus collected was periodically scattered at random over the plot, the idea being to ensure that the vegetation of the plots was affected by their preparation as little as possible. When the plots were ready to be used for experiments, strips of sheet metal were sunk vertically down into the ground to a depth of 0.25 metres, which left approximately 55 mm of the sheets exposed, all around the plot. This was done to prevent larvae escaping under the edges of the cages, and to prevent predators which live on or just below the ground from entering the cages. The cages were then placed inside this surround with the vertical struts sunk into the ground. The

cages were then completely covered with black muslin and the bottom edges of the muslin were attached to the metal sheeting. A number of canes, 2.5 metres long, were then sunk into the ground around the cages, and black cotton was stretched between these poles in an irregular pattern. The purpose of this black cotton was to prevent wild birds coming too close to the cages and pecking at larvae through the wire and muslin, which could produce anomalies in the results obtained, not only because these birds may have specific searching images, but also because such attacks would cause damage to the muslin cover which might allow larvae to escape.

Design 3. The third design was used to investigate various aspects of the predation, by wild birds, of various samples of dead P. meticulosa larvae presented to the birds on a number of types of background (e.g. grass lawn, unplanted flower beds and untended or rough grassland).

It would have been preferable to use living larvae for these tests, but problems involving the mobility of the larvae would then introduce errors, particularly if larvae migrated out of the area of study. Allen (1972b) noted that most predators do not normally eat their usual prey if these are dead, and quotes evidence involving frogs (Katz, 1937), and King penguins (Aptenodytes patagonia) (Gillespie, 1932), to illustrate this point; however, as will be evident from the results of experiments 5 and 6 (see pages 349-357), this is certainly not so in the case of many wild birds which prey upon the larvae of Lepidoptera. Larvae were killed using a fast

freezing method.

Areas of land which met the requirements of particular tests as far as vegetation was concerned, were marked out using black cotton and wooden pegs. Each area was 10m x 10m. The treatment of these plots prior to their use in experiments depended on the nature of the experiment concerned, and will, therefore, be described in the relevant part of the text.

General comments on the birds involved in the tests.

As Allen (1972b) notes, ground-feeding passerines are ideal for prey selection experiments. Apart from their abundance and ubiquity, they hunt almost exclusively by sight, and possess colour vision (Pumphrey, 1948; Kettlewell, 1955c), and they are very efficient at searching for prey (cf. Heppner, 1965, for Turdus migratorius).

The birds involved in experiments using designs 1 and 2 were all starlings, Sturnus vulgaris L.. This bird is one of the species which has established a more or less commensal relationship with man. The reaction of starlings to being kept in cages is, even initially, not too violent, and they very quickly "settle down" in captivity. They are extremely catholic in their choice of diet, and, in the wild, their diet includes ground living noctuid larvae (personal observation). Starlings are social feeders, and I have often watched as a group of a dozen or more moved across a bank or field in an unevenly spaced line abreast, feeding as they went. Their searching for food, particularly in unkempt grassland, seems to be carried out by poking their heads down into the foliage along the plant stems

and looking for prey in the low layers of herbage. This method of searching is particularly appropriate to the predation of P. meticulosa larvae in the later instars, as it has been noted that during the day these are to be found in the lower herbage layers.

Starlings were chosen for the caged experiments in preference to blackbirds because they take to captivity better, and because, as Allen (1972b) notes, their social feeding behaviour may make them more efficient "apostatic" predators than Turdus spp., because "social facilitation" may ensure that nearly all the individuals concentrate on common varieties of prey. Various workers have produced evidence to show that some animals may learn from the experience of others, a type of behaviour that has been called "observational learning". This phenomenon has been noted in the feeding behaviour of birds by a number of authors (e.g. Klopfer, 1961 working on Chloris chloris; Turner, 1965 on Passer domesticus and Fringilla coelebs; Murton, 1971b on Columba palumbus). These birds, like starlings, feed and roost in flocks, and it has been suggested that these communal activities improve the feeding efficiency of the individuals (Ward, 1965, Murton, 1971a).

The starlings used in tests employing experimental designs 1 and 2, were all captured at least six weeks before they were to be used for any test, and they were kept in cages of the type described in relation to design 1, two birds being kept in each cage. During periods when the birds were not being used for tests they were fed upon a diet of bread, rice, animal fat and chopped worms. This diet was chosen as it kept the birds

healthy, and because "cakes" made up of these materials bore no resemblence to any of the forms of larval prey, either in shape or colour.

The birds involved in tests, using the third experimental design, are given in Table 8Bi. This list includes all the species of birds which were noted entering, and apparently feeding on the ground, in the experimental plots during the tests. Of these birds, Jays, Woodpigeons and Pied Wagtails were each only noted in the plot areas during one test, in the case of the former two species one individual and in the case of the latter species two individuals being involved. Mistle thrushes and tree sparrows were both noted in plot areas during three tests, and at most one mistle thrush and three tree sparrows were noted in the plots at one time. Of the other birds, robins, hedge sparrows, and song thrushes were involved in all tests except one, one and two respectively, whilst the other three species were involved in all tests. Never more than two robins or song thrushes or more than four blackbirds were recorded in a plot at one time, whilst the maximum number of starlings, hedge sparrows or house sparrows on the plots at one time were 15, 6 and 17 respectively. Most of these species are very catholic in their choice of diet, the exceptions being the woodpigeons, tree sparrows and house sparrows which are all principally vegetarian or seed eaters. All the species except these three and the jay were actually observed at some time feeding on the dead larvae which were put out.

Table 8Bi.

List of those birds which were involved in the selection experiments in which dead larvae were put out on plots in the Ringwood area.

Common Names	Latin Names
Starling	STURNUS VULGARIS L.
Song Thrush	TURDUS PHILOMELOS HART.
Mistle Thrush	TURDUS VISCIVORUS L.
Blackbird	TURDUS MERULA L.
Robin	ERITHACUS RUBECULA L.
Hedge Sparrow (Dunnock)	PRUNELLA MODULARIS L.
Tree Sparrow	PASSER MONTANUS L.
House Sparrow	PASSER DOMESTICUS L.
Jay	GARRULUS GLANDARIUS L.
Pied Wagtail (Water Wagtail)	MOTACILLA ALBA L.
Wood Pigeon	COLUMBA PALUMBUS L.

C. The Experiments.

Experiment 1.

The aim of this experiment was to determine whether starlings showed a preference for eating larvae of a particular colour when larvae were offered on a white surface, upon which, to me at least, all the colour-types were very conspicuous. Experimental design 1 was used. The experiment consisted of four tests, and in all, eight starlings were involved.

Samples of 200 larvae were scattered at random over the bottoms of four cages, each of which contained two starlings. Each sample consisted of 50 larvae of each of the four colour-types; Gr, OL, Br and PY; those larvae put in cages A and B being in the 4th instar, and those put in cages C and D being in the 5th instar. The starlings were shut in the shelter boxes whilst the samples were put in the cages, and were let out to feed half an hour after this. The cages were observed using binoculars from the shelter of a shed, and tests were stopped when it was judged that approximately half the larvae had been eaten. The colour-types of the remaining larvae were then counted, and the number of larvae eaten was calculated by subtraction. To investigate the predation due to one particular starling, a red ring was put on one of those in cage A (starling 1), and the order in which this bird took larvae of different colour types was recorded by direct observation. Periods of more than 15 minutes during which starling 1 did not take a larva were also recorded. All these tests were carried out in October, 1976, and started within two hours of sunrise.

Results and analysis of data.

The results of these tests are given in Table 8Ci.

If the results of each test are considered separately, then chi-squared tests, comparing the number of larvae of each colour type eaten, taking the expected ratio between colour types to be 1:1:1:1, showed that the proportion of larvae eaten by the birds in cages B and D differed significantly from the expected, whilst those taken by birds in cages A and C did not. (These and other relevant chi-squared and probability values are given in Table 8Ci). If, on the other hand, the results of tests involving larvae of the same instar are summed for each colour type, chi-squared gives non-significant results for both 4th and 5th instars. A chi-squared test in which the summed figures for 4th instar larvae (cages A and B) were compared with those for 5th instar larvae (cages C and D), also gave a non-significant result ($X^2_3 = 4.637$, $p = 0.2$). Furthermore, if the results of each colour type from all four tests are summed and compared, the ensuing chi-squared value gives a probability value of between 0.9 and 0.8 indicating a considerable degree of homogeneity with the expected ratio. Finally, if a heterogeneity chi-squared test, (for method see Snedecor, 1956, p. 214), is carried out on the results, the value of chi-squared indicates that there is a high degree of heterogeneity between tests, ($X^2_2 = 26.692$, $p < 0.001$).

Considering the results from the two birds in cage A individually, then both birds show a significant departure from the expected, in the number of larvae of each colour type that

Table 8Ci.

Experiments on selection by birds. Results of Experiment 1, designed to discover whether starlings show a preference for eating larvae of a particular colour-type. In all tests 200 larvae were offered, the ratio between colour-types being 1:1:1:1.

Cage number	Starling numbers	Instar of larvae	Number of larvae of each colour type eaten				Total number of larvae eaten	Chi-squared values and probability values taking the expected ratio between colour-types taken as 1:1:1:1. Degrees of freedom = 3 in each case.	
			Gr	OL	Br	PY		Chi-squared	Probability
A	1 and 2	4th	27	16	27	32	102	5.373	0.2 - 0.1
B	3 and 4	4th	22	36	16	22	96	9.0	0.05 - 0.02
(A and B)		4th Totals	49	52	43	54	198	1.394	0.8 - 0.7
C	5 and 6	5th	32	27	24	17	100	4.72	0.2 - 0.1
D	7 and 8	5th	15	25	35	23	98	8.286	0.05 - 0.02
(C and D)		5th Totals	47	52	59	40	198	3.899	0.3 - 0.2
	Overall Totals		96	104	102	94	396	0.687	0.9 - 0.8
A	1	4th	6	14	8	19	47	9.383	0.05 - 0.02
A	2	4th	21	2	19	13	55	15.909	0.01 - 0.001

The colour types of larvae eaten by starling 1 in the order in which they were taken.

1st taken: Gr,Gr,PY,PY,PY,PY,PY,PY,PY,-PY,OL,OL,OL,OL,PY,PY,PY,-Br,OL,OL,OL,OL,-OL,Gr,Gr,Gr,OL,OL,OL,OL,-Br,Br,-PY, PY,PY,PY,-PY,PY,PY,OL,-Gr,-Br,Br,Br,Br,Br,-PY.*

* The hyphens in this series of results indicate a period of 15 minutes or more when the starling did not feed.

they ate. On the other hand, when the number of larvae taken by the two birds was summed, the results produced gave a non-significant chi-squared. Furthermore, a heterogeneity chi-squared test showed that the two birds acted very differently with respect to the number of larvae of each phenotype which they ate, ($X^2_1 = 19.919$, $p < 0.001$).

If the order in which larvae were taken by starling 1 is considered, it appears to be far from random, 33 of the 47 larvae taken being of the same colour type as the preceding one that was eaten. During the test there were nine periods of more than 15 minutes, (sometimes considerably more than 15 minutes), during which this starling did not feed. Changes in the type of larvae taken coincided with six of these. During these periods the starling generally sat upon one of the perches inside the cage.

Discussion

The results of this experiment indicate that, while in individual tests the starlings in a particular cage tended to take more larvae of some colours than others, this trend being significant in two of the four tests, overall the number of each colour type taken was very close to the expected ratio. There was considerable variation in the results from different tests. There is no evidence to indicate that in these experiments the starlings' reactions to 4th instar larvae was different to their reaction to 5th instar larvae, except that the larvae of the latter type were taken on average at a slower rate, probably because of their larger size.

The data recorded for starling 1 indicates that neither this starling nor starling 2 preyed upon larvae of different colour types in a random manner, and it is suggested that there is evidence that starling 1 was selecting a disproportionately large number of PY and OL larvae because it formed searching images for these two colour-types. It is notable that, in the majority of cases, larvae taken were of the same colour-type as the previous one eaten. This suggestion is supported by the fact that periods of over 15 minutes during which the starling did not feed tended to coincide with a change in the colour type taken, and it is thought that recommencement of feeding at the end of these periods may have been induced by the starling noticing a movement made by one of the larvae. The fact that the larvae were exposed throughout the tests introduces an anomaly with the situation which would be expected in the wild. As 4th and 5th instar larvae are negatively phototactic, then, even though many of the larvae used in these tests remained motionless for long periods, on the whole it is probable that they moved more in order to find a site where they were less exposed to the light than larvae in the wild would. However, if this is so, it would tend to bias the results towards a 1:1:1:1 ratio because larval movement is more likely to disrupt a searching image than reinforce it, as, in these experiments, it is likely to draw the starlings attention to unfamiliar colour types in three cases out of four. It is also notable that the data from starlings 1 and 2 do not support the idea of observational learning; however, as only two starlings were involved in the

test, and the feeding conditions were very artificial, the situation cannot be taken to constitute natural communal feeding.

Experiment 2.

The aim of this experiment was to determine whether starlings showed a preference for preying on 4th or 5th instar larvae of the same colour type in a comparison situation, because, as Morton Jones (1932, 1934) notes, other things being equal, birds generally select the largest insect prey available to them.

Experimental design 1 was used and 4 tests were involved. The same techniques as were employed in Experiment 1 were used, with the exception that the samples each consisted of 200 larvae of just one of the colour types Gr, OL, Br and PY, half the larvae being in the 4th instar and half in the 5th. The tests were stopped when approximately one quarter of the larvae had been eaten.

Results and data analysis

Table 8Cii gives the results of these tests, and the results of chi-squared tests comparing the number of 4th and 5th instar larvae eaten, taking the expected ratio between instars as 1:1.

In all four tests a highly significant excess of 5th instar larvae were taken.

Discussion

These tests then agree with Morton Jones' (loc cit) findings in that the larger insects (i.e. the 5th instar larvae) were

Table 8Cii.

Experiments on selection by birds. Results of Experiment 2, designed to discover whether starlings preferentially prey on 4th or 5th instar larvae. (Larvae presented in a 1:1 ratio)

Cage No.	Starling numbers	Colour types of larvae offered to starlings	The number of Larvae eaten 4th instar	5th instar	Total
A	1 and 2	Gr	4	46	50
B	3 and 4	OL	11	39	50
C	5 and 6	Br	1	51	52
D	7 and 8	PY	7	39	46
		Totals	23	175	198

For cage A results, $X_1^2 = 35.28$, $p < 0.001$;

for cage B results, $X_1^2 = 15.68$, $p < 0.001$;

for cage C results, $X_1^2 = 48.077$, $p < 0.001$;

for cage D results, $X_1^2 = 22.261$, $p < 0.001$.

taken in preference to the smaller insects (i.e. the 4th instar larvae) by birds.

Experiment 3.

The aim of this experiment was to determine whether individual starlings can become conditioned to searching for living larvae of a particular colour in artificial conditions.

Experimental design 1 was used, and in all, the experiment involved 12 tests. Six starlings, caged singly, were fed over a period of ten days on either green, olive or brown 4th instar larvae, each starling only being fed upon one type of prey. Each starling was then presented with a sample of 200 larvae of which half were of the colour familiar to them, the other half being one of the other two colours. Starlings were again shut in their shelter boxes while the samples were put out. Each test lasted until approximately one quarter of the larvae had been eaten. (If extremely strong searching images were formed, and initially only the familiar larvae were taken, then the proportion of this colour type would drop in relation to the other. If the ratio between the two changed drastically, then apostatic selection for the commoner colour-type may effect the results, as may the converse situation which would occur if the rarer colour was preferentially preyed upon, a preference which has been noted by Allen (1972b) when working with green and brown, flour and lard baits being offered to wild blackbirds in unequal proportions at high density. Therefore, the tests were designed so that the number of one colour type in the cage could never drop below half that of the other). After these

tests, starlings were again fed on the diet of bread, rice, animal fat and chopped worms, for six weeks, and the tests were then repeated in the same way with the exception that the two colour types of larvae which had been offered to starlings in the first series of tests were reversed, so that, if in the first test a starling was initially familiarised with Gr larvae, and then offered a mixture of Gr and OL larvae, in the second test that particular starling was initially given OL larvae.

Results and analysis of data

The results of these tests are given in Table 8Ciii.

In every test the number of larvae of the familiar colour type which were eaten exceeded the number of unfamiliar larvae taken. In each case this excess was highly significant, and in half the tests, only the familiar larvae were taken.

If the summed results of the two series of tests are compared, they prove to be homogeneous, (X^2_1 = 0.349, $0.7 > p > 0.5$).

Heterogeneity chi-squared tests carried out to investigate the variation in predation by individual starlings showed that, in the first series of tests, the variation was significant, although not highly so, (X^2_5 = 14.183, $0.02 > p > 0.01$). In the second series the variation between tests was not significant, (X^2_5 = 7.851, $0.2 > p > 0.1$).

Discussion

The first series of tests show that starlings do form searching images, and these may be very strong, in some cases

Table 8Ciii.

Experiments on selection by birds. Results of Experiment 3, designed to determine whether starlings can form searching images.

Test number	Starling number and details	Colour-type of larvae initially preferred to the starlings	Colour-types of larvae subsequently offered to the birds in a 1:1 ratio (200 larvae per sample	The number of larvae of the "familiar" colour-type, eaten	The number of larvae of the "unfamiliar" colour-type, eaten	Total number of larvae eaten	Chi-squared and probability values taking the expected ratios between colour-types as 1:1 in each test. (Degrees of freedom equals 1 in all cases) Chi-squared	Probability
1	9 male, juvenile	Gr	Gr and OL	46	3	49	37.735	< 0.001
2	10 male, adult	Gr	Gr and Br	53	0	53	53.0	< 0.001
3	11 female, adult	OL	OL and Gr	36	13	49	10.796	0.01 - 0.001
4	12 male, adult	OL	OL and Br	49	0	49	49.0	< 0.001
5	13 male, adult	Br	Br and Gr	50	0	50	50.0	< 0.001
6	14 female, adult	Br	Br and OL	41	11	52	17.308	< 0.001
			Total	275	27	302	203.656	< 0.001
7	9	OL	OL and Gr	54	0	54	54.0	< 0.001
8	10	Br	Br and Gr	49	0	49	49.0	< 0.001
9	11	Gr	Gr and OL	39	9	48	18.75	< 0.001
10	12	Br	Br and OL	41	6	47	26.064	< 0.001
11	13	Gr	Gr and Br	54	0	54	54.0	< 0.001
12	14	OL	OL and Br	43	8	51	24.020	<0.001
			Total	280	23	303	217.983	< 0.001
			Overall Total	555	50	605	421.529	< 0.001

birds eating larvae with which they were familiar to the exclusion of unfamiliar ones. The second series of tests show that, after an intervening period, starlings may be trained to form a searching image for larvae of the previously unfamiliar colour.

Some variation in the reactions of individual starlings to the prey is evident in the results, and it is worth noting that the two starlings which took most unfamiliar prey in the first set of tests also took more unfamiliar larvae than any of the other birds in the second series. It may be that this is a simple coincidence, but, on the other hand, two other possibilities which might explain this trend seem plausible. Firstly, some starlings may form stronger searching images than others, or, they may retain searching images, once formed, for a longer period. Brower (1960) notes there was some variation in the ability of the individual birds both to learn and to memorise the association of colour with edibility. Thus it may be that starlings 11 and 14 were not as efficient in forming or retaining searching images as the other starlings in the tests. There does not seem to be any obvious correlation between this feature and the age or sex of the starlings involved.

The second possibility is that, in the cases where no larvae of the unfamiliar type were taken, the two colour-types offered were more distinct than in tests when some were taken. It is notable that in the two tests of each series involving Gr and Br larvae, none of the unfamiliar larvae were taken, whilst in six of the other eight tests, when OL larvae, (which are intermediate in colour between the Gr and Br forms), were involved some of

the unfamiliar types were taken.

Experiment 4.

The aim of this experiment was to discover whether, when larvae of two colour types were offered at high density to starlings, in unequal proportions, the commoner or rarer form was eaten more often than expected, and whether the type of background, on which the larvae were offered, affected the results. If apostatic selection acts when prey are present at high density, then birds should take the commoner morph more than expected. However, the literature contains evidence suggesting that the opposite is the case, (e.g. Tinbergen, 1946; Pielowski, 1959, 1961; Pough, 1964; Allen, 1972b), and the thoery of apostatic selection outlined by Clarke (1962a), specifically excluded situations where prey occurred at very high densities.

The experimental design used incorporated some features of both design 1 and 2, and involved two series of four tests. The cages employed were those described in design 1, but the white base was removed, the cages being placed on the ground on an area of either grass lawn or bare soil. Plots on these surfaces were chosen because they provided natural backgrounds which were relatively uniform in colour, and, being green and brown respectively, they were similar in colour to those of the larvae used in the experiment. It may be mentioned here, that from my own subjective point of view, Br larvae on a soil background, and green larvae on a grass lawn background were equally well concealed. On the other hand, green larvae on a soil background were slightly more conspicuous than brown larvae

on a grass lawn background . The method used in experimental design 2,to prevent larvae from escaping from bottomless cages by sinking sheet metal into the ground around the cages, was employed.

Samples of 500 4th instar larvae were made up. In each of these samples two larval colour types (Gr and Br) were present, one being 9 times as common as the other. The starlings were shut in the boxes whilst samples were scattered at random on the ground in the cages, and were released into the main part of the cages one hour after the samples had been put out. After a suitable period of time had elapsed, the cages were removed and the plot areas were carefully searched for larvae, after which the top 4 cm of surface material in each plot were dug up using a turf lifter; this material then being sifted for any remaining larvae. This was done because the larvae, being negatively phototactic (see chapter 4), tended to move down amongst the grass stems or loose top soil. This process was repeated on the following three days, using new plots, but in each case, plots picked for a particular pair of birds were as similar as possible.

Six weeks after this first series of tests, a second series were undertaken. These were similar to the first series, with the exception that the backgrounds on which larvae were offered to a particular pair of starlings, were reversed.

The tests were carried out in January and February so that seasonal variation in the nature of the plots between the two series of tests was minimal. Tests always started early in the morning.

Results and analysis of data

The number of larvae of each form taken on each day of each test are given in Table 8Civ, together with other relevant details of the tests and the ratios between the number of the commoner and the number of the rarer forms of larvae taken, both for each day of all tests and for the total numbers of larvae in each test. If the starlings are preying at random, then the expected ratio between the number of the commoner and rarer colour types to be eaten is 9:1. A quick glance at the ratios for the totals of each test show that these ratios often differ radically from 9:1, and in one test (test 4), the number of larvae taken of the rarer colour type was greater than that of the common colour type.

When the total results for each test were compared with a 9:1 expected ratio using chi-squared, four of the tests gave probability values which indicate that the numbers of larvae taken differed significantly from the expected, whilst the other four gave non-significant probability values;

(for test 1, $X_1^2 = 547.086$, $p < 0.001$;
for test 2, $X_1^2 = 0.976$, $0.5 > p > 0.3$;
for test 3, $X_1^2 = 2.598$, $0.1 > p > 0.5$;
for test 4, $X_1^2 = 750.244$, $p < 0.001$;
for test 5, $X_1^2 = 0.708$, $0.5 > p > 0.3$;

Table 8Civ.

Experiments on selection by birds. Results of Experiment 4, designed to discover whether starlings feed preferentially on common or rare larval forms, when these are presented at a high density, and whether the type of background, on which larvae are offered, affects the results.

Test number	Day number	Starling numbers	Common colour type in the sample (450 per sample)	Rare colour type in the sample (50 per sample)	Background on which samples were offered	Number of larvae taken: common colour type	rare colour type	Total	Ratio between the number common and rare colour-types taken
1	1	1 and 2	Gr	Br	Grass	24	38	62	0.6 :1
	2	1 and 2	Gr	Br	Grass	38	41	79	0.9 :1
	3	1 and 2	Gr	Br	Grass	63	48	111	1.3 :1
	4	1 and 2	Gr	Br	Grass	41	32	73	1.3 :1
						Total 166	159	325	1.044:1
2	1	3 and 4	Br	Gr	Grass	50	7	57	7.1 :1
	2	3 and 4	Br	Gr	Grass	82	2	84	41.0 :1
	3	3 and 4	Br	Gr	Grass	84	16	100	5.3 :1
	4	3 and 4	Br	Gr	Grass	56	11	67	5.1 :1
						Total 272	36	308	7.556:1
3	1	5 and 6	Gr	Br	Soil	66	5	71	13.2 :1
	2	5 and 6	Gr	Br	Soil	86	7	93	12.3 :1
	3	5 and 6	Gr	Br	Soil	102	14	116	7.3 :1
	4	5 and 6	Gr	Br	Soil	81	1	82	81.0 :1
						Total 335	27	362	12.407:1
4	1	7 and 8	Br	Gr	Soil	34	48	82	0.7 :1
	2	7 and 8	Br	Gr	Soil	41	50	91	0.8 :1
	3	7 and 8	Br	Gr	Soil	75	49	124	1.5 :1
	4	7 and 8	Br	Gr	Soil	27	49	76	0.6 :1
						Total 177	196	373	0.903:1
5	1	1 and 2	Gr	Br	Soil	83	6	89	13.8 :1
	2	1 and 2	Gr	Br	Soil	88	16	104	5.5 :1
	3	1 and 2	Gr	Br	Soil	72	8	80	9.0 :1
	4	1 and 2	Gr	Br	Soil	74	0(1)*	74	74.0 :1
						Total 317	30	347	10.567:1
6	1	3 and 4	Br	Gr	Soil	45	47	92	1.0 :1
	2	3 and 4	Br	Gr	Soil	69	48	117	1.4 :1
	3	3 and 4	Br	Gr	Soil	19	50	69	0.4 :1
	4	3 and 4	Br	Gr	Soil	21	50	71	0.4 :1
						Total 154	195	349	0.790:1
7	1	5 and 6	Gr	Br	Grass	43	31	74	1.4 :1
	2	5 and 6	Gr	Br	Grass	42	40	82	1.1 :1
	3	5 and 6	Gr	Br	Grass	22	37	59	0.6 :1
	4	5 and 6	Gr	Br	Grass	37	29	66	1.3 :1
						Total 144	137	281	1.051:1
8	1	7 and 8	Br	Gr	Grass	65	6	71	10.8 :1
	2	7 and 8	Br	Gr	Grass	71	11	82	6.5 :1
	3	7 and 8	Br	Gr	Grass	62	11	73	5.6 :1
	4	7 and 8	Br	Gr	Grass	55	4	59	13.8 :1
						Total 253	32	285	7.906:1

* In this table and tables 8Cv, vi, vii and viii, an asterix signifies that for statistical purposes it was assumed that one larva was taken.

for test 6, X^2_1 = 817.047, $p < 0.001$;
for test 7, X^2_1 = 468.929, $p < 0.001$;
for test 8, X^2_1 = 0.478, $0.5 > p > 0.3$).

Before considering the results of the tests in any more detail, a number of potential sources of error inherent in the experiment should be noted. Because larvae could not be replaced during tests, once predation of larvae had commenced, the actual ratio between the colour types in a sample would rarely be 9:1. Furthermore, as only 50 larvae of the rarer colour type were present in the samples, then, if a larger number of larvae were allowed to be taken during a test, the results would not give a true reflection of a situation in which preferential predation of the rarer colour type was strong. This is particularly relevant to tests 4 and 6 where virtually all the larvae of the rarer colour type were eaten.

The nature of the substrates of the two backgrounds, on which tests were carried out, may also have had an effect on the results, apart from any effect produced by their colour. As mentioned previously, 4th instar larvae are negatively phototactic, and attempt to "hide" during the day. The grass lawn plots appeared to me to afford larvae greater shelter than the soil plots. The evidence from the tests support this view, in that, without exception, more larvae were taken by starlings when the background was soil. These points should be kept in mind when considering the results of the tests.

By considering the results of the eight tests together, a

theory to explain the results was developed. This incorporated six main points.

(i) Overall, starlings tend to preferentially eat larvae of the rarer colour type.

(ii) Generally on grass backgrounds starlings preferentially prey upon Br larvae.

(iii) Generally on soil backgrounds starlings preferentially prey upon Gr larvae.

(iv) The selection against Gr larvae on soil plots is more intense than that against Br larvae on grass lawn plots. This may be either because Gr larvae are relatively more conspicuous on soil than Br larvae are on a grass lawn, or because the lawn substrate enables larvae to hide more efficiently than the soil substrate. There is some circumstantial evidence to support both these possibilities (p. 341 and 345), and both are probably involved.

(v) On grass lawn plots, the tendency to feed preferentially on the rarer larvae is stronger than the tendency to take the more conspicuous Br larvae.

(vi) On soil backgrounds, the tendency to feed preferentially upon the rarer larvae is weaker than the tendency to take the more conspicuous Gr larvae.

We may now consider this theory in relation to individual tests.

Tests 1 and 7 were carried out on grass lawn plots, and Br larvae were rarer than Gr larvae. Therefore, on the basis of the hypothesis outlined above, the expectation would be that both

the selection against the rarer form, and the selection against the more conspicuous larvae, would cause Br larvae to be preyed upon more than expected. In fact in both these tests the ratios between the number of Gr and Br larvae taken were approximately 1:1. In other words, the number of Br larvae taken greatly exceeded the expectation based upon the 9:1 ratio of Gr to Br larvae in the samples put out.

In tests 2 and 8, grass lawn plots were again used, but Gr larvae were rarer than Br larvae in the samples put out. Therefore, the expectation is that selection against the more conspicuous larvae would favour Br larvae. Assuming the overall formation of searching images in each test to be at random with respect to the different morphs, then, if the two selective components are equal in strength, a 9:1 ratio between the numbers of the common and rare colour types eaten would be expected. In fact the ratios for tests 2 and 8 were 7.556:1 and 7.906:1 respectively, which implies that the selection due to preferential predation of the rarer morph is slightly stronger than that due to preferential predation of the more conspicuous morphs.

Tests 3 and 5 involve the reverse situation to the one just described for tests 2 and 8. That is to say, the background was soil, and the majority of larvae were Gr. The selection against the rare form of larvae should cause higher predation of Gr larvae than expected, whilst the selection against the more conspicuous form should cause the Br larvae to be taken in greater numbers than expected. Again, if these two components are of

equal strength, then the overall predation of larvae should be random, and thus produce a 9:1 ratio between the common and rare types. The actual ratios were 12.407:1 for test 3 and 10.567:1 for test 5, which indicates that the selection due to preferential predation of the more conspicuous form is stronger than that due to the preferential predation of the rare form.

Tests 4 and 6 were carried out on soil plots and the majority of the larvae in the samples were Br. Both the preferential predation of the more conspicuous morph, and the preferential predation of the rarer morph, should produce an unexpectedly high level of predation of Gr larvae. In fact, in these tests never more than 3 Gr larvae were left uneaten, and on three of the eight days, all the Gr larvae were taken. Overall in these tests the number of Gr larvae taken exceeded the number of Br larvae taken.

Conclusion

It seems from these tests that, when larvae of two colour types are presented to starlings at high density, in unequal proportions, the commoner morph is favoured due to preferential predation of the rarer morph. However, the background on which larvae are offered also affects the predation, and in tests where the rarer form is less conspicuous than the common form, selection against the more conspicuous form may reduce the preferential predation of the rarer form, or even confer an overall advantage upon the rare form.

The tendency for taking the rarer type of prey supports the theory that, when prey are dense, predators tend to exert

'unifying selection' (Pielowski, 1959, 1961). The behavioural cause of such an effect is probably quite straightforward. In close groups of prey, any different or odd types are likely to be conspicuous, whether this difference is due to colouration, location, or behaviour. It is reasonable to conclude that the birds tended to take these types because they were relatively more conspicuous. This is in accordance with our knowledge that predators preferentially choose non-cryptic prey (see, e.g. Sumner, 1934, Kettlewell, 1958).

Experiment 5.

The aim of this experiment was to discover whether, over a period of time, wild birds feeding on a "population" of dead larvae of two colour types, presented in unequal proportions, preferentially preyed upon the commoner or rarer type, and whether the background on which the samples were offered affected the results.

Experimental design 3 was used, and three series of two tests, each of five days duration, were involved. Each test involved a mixed sample of 200 5th instar larvae. In each case Gr and Br larvae were used, with larvae of one colour nine times as common as the other. The larvae were distributed at random over a 100 square metre plot of land, which meant that the larvae were presented at a density of two larvae per square metre. Natural populations have been recorded in which P. meticulosa larvae were present in both higher and lower densities than this.

Three types of background were used in the tests. These were grass lawn (series 1 tests), bare soil (series 2), and rough

grassland (series 3) of the type described in experimental design 2 (see page 324). All the plots were situated in gardens around the Ringwood area, and they were never less than 0.6 km apart.

The method used to randomise the distribution of prey on the plots was similar to that used by Allen (1972b pp. 58 - 60). In short, this method involved drawing up a square grid of 400 equal squares, each square representing a 0.25 square metre quadrant. A number of photocopies of this grid were produced. 400 playing cards (equal numbers of reds and blacks) were then dealt one by one. Black cards represented quadrants in which larvae were to be absent, whilst red cards represented quadrants in which larvae were to be present. The grid was filled in row by row. Then a second set of 200 playing cards 180 black and 20 red were dealt to give the distribution of the common and rare colour types in the quadrants in which larvae were to be present.

The tests were carried out in November and early December. Plots were prepared by marking them out with black cotton and pegs. They were then thoroughly swept, using a standard sweep-net, on each of the three nights immediately preceding the test, and all larvae of any sort taken were removed. The areas immediately surrounding the plots were also swept. On the evening before the tests were to commence, pegs were sunk into the ground around the perimeter of the plots at 0.5 m intervals, and black cotton was strung between opposite pegs in such a way that a grid of 400 quadrants each 0.25 square metres in area resulted. The

following morning larvae were put out in a distribution determined by the method noted previously. To eliminate possible bias in the positioning of baits relative to ground cover, they were dropped into their appropriate quadrants from waist height. All the black cotton was then removed, the pegs being left in place. The plots were then kept under observation for most of the test, and all birds which were seen to alight on the ground during the test were recorded. At the end of the test cotton was replaced and the numbers of larvae of each colour type remaining were recorded. The process was repeated on each of the four subsequent days, a new prey distribution grid being used each time. Although the amount of time that the samples were out on the plots varied slightly from day to day, the overall duration of all tests was the same.

The results.

Due to the difficulty in estimating the number of birds in the plot at some periods when a large number of birds were present in the proximity of the plots, and in estimating the amount of time that birds actually spent searching for prey whilst they were on the plots, the average predator density on the plots per period of time could not be assessed with any degree of accuracy. However, notes taken on the number of "bird visits", and their average duration, indicated that the bird populations in the proximity of the six plots were fairly similar in constitution with respect to the ground feeding insectivorous birds which visited the plots, particularly in the cases of the more important predators in the tests (i.e. starlings, hedge

sparrows and blackbirds).

The overall numbers of larvae taken during each day of each test are given in Table 8Cv. The ratio between the numbers of the common and rare morphs are also given, (on days when no larvae of one of the morphs were eaten, it was assumed that one was taken to obtain a ratio).

The method of this experiment incorporates a number of variables which could lead to inaccuracies in the results, either when considering data from different tests, or when relating the results to the natural predation of living larvae by birds. These include; (a) variations in the constitution of the bird populations. (b) The immobility of the prey and the actual method used to place larvae in quadrants, which may have led to the dead larvae being more exposed than living larvae which climb down into the low vegetation layers, would normally be. (On the other hand, larval movement which may attract the attention of predators was negligible). (c) The variation in the plots with season. If the basic or average component colours of the vegetation on a plot changed from season to season, then the conspicuousness of the different larval colour types might also change.

To overcome these sources of error, or at least obtain some idea of their effect, three further experiments using Gr and Br larvae, with one morph being nine times as common as the other, were carried out. One of these (experiment 6), involved the same experimental procedure as experiment 5, but was carried out in July and August. The other two used design 2, which involves caged starlings and living larvae, one being carried out

Table 8Cv.

Experiments on selection by birds. Results of Experiment 5, designed to determine whether wild birds preferentially prey upon commoner or rarer larval colour-types, when these are presented at low density, and whether the type of background, on which larvae are offered, affects the result. (Samples of 200 dead larvae were used). (Tests were carried out in November and December, 1976).

Test number	Day number	Common colour type in the sample (180 per sample)	Rare colour-type in the sample (20 per sample)	Nature of plot	Number of larvae taken: common colour type	rare colour type	Total	Ratio between the number of common and rare colour types taken
1	1	Gr	Br	Grass lawn	37	13	50	2.8 :1
	2	Gr	Br	Grass lawn	46	10	56	4.6 :1
	3	Gr	Br	Grass lawn	51	7	58	7.3 :1
	4	Gr	Br	Grass lawn	59	15	74	3.9 :1
	5	Gr	Br	Grass lawn	23	17	40	1.4 :1
					Total 216	62	278	3.484:1
2	1	Br	Gr	Grass lawn	61	8	69	7.6 :1
	2	Br	Gr	Grass lawn	59	6	65	9.8 :1
	3	Br	Gr	Grass lawn	60	5	65	12.0 :1
	4	Br	Gr	Grass lawn	83	4	87	20.8 :1
	5	Br	Gr	Grass lawn	107	3	110	35.7 :1
					Total 370	26	396	14.231:1
3	1	Gr	Br	Soil bed	98	6	104	16.3 :1
	2	Gr	Br	Soil bed	113	4	117	38.3 :1
	3	Gr	Br	Soil bed	165	2	167	82.5 :1
	4	Gr	Br	Soil bed	136	2	138	68.0 :1
	5	Gr	Br	Soil bed	120	0(1)*	120	120.0 :1
					Total 632	14	646	45.143:1
4	1	Br	Gr	Soil bed	48	11	59	4.4 :1
	2	Br	Gr	Soil bed	69	16	85	4.3 :1
	3	Br	Gr	Soil bed	32	20	52	1.6 :1
	4	Br	Gr	Soil bed	81	14	95	5.8 :1
	5	Br	Gr	Soil bed	60	20	80	3.0 :1
					Total 290	81	371	3.580:1
5	1	Gr	Br	Rough grassland	48	6	54	8.0 :1
	2	Gr	Br	Rough grassland	52	3	55	17.3 :1
	3	Gr	Br	Rough grassland	69	1	70	69.0 :1
	4	Gr	Br	Rough grassland	56	0(1)*	56	56.0 :1
	5	Gr	Br	Rough grassland	69	0(1)*	69	69.0 :1
					Total 294	10	304	29.4 :1
6	1	Br	Gr	Rough grassland	39	8	47	4.9 :1
	2	Br	Gr	Rough grassland	43	4	47	10.8 :1
	3	Br	Gr	Rough grassland	41	0(1)*	41	41.0 :1
	4	Br	Gr	Rough grassland	56	2	58	28.0 :1
	5	Br	Gr	Rough grassland	62	0(1)*	56	62.0 :1
					Total 241	14	255	17.214:1

in January and February (experiment 7), the other being carried out in July and August (experiment 8). As these four experiments are inter-related, to avoid repetition I will discuss the results of all four tests together at the end of the explanation of experiment 8. However, I think it would be profitable to the reader if some of the general trends, evident in the results of experiment 5, were mentioned here.

Although there is considerable variation in both the overall predation, and the ratios between the number of common and rare morphs taken between days of a particular test, a cursory glance at the totals from each test indicates that there were also considerable variations in these figures between tests. Generally, the predation of Br larvae was significantly greater than expected on the grasslawn plots, (for test 1, $X_1^2 = 46.748$, $p < 0.001$; for test 2, $X_1^2 = 5.190$, $0.05 > p > 0.02$), whilst the predation of Gr larvae was significantly greater than expected on soil backgrounds, (for test 3, $X_1^2 = 44.038$, $p < 0.001$; for test 4, $X_1^2 = 57.718$, $p < 0.001$). In rough grassland, predation of larvae of the commoner colour type was significantly higher than expected, (for test 5, $X_1^2 = 15.210$, $p < 0.001$; for test 6, $X_1^2 = 5.762$, $0.02 > p > 0.01$).

Experiment 6.

The aim of this experiment was similar to that of experiment 5, but it was carried out in July and August, the intention being to give results which could be compared with those of experiment 5 which was carried out in the winter.

The method used was exactly the same as that employed for

experiment 5, with the following exceptions. (i) When grasslawn plots were used, these were mown nine days prior to the start of the tests so that the depth of grass was similar to that of plots in November and December. (ii) The soil plots were thoroughly weeded prior to the tests. (iii) The overall duration of the tests was greater.

Results.

The results of the number of larvae taken and the ratio between the morphs are given in Table 8Cvi.

The comments made in relation to the general constitution of the bird populations in the proximity of the plots in experiment 5 are also applicable to this experiment (see page 351).

As mentioned previously, the detailed discussion of the results of this experiment will be left until later, but again it is appropriate to mention some of the general trends in the results here.

Again there is considerable variation between tests in both the overall numbers of larvae taken, and in the ratios between the number of the common and rare morphs taken. Generally, on grass lawn backgrounds the number of Br larvae taken was significantly greater than expected, (for test 1, $X_1^2 = 59.394$, $p < 0.001$; for test 2, $X_1^2 = 15.981$, $p < 0.001$), whilst on soil plots the number of Gr larvae eaten was significantly greater than expected, (for test 3, $X_1^2 = 42.561$, $p < 0.001$; for test 4, $X_1^2 = 49.683$, $p < 0.001$). On rough grassland, when Gr larvae were present in excess (test 5), the

Table 8Cvi.

Experiments on selection by birds. Results of Experiment 6, designed to determine whether wild birds preferentially prey upon commoner or rarer larval colour-types, when these are presented at low density, and whether the type of background on which larvae are offered, affects the results. (Samples of 200 dead larvae were used). (Tests were carried out in July and August, 1977).

Test number	Day number	Common colour-type in the sample (180 per sample)	Rare colour-type in the sample (20 per sample)	Nature of plot	Number of larvae taken: common colour type	rare colour type	Total	Ratio between the number of common and rare colour-types taken
1	1	Gr	Br	Grass lawn	51	17	68	3.0 :1
	2	Gr	Br	Grass lawn	40	9	49	4.4 :1
	3	Gr	Br	Grass lawn	50	15	65	3.3 :1
	4	Gr	Br	Grass lawn	68	18	86	3.8 :1
	5	Gr	Br	Grass lawn	46	16	62	2.9 :1
					Total 255	75	330	3.4 :1
2	1	Br	Gr	Grass lawn	78	11	89	7.1 :1
	2	Br	Gr	Grass lawn	91	5	96	18.2 :1
	3	Br	Gr	Grass lawn	67	3	70	22.3 :1
	4	Br	Gr	Grass lawn	84	0(1)*	84	84.0 :1
	5	Br	Gr	Grass lawn	129	2	131	64.5 :1
					Total 449	21	470	21.381:1
3	1	Gr	Br	Soil bed	130	5	135	26.0 :1
	2	Gr	Br	Soil bed	106	4	110	26.5 :1
	3	Gr	Br	Soil bed	99	2	101	49.5 :1
	4	Gr	Br	Soil bed	169	2	171	84.5 :1
	5	Gr	Br	Soil bed	143	0(1)*	143	143.1 :1
					Total 647	13	660	49.769:1
4	1	Br	Gr	Soil bed	44	20	64	2.2 :1
	2	Br	Gr	Soil bed	56	13	69	4.3 :1
	3	Br	Gr	Soil bed	84	19	103	4.4 :1
	4	Br	Gr	Soil bed	71	20	91	3.6 :1
	5	Br	Gr	Soil bed	89	15	104	5.9 :1
					Total 344	87	431	3.954:1
5	1	Gr	Br	Rough Grassland	36	13	49	2.8 :1
	2	Gr	Br	Rough Grassland	41	9	50	4.6 :1
	3	Gr	Br	Rough Grassland	50	4	54	12.5 :1
	4	Gr	Br	Rough Grassland	56	2	58	28.0 :1
	5	Gr	Br	Rough Grassland	50	1	51	50.0 :1
					Total 233	29	262	8.034:1
6	1	Br	Gr	Rough Grassland	48	5	53	9.6 :1
	2	Br	Gr	Rough Grassland	58	5	63	11.6 :1
	3	Br	Gr	Rough Grassland	63	5	68	12.6 :1
	4	Br	Gr	Rough Grassland	56	1	57	56.0 :1
	5	Br	Gr	Rough Grassland	71	1	72	71.0 :1
					Total 296	17	313	17.412:1

number of the rarer Br larvae taken was slightly higher than expected, in relation to the total number of larvae eaten (cf. experiment 5 test 5 p 354), but not significantly so (X^2_1 = 0.332, $0.7 > p > 0.5$). When Br larvae were present in excess (test 6) they were preyed upon to a significantly greater extent than was expected (X^2_1 = 7.259, $0.01 > p > 0.001$).

Experiments 7 and 8.

The aims of these experiments were, firstly, to determine the reactions of caged starlings to mixed samples of living 5th instar Gr and Br larvae, offered in a variety of different environments, at an average density of two larvae per square metre, and secondly, to produce results from experiments which eliminated many of the variables inherent in the methods used in experiments 5 and 6.

Experimental design 2 was used for both experiments. Each experiment consisted of six pairs of tests, (an initial test notified by (a), and a replicate test notified by (b). The initial tests were all carried out on the same day, the replicate tests being carried out 14 days later.

The methods employed in the two experiments were identical, except that experiment 7 was carried out in January and experiment 8 in August. In experiment 8, the grass lawn plots were mown nine days prior to the tests, and the soil plots were weeded prior to the tests, whilst with experiment 7 plots these operations were not required.

Four starlings were used for each test. These had been captured at least six weeks previously and had not been used in any other experiments. Samples of 100 larvae were used. 90 of

one colour and 10 of the other. The larvae were put out by splitting the sample into 4 equal groups, each with approximately equal proportions of Gr and Br larvae, and these were scattered at random over each of the four quarters of the plot area.

A cage with four starlings shut in the shelter box was then placed over the plot areas, and muslin was spread over the cages and fastened to the metal sheeting sunk into the ground around the base of each cage. The starlings were released one hour after the samples were released. Each test lasted for seven hours.

The method of re-collecting samples varied. Preliminary tests had shown that when re-collecting larvae put out on plots of the three types used in these experiments, by sweep-netting and searching, the recapture rate from samples on grass lawn plots was 93.5%, from samples on soil plots 99%, and from samples on rough grassland plots 67.5%, searching at an average rate of two square metres per five minutes. These preliminary tests also showed that re-collection by this method did not favour one colour or another. Therefore, on grass lawns, and soil plots, samples were re-collected using this method, whilst those on rough grassland plots, were re-collected by initial sweeping and subsequent removal of the herbage and top layer of soil with a turf-lifter. The material obtained was then sifted carefully for larvae. This latter method gave a 99.5% recovery rate.

The replicate tests were carried out two weeks after the first series on new plots. In both series of tests, the starlings

in a particular group were given larvae of the two colour types in the same proportions on the same plot type.

Results.

The results of experiment 7 are given in Table 8Cvii, and those of experiment 8 in Table 8Cviii. In both cases the ratio between the common and rare morphs are given, both for the initial and the replicate tests, and for the total results of each test.

Heterogeneity chi-squared tests indicate that in no case was the variation in predation between the runs of a particular test significant.

The results of experiment 7 show that on grass lawn plots, when Gr larvae were in excess, the number of Br larvae taken was greater than expected, although not significantly so, whilst when Br larvae were in excess the number of Br larvae preyed upon was significantly higher than expected. In tests on soil plots, significantly greater numbers of Gr larvae were taken than expected both when these were the common and the rare morph. On rough grass plots, the number of Gr larvae taken was greater than expected, though not significantly so, irrespective of whether Gr was the common or rare morph. (For chi-squared values see Table 8Cvii).

The results for experiment 8 show the same trends for tests 2, 3 and 4 as the equivalent tests of experiment 7. For test 1, as in experiment 7, the number of Br larvae taken is greater than expected, but in this case, the deviation from the expected is significant at the 5% level. In tests 5 and 6, the

Table 8Cvii.

Experiments on selection by birds. Results of Experiment 7, designed to determine whether caged starlings preferentially prey upon commoner or rarer larval colour-types, when these are presented at low density, and whether the type of background, on which larvae are offered, affects the result. (Samples of 100 living larvae were used). (Tests were carried out in November and December, 1976).

Test number	Common colour type in the sample (90 per sample	Rare colour type in the sample (10 per sample	Nature of the plot	Number of the common colour type taken	Number of the rare colour type taken	Total	Ratio between the number of common and rare colour-types taken	Chi-squared and probability values taking the expected ratio between the common and rare forms to be 9:1 (Degrees of freedom equals 1) Chi-squared	Probability
1(a)	Gr	Br	Grass lawn	66	9	75	7.3 :1	0.333	0.7 - 0.5
(b)	Gr	Br	Grass lawn	57	8	65	7.1 :1	0.385	0.7 - 0.5
			Total	123	17	140	7.235:1	0.714	0.5 - 0.3
2(a)	Br	Gr	Grass lawn	79	2	81	39.5 :1	5.104	0.05 - 0.02
(b)	Br	Gr	Grass lawn	86	2	88	43.0 :1	5.838	0.02 - 0.01
			Total	165	4	169	41.25 :1	10.941	< 0.001
3(a)	Gr	Br	Soil bed	89	0(1)*	89	89.0 :1	9.889	0.01 - 0.001
(b)	Gr	Br	Soil bed	87	1	88	87.0 :1	7.682	0.01 - 0.001
			Total	176	1	177	176.0 :1	17.507	< 0.001
4(a)	Br	Gr	Soil bed	51	10	61	5.1 :1	2.770	0.1 - 0.05
(b)	Br	Gr	Soil bed	38	10	48	3.8 :1	6.259	0.02 - 0.01
			Total	89	20	109	4.45 :1	8.441	0.01 - 0.001
5(a)	Gr	Br	Rough grassland	37	2	39	18.5 :1	1.028	0.5 - 0.3
(b)	Gr	Br	Rough grassland	31	1	32	31.0 :1	1.681	0.2 - 0.1
			Total	68	3	71	22.667:1	2.631	0.2 - 0.1
6(a)	Br	Gr	Rough grassland	22	4	26	5.5 :1	0.571	0.5 - 0.3
(b)	Br	Gr	Rough grassland	27	3	30	9.0 :1	0.014	0.95 - 0.90
			Total	49	7	56	7.0 :1	0.389	0.7 - 0.5

Table 8Cviii.

Experiments on selection by birds. Results of Experiment 8, designed to determine whether caged starlings preferentially prey upon commoner or rarer larval colour-types, when these are presented at low density, and whether the type of background, on which larvae are offered, affects the result. (Samples of 100 living larvae were used). (Tests were carried out in July and August, 1977).

Test number	Common colour type in the sample (90 per sample	Rare colour type in the sample (10 per sample	Nature of the plot	Number of the common colour type taken	Number of the rare colour type taken	Total	Ratio between the number of common and rare colour-types taken	Chi-squared and probability values taking the expected ratio between the common and rare forms to be 9:1 (Degrees of freedom equals 1) Chi-squared	Probability
1(a)	Gr	Br	Grass lawn	49	10	59	4.9 :1	3.166	0.1 - 0.05
(b)	Gr	Br	Grass lawn	54	10	64	5.4 :1	2.25	0.2 - 0.1
			Total	103	20	123	5.15 :1	5.356	0.05 - 0.02
2(a)	Br	Gr	Grass lawn	86	1	87	86 :1	7.572	0.01 - 0.001
(b)	Br	Gr	Grass lawn	82	2	84	41 :1	5.418	0.02 - 0.01
			Total	168	3	171	56 :1	12.918	< 0.001
3(a)	Gr	Br	Soil bed	90	2	92	45 :1	6.261	0.02 - 0.01
(b)	Gr	Br	Soil bed	87	0(1)*	87	87 :1	9.667	0.01 - 0.001
			Total	177	2	179	88.5 :1	15.692	< 0.001
4(a)	Br	Gr	Soil bed	42	10	52	4.2 :1	4.923	0.05 - 0.02
(b)	Br	Gr	Soil bed	49	10	59	4.9 :1	3.166	0.1 - 0.05
			Total	91	20	111	4.55 :1	7.929	0.01 - 0.001
5(a)	Gr	Br	Rough grassland	27	5	32	5.4 :1	1.125	0.3 - 0.2
(b)	Gr	Br	Rough grassland	24	3	27	8.0 :1	0.037	0.9 - 0.8
			Total	51	8	59	6.375:1	0.831	0.5 - 0.3
6(a)	Br	Gr	Rough grassland	36	1	37	36 :1	2.189	0.2 - 0.1
(b)	Br	Gr	Rough grassland	35	1	36	35 :1	2.086	0.2 - 0.1
			Total	71	2	73	35.5 :1	4.275	0.05 - 0.02

situation is almost exactly the reverse of that in the equivalent tests of experiment 7, predation of brown larvae being greater than expected. In test 5, this deviation is not significant, whilst in test 6 it is. (For chi-squared values see Table 8Cviii).

Discussion of experiments 5 - 8 (inclusive).

Experiments 7 and 8 were designed in part to reduce some of the errors inherent in experiments 5 and 6 relating to the day to day variation in the composition of the predator populations of tests in these experiments, and the homogeneity between results of the two "runs" for each test in experiments 7 and 8 indicates that this aim was achieved.

The results of these four experiments are rather complex and include six basic variables which must be taken into account, these being; (i) the colours of the larvae; (ii) the frequency of the larval colour types in the samples; (iii) the state of prey, i.e. whether larvae were living or dead; (iv) the number and type of predator involved; (v) the type of background upon which the prey samples were offered; (vi) the season during which the experiments were carried out.

To make the explanation of the results easier for the reader to comprehend, I will initially outline the hypothesis which was formed as a result of these experiments, thereafter considering the potential errors in the experimental procedure, and the results of the experiments, in relation to this theory.

The hypothesis incorporates four main points.

(1) On grass lawn and soil plots, larvae of the more

conspicuous colour type (i.e. Br and Gr respectively), are preyed upon in greater numbers than expected, irrespective of season, larval stage, or the constitution of the predator population.

(2) On grass lawn and soil plots, when the more conspicuous colour type is nine times as common as the other colour type in the sample, the ratio between the number of this common form which are eaten and the number of the rarer, less conspicuous morph which are eaten, increases over a period of days, when wild birds are predators, irrespective of season. It is suggested that this is because birds become relatively more efficient in their predation of the common conspicuous larvae due to the formation of searching images for this morph. On the other hand, in similar conditions, when the less conspicuous larvae are nine times as common as the more conspicuous morph no such trend is apparent, which implies that the preferential predation of the common morph is balanced by the preferential predation of the more conspicuous morphs when these are dissimilar.

(3) On rough grassland plots, in winter, Gr larvae are slightly more conspicuous to birds than Br larvae, the reverse being true in summer.

(4) On rough grassland plots, initially predation of larvae of the more conspicuous colour type is greater than expected, whether this is the commoner or rarer morph. However, over a period of consecutive days, the predation of the commoner form by wild birds increases, whilst that of the rare form decreases. This happens even when the common morph is slightly

less conspicuous than the rare form. This indicates that, when the difference in the crypsis of the two forms is slight, searching image formation over-rides the camouflage effect, so that the rarer form obtains a selective advantage.

Two very important inter-related sources of error inherent in the experimental method used for tests in experiments 5 and 6 have not yet been mentioned and should be considered here. Firstly, although the plots used in the experiment were swept and searched for larvae before the tests, there would have undoubtedly been some natural Lepidoptera larvae and other types of potential prey for the birds on the plots, particularly those in which the vegetation was of rough herbage. Furthermore, no account was taken of potential prey in the surrounding areas. As the wild birds did not feed only inside the plots, then, depending upon the nature of prey outside the plots and non-sample prey inside the plots, these might cause birds to form searching images not correlated to the ratio of the larval colour types in the samples.

Secondly, the possibility that the birds may have had preformed searching images at the start of tests is not taken into account in the hypothesis.

In fact, the day 1 results of tests 5 and 6 in experiments 5 and 6, upon which point 3 of the hypothesis was based, could be accounted for by this second source of error, rather than on the basis of the conspicuousness of prey, if, in winter the wild birds initially had a slight bias towards taking Gr larvae, and in summer towards taking Br larvae, due to preformed searching images. Experiments 9 and 10, which will be outlined

later, were designed to confirm point 3 of the hypothesis, and the results obtained did so (see pages 367 - 370). Furthermore, the results of tests 5 and 6 in experiments 7 and 8 support the 3rd point of the hypothesis, (although, due to the smallness of the samples, not conclusively), and in these tests the errors outlined previously would not be relevent.

Tests 1, 2, 3 and 4 of experiments 5 and 6 indicate that whilst birds may form searching images for larvae of the common colour type, they do not do so when the common colour type is markedly less conspicuous than the rare colour type.

The most important results in relation to the situation of P. meticulosa larvae in the wild are undoubtedly those of the fifth and sixth tests in each experiment, as these were carried out on plots with vegetation of the type in which P. meticulosa larvae are usually found.

From the results of these tests it may be deduced that birds may form searching images for larvae of a common colour type, even when these are marginally less conspicuous than larvae of a rarer colour type. This is indicative of apostatic selection, which may, as Clarke (1962a) has pointed out, maintain a colour polymorphism in balance. Furthermore, the fact that Gr larvae were preyed upon more than expected in winter, and Br larvae were preyed upon more than expected in summer, suggests that Br larvae are more cryptic than Gr larvae in winter, the reverse being the case in summer. As will be seen, the results of experiments 9 and 10 support this suggestion. This feature may explain the seasonal variation in the frequencies of the alleles of genes A and B. In chapter 5 it was shown that the

frequencies of alleles a^+ and b^o fall between the spring and autumn main emergences, and rise between the autumn and spring main emergences. As both b^o when homozygous and a^+ produce brown larvae, whilst b' when homozygous causes larvae to be green in the 4th instar, and a when homozygous leaves the larval colour under the control of genes B, D and E, in the 5th instar, then the seasonal variations in the frequencies of these alleles are consistent with the suggestion that selection by bird predation favours Br larvae in the winter and Gr larvae in the summer.

It may also be noted from the results of tests 5 and 6, that the preferential initial predation of Br larvae in summer appears to be relatively stronger than that of green larvae in winter, though not significantly so. This feature will be discussed later in relation to experiments 9 and 10 (pp. 367-370).

It appears then, that the maintenance of the polymorphisms and the seasonal variations in phenotypic frequencies may depend, to a large extent, on two aspects of bird predation, these being searching image formation leading to apostatic selection, and the tendency for birds to preferentially prey upon more conspicuous larvae. Experiments 5 to 8 deal with an artificial situation in that only two of the larval colour types were present in the samples offered to the birds and the ratios of these two colour types in the samples were not representative of the ratio between these colour types in the wild. It thus became imperative to discover the effect of birds on samples of larvae in which all the larval colour types were represented, at phenotypic frequencies comparable with those found in the

wild. Furthermore, the larvae used were all in the 5th instar, and, although the results of experiment 1 show that birds do not treat 4th instar larvae differently from 5th instar larvae with respect to prey colour type preferences, the reaction of birds to 3rd instar larvae is not known, and the treatment of 4th and 5th instar larvae may be different in more natural conditions than those prevalent in experiments 1 and 2. Thus, when testing the effect of birds on samples of larvae in which morph frequencies were similar to those found in natural populations, tests were carried out on 3rd, 4th and 5th instar samples individually, (experiments 11 and 12).

Experiments 9 and 10.

These experiments were designed to discover whether caged birds prey upon green larvae more heavily than brown larvae in winter, and brown larvae more heavily than green in summer, when mixed samples of equal numbers of these two larval types were offered to the birds, on rough grassland plots.

The methods used for experiments 9 and 10, were the same as those used in experiments 7 and 8 respectively, with the exception that, in all cases, the samples were made up of equal numbers of Gr and Br larvae, and, that tests were only carried out on rough grassland plots. In each experiment, two tests (tests 1 and 2) each using four birds, were carried out simultaneously, and duplicate tests (tests 3 and 4) were carried out 14 days later. Experiment 9 was carried out in February and experiment 10 at the end of August.

Results.

The results of both these experiments are given in Table 8Cix/x.

In every test in experiment 9, the number of green larvae taken was greater than expected, whilst in experiment 10 tests, the number of Br larvae taken was greater than expected. The departure from the expected 1:1 ratio, based on random predation, was only significant for one of these tests (experiment 10 test 3). (The chi-squared and probability values for these and other tests mentioned in the text relating to these experiments are given in Table 8Cix/x). However, as heterogeneity chi-squared tests showed that the results of tests in a particular experiment did not vary significantly, it is reasonable to compare the total number of larvae of each colour type taken with the expected for each experiment. The results of experiment 9 show that Gr larvae are taken significantly more often than Br larvae in winter, whilst experiment 10 shows that Br larvae are taken significantly more often than Gr in summer. The only realistic explanation of this feature is that Br larvae are less conspicuous than Gr larvae in winter, in a rough grassland habitat, the reverse being the case in summer.

The suggestion made, in relation to the results of tests in experiments 5 to 8 inclusive, that the preferential predation of Br larvae in summer is relatively stronger than that of Gr larvae in winter, (see p. 363) is not reinforced by the results of experiments 9 and 10. Indeed, analysis of these results shows that the preferences for different colour-types at different

Table 8Cix/x.

Experiments on selection by birds. Results of experiments 9 and 10, designed to investigate predation by starlings on samples of equal numbers of green and brown 5th instar larvae, in winter and summer.

Experiment/ test numbers	Number of larvae taken			Ratio between numbers of green and brown larvae taken	Chi-squared and probability values taking the expected ratio between colour types as 1:1		
	Green	Brown	Total		Chi-squared	D.F.	Probability
Winter							
9/1	39	27	66	1:0.692	2.182	1	0.2 - 0.1
9/2	33	22	55	1:0.667	2.2	1	0.2 - 0.1
9/3	28	21	49	1:0.75	1.0	1	0.5 - 0.3
9/4	36	26	62	1:0.722	1.613	1	0.3 - 0.2
Totals	136	96	232	1:0.706	6.897	1	< 0.001
					heterogeneity 0.098	3	> 0.99
Summer							
10/1	24	31	55	1:1.292	0.891	1	0.5 - 0.3
10/2	21	33	54	1:1.571	2.667	1	0.2 - 0.1
10/3	17	32	49	1:1.882	4.592	1	0.05 - 0.02
10/4	20	28	48	1:1.4	1.333	1	0.3 - 0.2
Totals	82	124	206	1:1.512	8.563	1	0.01 - 0.001
					heterogeneity 0.920	3	0.9 - 0.8

seasons are almost exactly equivalent. This can best be shown by a heterogeneity chi-squared test in which the pooled chi-squared value is obtained by summing the total number of larvae of the more commonly taken morph from each experiment to produce one class, and summing the numbers of larvae of the less commonly taken morphs from each experiment to produce a second class, and then comparing these two taking the expected ratio between the two as 1:1. This gives a heterogeneity chi-squared value of 0.108 which with 1 degree of freedom gives a probability of between 0.8 and 0.7.

Experiments 11 and 12.

The aim of these experiments was to determine whether starlings preyed differently upon larvae of various colour types in rough grassland, when the larval colour types were present on plots at frequencies approximately equal to those found in wild populations.

Materials and Methods for Experiment 11.

Experimental design 2 was used. The experiment involved three series of four tests. In series A tests, 3rd instar larvae were used, 4th and 5th instar larvae being used in series B and C tests respectively. 12 rough grassland plots were prepared. (For a description of this preparation see p. 182 and pp. 323-325). Three starlings were used in each test. It was decided to use caged rather than wild birds because experimental design 2 allows greater control of the experiment to be maintained, and reduces many of the variables inherent in experimental design 3. Furthermore, due to the behavioural

difference between larvae with c^+ and those homozygous for c, it was important to use living larvae for tests.

The proportions of the larval colour types in the samples were based on the proportions of phenotypes in the AF2 samples obtained by sweep-netting in the Ringwood area between June and August, 1977 (see Table 5Biii p 236). In the 3rd instar samples, two types of green larvae were present in the samples. Three-quarters were known to contain c^+ in their genotype, whilst the other quarter were homozygous for c, (being green due to the fact that they were also homozygous for b'). To distinguish these two types of larvae, one type in each sample was marked with a small spot of green cellulose paint[1]. To reduce the chance of this spot making larvae more conspicuous to the birds, the spot was placed on the underside of the larvae between the first pair of prolegs, and the type of larva marked was alternated in the four tests.

Due to the rarity of the three yellow larval colour-types, and particularly that of YG and YB larvae, the phenotypic frequencies of all three yellow colour types were summed, and the resulting frequency was represented in the samples by PY larvae only. The exact constitution of each sample is given in Table 8Cxi.

In experiments 5 - 10 inclusive, larvae were distributed over plot areas at an average density of two per square metre; however, during sweep-netting and "hands and knees" searching

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1 Brower (1960) working on starlings and meal worms showed that marking meal worms with green cellulose paint did not reduce their palatability to starlings.

operations in the spring and summer of 1977, it was discovered that larvae of P. meticulosa may occur at an average density as high as five larvae per square metre over an area of 100 square metres, and thus, 200 larvae were used in each sample which is equivalent to an average larval density of four per square metre on the plots.

Each test started at 7.30 a.m. and continued until 4.30 p.m. the following day. The four tests in a series all used the same starlings and were carried out over a period of eight consecutive days between 15th and 30th August, series A commencing on the 15th, series B on the 19th and series C on the 23rd.

Again the distribution of larvae was randomised by splitting the samples into four groups with the colour types in roughly equal proportions in each group and then scattering one group over each quarter of the plots.

Materials and Methods for experiment 12.

The materials and methods used for experiment 11 were also used for experiment 12 with the following exceptions. (a) The proportions of the larval colour types in samples were based on the proportions of larval colour types observed in the data from larvae obtained by sweep-netting in the Ringwood area between December, 1976 and April, 1977. (b) The tests were carried out between 26th December, 1977 and 10th January, 1978, series A commencing on the 26th December, series B on 30th December and series C on 3rd January.

Results, analysis of data and preliminary discussion.

The results of experiment 11 are given in Table 8Cxi, and those from experiment 12 in Table 8Cxii. In both tables the number of larvae of each colour type in samples is given, together with the percentage of the sample that this number represents given in parentheses. The total number of larvae which were homozygous recessive is also given for 3rd instar samples. The number of larvae of each colour type taken by birds in each test is then given, (the number of larvae homozygous recessive for gene C which were taken in series A tests, also being given), followed by the total number of larvae taken. There then follow two sets of proportions. The first of these gives the number of each colour type taken as a percentage of the number of the relevant colour type in the sample. The second gives the number of larvae of each colour type taken as a percentage of the total number of larvae taken.

The results of experiments 11 and 12 will be considered side by side in the following text, and, when referring to results from a particular test, the experiment number and series letter will be given with the test number, (e.g. the first test involving 3rd instar larvae in summer will be written as test 11-A-1).

Throughout the explanation of the data analysis, and the subsequent discussion of the two experiments, reference is made, in the main, to the predation of Gr, OL, and Br larval colour types. Little reference is made to the predation of PY larvae. This is because the numbers of PY larvae were so small

Table 8Cxi.

Experiments on selection by birds. Results of Experiment 11, designed to investigate predation by starlings, in summer, on samples of larvae, in which colour types were present at natural frequencies, on rough grassland plots.

Series number	Constitution of samples: Number of larvae of colours: Gr (with c^+)	Gr (with cc)	OL	Br	PY	Test Number	Number of larvae of each colour type taken: Gr (with c^+)	Gr (with cc)	OL	Br	PY	(Total with cc)	Total taken	Percentage of larvae put out taken for each colour: Gr (with c^+)	Gr (with cc)	OL	Br	PY	(Total for cc)	Number of each colour taken as a percentage of the total number taken: Gr (with c^+)	Gr (with cc)	OL	Br	PY	(Total for cc)
A (3rd instar larvae)	128 (64%)	43 (21.5%)	22 (11%)	6 (3%)	1 (0.5%)	(1	4	6	3	2	0	11	15	3.1	14.0	13.6	33.3	0	15.3	26.7	40	20	13.3	0	73.3
						(2	4	3	3	0	0	6	10	3.1	7.0	13.6	0	0	8.3	40	30	30	0	0	60
						(3	11	3	0	0	0	3	14	8.6	7.0	0	0	0	4.2	78.6	21.4	0	0	0	21.4
						(4	14	2	0	1	0	3	17	10.9	4.7	0	16.7	0	4.2	82.4	11.8	0	5.9	0	17.6
Totals	512	172	88	24	4		33	14	6	3	0	23	56	6.4	8.1	6.8	12.5	0	8.0	58.9	25	10.7	5.4	0	41.1
B (4th instar larvae)	81 (40.5%)		91 (45.5%)	24 (12%)	4 (2%)	(1	23		28	15	0		66	28.4		30.8	62.5	0		34.8	42.4	22.7	0		
						(2	28		33	10	1		72	34.6		36.3	41.7	25		38.9	45.8	13.9	1.4		
						(3	38		39	9	0		86	46.9		42.9	37.5	0		44.2	45.3	10.5	0		
						(4	42		45	8	0		95	51.9		49.5	33.3	0		44.2	47.4	8.4	0		
Totals	324		364	96	16		131		145	42	1		319	40.4		39.8	43.75	6.25		41.1	45.5	13.2	0.3		
C (5th instar larvae)	34 (17%)		37 (18.5%)	124 (62%)	5 (2.5%)	(1	10		12	57	2		81	29.4		32.4	46.0	40		12.3	14.8	70.4	2.5		
						(2	5		6	68	1		80	14.7		16.2	54.8	20		6.3	7.5	85	1.3		
						(3	2		3	79	0		84	5.9		8.1	63.7	0		2.4	3.6	94.0	0		
						(4	0		1	85	0		86	0		2.7	68.5	0		0	1.2	98.2	0		
Totals	136		148	496	20		17		22	289	3		331	12.5		14.9	58.3	15		5.1	6.6	87.3	0.9		

Table 8Cxii.

Experiments on selection by birds. Results of Experiment 12, designed to investigate predation by starlings, in winter, on samples of larvae, in which colour types were present at natural frequencies, on rough grassland plots.

Series number	Constitution of samples: Number of larvae of colours: Gr+ (with c+)	Gr (with cc)	OL	Br	PY	Test Number	Number of larvae of each colour type taken: Gr (with c+)	Gr (with cc)	OL	Br	PY	(Total with cc)	Total taken	Percentage of larvae put out taken for each colour: Gr (with c+)	Gr (with cc)	OL	Br	PY	(Total for cc)	Number of each colour taken as a percentage of the total number taken: Gr (with c+)	Gr (with cc)	OL	Br	PY	(Total for cc)
A (3rd inster larvae)	130 (65%)	43 (21.5%)	20 (10%)	5 (2.5%)	2 (1%)	(1	7	8	4	1	0	13	20	5.4	18.6	20	20	0	18.6	35	40	20	5	0	65
						(2	16	4	2	0	0	6	22	12.3	9.3	10	0	0	8.6	72.7	18.2	9.1	0	0	27.3
						(3	23	2	1	0	0	3	26	17.7	4.7	5	0	0	4.3	88.5	7.7	3.8	0	0	11.5
						(4	34	2	0	0	0	2	36	26.2	4.7	0	0	0	2.9	94.4	5.6	0	0	0	5.6
Totals	520	172	80	20	8		80	16	7	1	0	24	104	15.4	9.3	8.8	5	0	8.6	76.9	15.4	6.7	1	0	23.1
B (4th instar larvae)	86 (43%)		88 (44%)	22 (11%)	4 (2%)	(1	42		36	8	1		87	48.8		40.9	36.4	25		48.3		41.4	9.2	1.1	
						(2	44		39	3	0		86	51.2		44.3	13.6	0		51.2		45.3	3.5	0	
						(3	48		49	0	0		97	55.8		55.7	0	0		49.5		50.5	0	0	
						(4	57		52	0	0		109	66.3		59.1	0	0		52.3		47.7	0	0	
Totals	344		352	88	16		191		176	11	1		379	55.5		50.0	12.5	6.3		50.4		46.4	2.9	0.3	
C (5th instar larvae)	33 (16.5%)		36 (18%)	127 (63.5%)	4 (2%)	(1	20		19	50	2		91	60.6		52.8	39.4	50		22.0		20.9	54.9	2.2	
						(2	17		13	58	1		89	51.5		36.1	45.7	25		19.1		14.6	65.2	1.1	
						(3	15		8	67	0		90	45.5		22.2	52.8	0		16.7		8.9	74.4	0	
						(4	15		2	80	0		97	45.5		5.5	63.0	0		15.5		2.1	82.5	0	
Totals	132		144	508	16		67		42	255	3		367	50.8		29.2	50.2	18.8		18.3		11.4	69.5	0.8	

that no statistically valid deductions could be drawn from the results involving PY larvae. However, it is perhaps worth noting that the average proportion of PY larvae put out which were taken in all the tests (8 out of 80) was lower than that for any other colour type. The reason for this could be either (a) that the birds do not associate items which are yellow with edibility, (although the results of experiments 1 and 2 indicate that this is not the case);or(b) yellow larvae are less conspicuous to birds than other morphs in rough grassland vegetation, (my own subjective view is that yellow larvae were more conspicuous than other larval colour types, in this type of vegetation);or(c) that birds prey on other commoner morphs in preference to PY larvae because they form searching images for the commoner larval types.

Looking first mainly at the results of the A series of tests, it is notable that far fewer larvae were taken in these tests than in the B or C series. This is probably due to the size of the 3rd instar larvae. These larvae, being relatively small, would be more difficult to find than those of later instars used in Series B and C tests. Furthermore, because their small size would not make them very profitable prey items for the birds, it seems logical to assume that the birds are less likely to form searching images for these larvae than for larger larvae. Nevertheless, the results of the 11-A and 12-A tests do provide some indication that the starlings did form searching images for the commoner type of larvae. In tests 11-A-1 and 12-A-1, the number of larvae, homozygous for cc,

which were taken was significantly higher than expected (for 11-A-1, X_1^2 = 9.689, $0.01 > p > 0.001$; for 12-A-1, X_1^2 = 7.912, $0.01 > p > 0.001$). However, in the following tests there was a progressive drop in the proportion of larvae of all the colour types that were homozygous recessive for gene C that were eaten, and a progressive rise in the proportion of c^+ larvae taken, in relation to both the number of the relevant larval type put on the plots, and the total number of larvae taken, so that, in tests 11-A-4 and 12-A-4, the number of larvae homozygous recessive for gene C which were taken was lower than expected, although, in the case of test 11-A-4, not significantly so, (for 11-A-4, X_1^2 = 2.251, $0.2 > p > 0.1$; for 12-A-4, X_1^2 = 13.719, $p > 0.001$).

This feature is not caused totally by a switch in the preferential predation from larvae of non-green colour types to green larvae, because, although the proportion of the total number of larvae eaten in each test that were non-green in general did drop from one test to the next, the proportion of green larvae homozygous for gene C which were taken also dropped. It therefore seems probable that the results of these tests do not involve a change in searching image with respect to the colour of the prey being sought, but rather, they involve a change in the basic searching behaviour, so that the birds change from looking for larvae in the low herbage layers to seeking them in the higher herbage layers.

One of the behavioural characteristics of P. meticulosa larvae may be partly the cause of such a change. When vegetation upon which a larva is resting is disturbed, the larva immediately

curls up and drops from the vegetation. If a starling feeding on the ground were to brush against a piece of vegetation on which a larva was situated, the ensuing movement produced by the larva's drop to the ground, might attract the starling's attention, and then induce the bird to start searching for similar prey higher up in the herbage. Direct observation of the birds feeding behaviour in the rough vegetation was virtually impossible, and so obviously this idea is pure speculation.

It is also significant to note that only 1 out of 104 larvae taken in the winter (11-A) tests was brown whilst three brown out of a total of 56 larvae were taken in summer (12-A tests). Whilst these figures are too small to be taken as conclusive evidence, they lend support to the hypothesis that brown larvae are less conspicuous in winter than they are in summer in rough grassland vegetation.

These results suggest that larvae which inhabit the lower herbage layers during the day, obtain an advantage from this habit when compared with the commoner larvae which remain in the higher herbage layers during the day. On the other hand, predation was comparatively slight when compared with the predation in other tests. Furthermore, it was in part "forced", as the area over which the starlings could search for prey was severely limited, and many of the other species of organism, which the starlings might have utilized as prey items in a rough grassland habitat, were eliminated from the plot areas by the nature of the plot preparation. Therefore, it is doubtful whether predation by starlings contributes significantly to the mortality rate in 3rd instar larvae of <u>P. meticulosa</u> in the wild.

Turning now to the results of the 11-B and 12-B tests: again we have evidence that the birds form searching images. In both these series of tests the commoner larval colour types (Green and Olive) are taken in greater numbers in the 4th test of the series than in the first, indicating that the starlings have formed searching images for these colour types. On the other hand, there is a progressive drop in the number of brown larvae taken in consecutive tests. In the case of the 12-B experiments, initially more Br larvae were taken than was expected, the other three morphs being taken in smaller numbers than expected. This indicates that not only are Br larvae significantly more conspicuous than green larvae in the summer ($X^2_3 = 8.327$, $0.05 > p > 0.02$), but also that they are more conspicuous than OL larvae. The results of test 11-B-1 also indicate that OL larvae are more conspicuous than Gr larvae, the proportion of the OL larvae taken by birds being greater than the proportion of the Gr larvae taken. However, the figures in this case are not significant ($X^2_1 = 0.079$, $0.8 > p > 0.7$).

Furthermore, if the advantage conferred upon Gr larvae over OL larvae, by being less conspicuous, is real, it is lost when birds form searching images for these two forms, as, in tests 11-B-3 and 11-B-4, the proportion of Gr larvae taken was greater than the proportion of OL larvae taken in relation to the numbers of these colour types put out. This indicates that the starlings form "stronger" searching images for Gr larvae than for OL larvae. The reason for this is not known and I can not think of a plausible theory for its cause. However, as more

OL than Gr larvae were present in the 11-B samples, we are not dealing with a situation where the commoner colour-type promotes stronger searching images. Indeed, as the differences between the proportions are not significant (heterogeneity chi-squared considering all 11-B tests; $X^2_3 = 0.293$, $0.98 > p > 0.95$; heterogeneity considering tests 11-B-1 and 11-B-4, $X^2_1 = 0.125$, $0.8 > p > 0.7$), it is probable that these results are due to chance.

In the 11-B tests 12% of larvae put out were Br, and, whilst in the first two tests (11-B-1 and 11-B-2) more Br larvae were taken than expected, in the later tests (11-B-3 and 11-B-4), less Br larvae were taken than expected. It is notable that the excess of larvae taken in test 11-B-1 over the expected number is highly significant ($X^2_1 = 7.249$, $0.01 > p > 0.001$), and that other experiments, notably experiments 6, 8 and 10, have indicated that Br larvae are more conspicuous than Gr larvae in the summer months on rough grassland. These facts, coupled with the increase in the predation of Gr and OL larvae, strongly suggest that we have here a demonstration that the formation of searching images for the common colour types of a species can confer an advantage on more conspicuous but rarer colour types, and, therefore, this is an example of apostatic selection. This suggestion is further endorsed by the results of the 12-B tests.

In 12-B-1, less Br larvae were taken than expected on the basis of random predation, 9.2% of larvae taken being Br, whereas 11% of the sample were of this colour. This result may be explained by the suggestion, based on earlier

experiments, that Br larvae are less conspicuous than Gr larvae in winter on a rough herbage background. If the numbers of larvae of each colour type which were taken are considered as proportions of the number of larvae of that colour type put out, we can see that the proportion of Gr larvae taken was greater than that of OL larvae, which in turn was greater than the proportion of Br larvae taken. This implies that OL larvae are more conspicuous than Br larvae, and less conspicuous than Gr larvae in winter.

In subsequent 12-B tests the number of Br larvae which were eaten fell, so that, in tests 12-B-3 and 12-B-4, none were taken. At the same time there was a rise in the number of both Gr and OL larvae eaten. Here again, we have the formation of searching images for the two common colour types. This situation again constitutes apostatic selection as the formation of these searching images confers an advantage on the colour type which to the predator stands out from the effective norm (i.e. brown). If the predation levels of Br larvae in tests 11-B-4 and 12-B-4 are compared, then the predation of this colour type is shown to be significantly greater in summer than winter once the birds have formed searching images, ($X_1^2 = 9.624$, $0.01 > p > 0.001$). Furthermore, the overall predation of Br larvae was significantly greater in the 11-B tests than in the 12-B tests ($X_1^2 = 26.073$, $p < 0.001$).

On the other hand, the overall predation of Br larvae in the first tests of these series (i.e. 11-B-1 and 12-B-1) is not significantly different from that expected on the basis that predation is random with respect to colour type ($X_1^2 = 1.949$,

$0.2 > p > 0.1$). This feature, coupled with the fact that the results of 11-B-1 and 12-B-1 are heterogeneous with respect to the number of Br larvae taken in relation to the proportion in the sample which were Br. ($X^2_1 = 5.613$, $0.02 > p > 0.01$), indicates that, if the effects of searching image formation are disregarded for the moment, the seasonal variation in the allelic frequencies of b' and b^o could be explained by the relative conspicuousness of Br, OL and Gr larvae in winter and summer. However, it has been noted that selection on this type of basis could not maintain a polymorphism, since it would be frequency independant (Cain et al., 1960). It may then be argued that apostatic selection, coupled with this system, may maintain the polymorphism, because, although on the basis of the 11-B and 12-B results it would appear that Br larvae become selectively favoured in both summer and winter, in the wild larvae of other species would influence the strength of search image formation. Furthermore, as has already been noted, the larvae of P. meticulosa present in a population at one time are not all in the same instar, and as the colour type frequencies vary between instars, then larvae of other instars, particularly the 5th instar will affect the formation of searching images. Birds certainly do prey on these larvae in the wild, and Experiments 5 and 6 indicate that wild bird populations can cause apostatic selection. However, before pursuing this dicussion of the selective advantages and disadvantages conferred upon the colour-types controlled by b' and b^o in the 4th instar by bird predation any further, we should consider the situation in the 5th instar where these

alleles are also expressed phenotypically in some circumstances.

Therefore, we now move to a consideration of the results of the 11-C and 12-C tests.

In natural populations, the majority of 5th instar larvae are Br. The results of 11-C-1 and 12-C-1 again show that the number of Br larvae taken in the first test of a series was greater than expected in summer, and less than expected in winter, the deviation from the expected being close to significance in both cases (for 11-C-1, $X_1^2 = 2.4087$, $0.2 > p > 0.1$; for 12-C-1, $X_1^2 = 2.8734$, $0.1 > p > 0.05$). These results agree with the suggestion that Br larvae are more conspicuous than Gr larvae in summer and less conspicuous in winter. These initial tests also indicate that the relative conspicuousness of OL larvae is intermediate to those of Gr and Br larvae in both winter and summer. Furthermore, both series of tests show a progressive rise in the number of Br larvae taken in consecutive tests, and a progressive drop in the number of other colour types taken, which indicates that the starlings formed searching images for larvae of the commonest colour type.

It is notable when considering the drops in the numbers of OL and Gr larvae taken, that the drop in Gr larvae is greater in the 11-C tests than in the 12-C tests, in the former case the drop being from 29.4% to 0% of the number put out, and in the latter, from 60.6% to 45.5%. This would be because Gr larvae are more conspicuous in winter than other colour types, and thus, they would have appeared to the starlings to be commoner than they really were in the 12-C tests. On the other hand, because

Gr larvae are less conspicuous than other colour types in summer, they would have appeared to be less common than they were in the 11-C samples.

If it is assumed, as the results of test 12-C-1 suggest, that the conspicuousness of OL larvae in winter is greater than that of Br larvae and less than that of Gr larvae in rough herbage vegetation, then, although the numbers of larvae of these two colour types were approximately equal in the 12-C samples (33 green and 36 olive), to the birds, the apparent frequency of the OL larvae would probably be smaller than that of Gr larvae. It then follows from the definition of apostatic selection, that this selection would confer a greater advantage upon OL larvae than Gr larvae in winter, unless, of course, the greater similarity between Br and OL larvae than between Br and Gr causes birds which have formed searching images for Br larvae, to take OL larvae "by mistake" more often than Gr larvae. The drop in the proportion of OL larvae taken in the 12-C tests was substantially more pronounced than the drop in Gr larvae. Therefore, these results endorse the theory of apostatic selection still further, and also suggest that any increase in the predation of OL larvae "by mistake" was not substantial.

If we consider the results of the B and C series of tests from experiments 11 and 12 together, we seem to have a situation in which the conspicuousness of the colour types Gr, OL and Br confers an advantage on Br larvae, and thus alleles b^{o} and a^{+} in winter, and an advantage on Gr larvae, and thus alleles b' and $\underline{a}$ in summer, the survival value of OL being in general

intermediate to those of Gr and Br in both winter and summer. The formation of searching images by birds preying upon these larvae seems to favour Br larvae, and thus the allele b^{o} in the 4th instar, whilst favouring Gr and OL larvae and thus the alleles b' and a in the 5th instar.

The situation may be further complicated by various factors such as (a) the variation in the basic predation habits of individual birds and different species of birds, and their ability to both learn and memorise searching images. For example, Allen (1972a) showed that blackbirds tend to preferentially eat brown prey rather than green prey. (b) The frequencies of other prey items which are similar in colour and shape to one or other of the P. meticulosa larval morphs. (c) The possibility that, if a significant number of larvae ecdyse and change colour over a short period of time, these will gain an advantage because they are overlooked until birds adjust their searching images to the "new colour". This situation is in some ways analagous to that noted by Tinbergen (1960, see p. 316), and is particularly relevant to the changes in colour between 4th and 5th instars.

Rather than discussing these points and the results of experiments 11 and 12 further in comparative isolation to the results of other experiments described in this and other chapters, I will move now to a general discussion in which I will correlate and integrate the facts and hypotheses which have resulted from the work described in chapters 3 - 8 inclusive, hopefully making constructive critisisms of the work, and suggesting further work which may be done, particularly with respect to the possible maintenance of polymorphism by bird predation.

CHAPTER 9 GENERAL DISCUSSION

The intention of this chapter is to draw together evidence from the work on various aspects relating to larval colour variation in *P. meticulosa*, which has been described in chapters 3 - 8 inclusive. The findings will be used as a basis for discussing the maintenance of the variation in natural populations, and I shall stress likely areas of future research. To avoid too much repetition, experimental evidence and points discussed in previous chapters will generally be referred to but not quoted.

Before doing this, however, some points should be made on what I believe was one of the most critical decisions made during this study, that is to say, the initial choice of moth around which the work was to be based.

A. The choice of *P. meticulosa* as subject material.

The choice of *P. meticulosa* as the basis of this study has proved to be fortunate in many ways, but it has also had a number of drawbacks.

The complex system controlling the colour variation which emerged, meant that the ensuing work had to be limited to particular areas of study. Thus, once the system had been outlined, later work was designed to investigate some of the ecological consequences of the system and in particular the way in which the system is, or may be, maintained in the wild. The biochemistry of the system has not been scrutinized, and, although some speculative theories on the nature of the colour

variation may be suggested on the basis of the work described in chapter 3, work, on the basic nature of the colour variation, and on the way in which the genetic system invokes colour changes, is badly needed. It is suggested that this work should be designed to investigate the pigmental make-up of the various colour types, particularly to discover whether the olive colour type is produced by a mixture of some or all of the pigments present in the green and brown colour types, and to discover the way in which the alleles e' and e^{o} prevent the phenotypic expression of particular alleles of genes A and B. It is also suggested that an investigation into the effect of developmental hormones, such as ecdyson and juvenile hormone, on the stage at which colour changes occur, should be carried out. This should consider particularly the way in which allele c, when homozygous, causes the phenotypic expressions of genes B, D and E to become apparent in the 3rd instar.

The complexity of the system, and the rarity of some of the colour types, has also led to problems in studying larval populations in the wild. For example, the frequencies of the alleles of genes D and E could not be calculated because of the epistatic and hypostatic interactions between these alleles (pp. 210-217). The rarity of the yellow colour types also prevented results of any great statistical significance being obtained from the lat er predation experiments, in which yellow larval types were presented to birds at natural frequencies.

On the other hand, the choice of P. meticulosa has had many beneficial consequencies. It is a remarkably easy species to rear in large numbers and it suffers a wide range of

environmental conditions with few adverse effects. Furthermore, the fact that it shows no true diapause at any stage enabled the large numbers of insects, of particular genotypes and at specific stages of development, required in certain experiments, to be attained by the manipulation of the life cycle length.

Because of its complexity, the system has also led to the investigation of a wide spectrum of problems under the broad heading of ecological genetics, and this in turn has induced new techniques to be developed and old techniques to be adapted to study a varied range of subjects.

B. The control of larval colour variation.

Normally, the colour of P. meticulosa larvae in early instars is green, whilst in later instars six main colour types occur (chapter 3A).

The colour of the larvae is under the control of two systems, being affected by foodplant in the early instars, and being controlled by five unlinked major genes in the later instars, (chapters 3B and 3C, pp. 98-157), the "switch" between the two mechanisms acting at the commencement of the 4th or in some cases the 3rd instar. The genetic system controlling larval colour in the later instars is summarised in Tables 3Di and 3Dii (pp. 158-162). Evidence has also been presented to support the contention that environmental factors such as temperature and light do not affect larval colour, nor do larval density or the rate of larval development, (chapter 3B, pp. 58 - 98).

C. The behaviour of larvae in response to light.

The switch from foodplant to genetic control of larval colour coincides with a change in larval behaviour. When their colour is foodplant controlled, larvae have a positive tactic response to light, whilst when larval colour is genetically controlled, a negative phototactic response is apparent (chapter 4). Previous suggestions, that larvae of different colour types habitually rest upon backgrounds which they match, appear to be unfounded. However, as larvae in early instars take on the colour of their food, and because larvae, in which colour is foodplant controlled, stay in the upper herbage layers all the time, the colour of these larvae is almost always the same as that of the material they are resting upon.

We must now consider three rather different questions which the control of colour variation and the correlated phototactic responses invoke.

Firstly, why has the system, which involves a change in both the basic mechanism controlling larval colour, and the behaviour of larvae, approximately half way through the larval stage, evolved?

Secondly, why is there no linkage between the loci involved in the control of colour variation? This question is posed in relation to the fact that a number of authors have pointed out that disruptive selection will produce a tendency for increased linkage (Mather, 1955, Sheppard, 1959, Ford, 1964).

Thirdly, why and how is the genetic polymorphism maintained

in the wild?

D. The switch between foodplant and genetic systems of colour control.

Two hypothetical explanations will be put forward to account for the switch between foodplant and genetic systems of colour control.

The first of these hypotheses assumes that predation of larvae by animals, which find larvae by visual cues, is significant in all instars. If this is so, then it is suggested that, whilst larvae are relatively small, they will be well concealed on foodplants if they match the colour of these foodplants, and, whilst larvae are small, shadow effects will be minimal. However, as larvae grow shadow effects will become more pronounced, and, therefore, larvae in the later instars, even if their colour was foodplant controlled, would become more conspicuous. There are two ways in which this development could be overcome. Larvae could develop counter-shading markings, or alternatively a mechanism could evolve by which larvae would move to a position where shade effects would not be important. It is suggested that P. meticulosa has adopted this second course of action, and the change in phototactic behaviour response which occurs at the 2nd or 3rd larval ecdysis is the outcome of such a development. It is further suggested that once the behavioural switch had evolved, larval colour might evolve in response to apostatic selection and selection against those larval forms which are least well concealed in the low herbage layers, due to predators which search for food

using colour vision. It is relevant to note that larvae of other noctuid species, such as Noctua pronuba, which are virtually monomorphic for colour characters, are also positively phototactic in early instars and negatively phototactic in later instars (Madge, 1962, 1964 a, b). This indicates that such a change in the response of larvae to light may come about without associated changes in larval colour.

It may also be noted that the dark v-shaped markings along either side of the dorsal line generally only become apparent in the later instars. Whilst the larval markings were not closely scrutinised, it may be suggested that these would be an advantage to larvae in which crypsis is dependant on a "match" with a composite environment where shadows from the overlying herbage would be a significant feature of the overall colour and light effect, because the v-shaped markings would tend to break up the outline of the larvae. On the other hand, to larvae in which crypsis is dependant on a close match with a fairly uniformly coloured surface, such as a sorrel or dock leaf, in an exposed position, the dark v-shaped marks would be a disadvantage.

One apparent flaw in this hypothesis is that, if it is true, it suggests that N. pronuba larvae ought to be polymorphic as they live in the same type of environment as P. meticulosa larvae and also show the switch from positive to negative phototaxis. However, the larvae of N. pronuba bury themselves below and remain in the soil surface during the day (Madge, 1962, 1964a). They would, therefore, not be subject to visual predation to the same extent as P. meticulosa larvae which remain

above the ground. It would be interesting to discover, in Lepidoptera whose larvae live in the same type habitat as P. meticulosa and N. pronuba, and exhibit the same switch in their behavioural response to light as these two species, whether or not species which have monomorphic larvae generally exhibit the burying habit whilst polymorphic larvae generally remain on top of the soil surface.

The second hypothesis assumes that selection by predators, which hunt by sight, and have colour vision, is relatively unimportant in the early instars, but increases in importance in subsequent instars as larval size increases. It is suggested that, if young larvae were distributed at random throughout the herbage layers, predation by ground feeding predators which find prey mainly by non-visual cues (such as ground beetles, small mammals etc), would be greater than predation by those predators which feed more in the higher herbage layers. Therefore, a selective advantage would be conferred upon young larvae which tend to live in the higher strata. However, if predation by birds increases as larval size increases, then, this advantage will diminish as larvae reach the later instars, and this hypothesis suggests that once larvae reach the 4th instar, those which rest in the higher herbage strata throughout the day, when the majority of birds feed, are at a selective disadvantage. Thus, any mechanism which leads to larvae staying in the low herbage layers, where they would be less exposed to bird predation, during the day, will bestow an advantage on larvae which have it. Larvae would move back into the higher herbage layers at night to feed. Furthermore,

as birds which prey on larvae do not generally feed at night, whilst most ground feeding predators are partly or wholly nocturnal, predation in the lower herbage layers may well be greater at night than in the higher herbage layers.

Once the switch in the response to light had developed, the colour control mechanism could evolve along the lines noted in the first hypothesis.

One critisism of this hypothesis is that there is no evidence to indicate that birds search for food in the upper herbage layers more than in the lower herbage layers, and in fact tests 11-A and 12-A (chapter 8) provide some indications that the converse may be true. However, it may be argued that, were larvae of later instars to remain in exposed situations in the higher herbage layers, and their colour was foodplant controlled, shadow effects would make the larvae conspicuous. Moreover, tests 11-A and 12-A also indicate that birds may adjust their feeding behaviour very rapidly with respect to where they search for food.

Although speculative, both hypotheses seem more or less credible, but, I think the second holds more substance as I suspect that predation of young larvae by organisms which hunt by sight is slight. Indeed, there is some evidence from experiments 11 and 12 (chapter 8) that predation by birds of 3rd instar larvae is not as heavy as that of 4th or 5th instar larvae, and experiment 2 (chapter 8) shows that birds preferentially prey upon larger larvae when given a choice of two sizes. Furthermore, Den Boer (1971) notes that bird predation of the larvae of Bupalus piniarius L. is only important from the 4th

instar onwards because of the larval size. On the other hand, it may be noted that the two hypotheses are not totally mutually exclusive, and both may have had some effect on the evolution of the switch in question.

One final factor which may have contributed to the evolution of the switch should be noted. The evidence from the experimental studies of selection by parasites and viruses (chapter 7) indicates that 3rd instar larvae which spend the day close to the ground seem more susceptible to viral infection than those of the 4th or 5th instars, whilst the incidence of attacks by hymenopteran and dipteran parasites is less in these 3rd instar larvae than in 4th or 5th instar larvae. This may imply that younger larvae are more susceptible to viral infection, whilst parasites tend to attack older larvae. It has already been noted that viral disease selectively favours 3rd instar larvae which stay in the upper herbage layers all the time, and that the reverse is true with respect to parasite attacks (chapter 7 pp. 293 and 304). Thus behaviour which induces larvae to remain in the high herbage layers will be advantageous whilst larvae are young and particularly susceptible to viral diseases; however, the overall increase in the incidence of parasite attacks on older larvae will confer an advantage on those larvae which move to the lower herbage layers during the day when in these later instars.

E. The apparent lack of linkage in the colour system.

It has been pointed out by a number of authors (Fisher, 1930a; Sheppard, 1953, 1955, 1959; Mather, 1955; Kimura, 1956;

Ford, 1964) that, if two or more allelomorphs of a number of genes at different loci are advantageous in some combinations, but disadvantageous in others, there will be strong selection for increased linkage between these loci. Mather (1955) notes that the tendency for increased linkage will be particularly evident when there is strong disruptive selection acting on the forms of a species.

The colour variation in later instars of P. meticulosa is discontinuous and must, therefore, be the outcome of disruptive selection. Why, then is there no linkage between the five major genes which control this variation?

The answer to this question is rather complex and may be considered in three parts. Firstly, suggestions relating to the correlation between genetic control of colour and negative phototaxis will be considered. Secondly, the lack of linkage between the five major genes will be viewed with respect to the question; "Why should linkage between these loci evolve"? Finally, I will argue that selection acts against the evolution of linkage between loci A, B and C.

It has been argued that the evolution of the switch between positive and negative tactic responses to light, which occur at the 2nd or 3rd larval ecdyses, preceded the evolution of the genetic system controlling larval colour in the later instars. It was suggested that the genetic polymorphism evolved because, if larval colour were foodplant controlled, then once larvae left the foodplant in the day to "hide" in the low herbage layers, they would not necessarily match their background. (The

evolution of the genetic system controlling colour will be discussed later). It seems then obvious that, once colour became genetically rather than foodplant controlled in the later instars, any larvae which remained in the upper herbage layers on the leaves of the foodplant would be more conspicuous than if their colour were foodplant controlled. Therefore, a mechanism causing the colour of larvae which are negatively phototactic to be genetically controlled, and the colour of larvae which are positively phototactic to be foodplant controlled, would be advantageous. This mechanism obviously has developed because these connections do occur.

It is suggested that the correlation between the two aspects, (i.e. changes in the response to light and the onset of genetic control of colour), may be induced by a particular state of hormonal balance which normally exists at the beginning of the 4th larval instar. The fact that larvae homozygous for allele c are both negatively phototactic, and have genetically controlled colour may be simply explained if this allele affects the hormonal balance in the 3rd instar.

Alternatively, it may be argued that gene B is a "supergene", which has evolved due to selection for very close linkage between the genes causing the change from positive to negative phototaxis and the allelomorphs b' and b^{o}. Under this system e' and e^{o} (in the absence of both d^{+} and e^{+}) may prevent the phenotypic expression of b' and b^{o} respectively, without preventing the expression of the gene causing the switch to negative phototaxis. The occurrence of 3rd instar negatively phototactic larvae in which colour is genetically controlled may.

be explained either by a hormonal system, or by allele c, when homozygous, causing the phenotypic expression of genes B, D and E, and the change to negative phototaxis, to arise in the 3rd instar.

We now turn to a consideration of the lack of linkage between the 5 major genes.

If we consider three of the classic cases in which the existence of super-genes has been demonstrated, i.e. Papilio dardanus, Cepaea nemoralis and Primula vulgaris, in each case there are two or more distinct characters involved in the polymorphisms. In P. dardanus the mimetic patterns are composed of a limited number of elements in different combinations. (Clarke and Sheppard, 1960c). In C. nemoralis the main components of the external variation are shell colour and shell banding (Cain and Sheppard, 1954 and others). In P. vulgaris, (and many other Primulaceae) there are four forms of flowers, the pin and thrum forms, and the long and short homostyle forms. The polymorphism involves not only the positions of the stigma and anthers in the corolla-tube, but also legitimacy mechanisms (penetration and rate of pollen tube growth), together with pollen-size and length of papillae on the stigma (Ernst, 1933). In each of these cases some combinations of characters are advantageous and others disadvantageous. The main reason for the evolution of linkage seems to be to hold together advantageous combinations of characters, whilst maintaining the potential for chiasma formation, within the "super-genes" or linkage groups, to give rise to other combinations from time to time. However, it is important to note that disruptive selection need not always

lead to the evolution of a super-gene, as a similar effect, (i.e. one which ensures that advantageous combinations are promoted whilst the potential for other combinations is retained in the gene pool but is rarely expressed phenotypically), may be produced by selection for a gene complex incorporating appropriate epistatic and dominance effects. The Batesian mimics Papilio polytes L. and Papilio memnon L. may be cited as examples of this (Clarke and Sheppard, 1960c).

The genetic system in P. meticulosa incorporates a number of dominance and epistatic effects, and it is my contention that when considering larval ground colour alone, the evolution of dominance and epistatic effects retains the genotypic variability of the system without the need for linkage. Moreover, as the character under consideration has only one basic element, there is no necessity for linkage to have evolved, as the effects of linkage would be nullified by epistatic effects. (On the other hand, were larval markings to be studied in relation to larval ground colour, linkage may well be involved).

Furthermore, it will be suggested (p. 416) that due to the effects of bird predation on larvae, linkage between genes A, B and C would be a positive disadvantage. However, for this to be explained comprehensively we must first consider the maintenance of the colour polymorphism in the later instars.

F. The maintenance of the genetic polymorphism.

The results in chapter 5 have shown that the allelic frequencies of three major genes (A, B and C) are stable from

year to year, and whilst the frequencies of D and E could not be calculated, the phenotypic frequencies of the yellow colour types indicate that these are also balanced. The polymorphism then appears to be balanced. However, there is considerable seasonal variation in some allelomorph frequencies, particularly those of genes A and B.

We must now consider the selection experiments designed to look into the ways in which the polymorphism may be maintained, (chapters 6 - 8 inclusive). Most of the experiments concentrated on the Gr, Br, OL complex, and were thus concerned with genes A, B and C.

i. Gene C

The alleles of gene C control whether genes B, D and E are expressed phenotypically in the 3rd instar, and whether 3rd instar larvae are positively or negatively phototactic, (chapters 3 and 4). The frequencies of the two alleles appear to be fairly constant, that of c^+ being slightly greater than 0.5, and that of c being slightly less than 0.5. There appears to be a slight increase in the frequency of c during the winter, and a similar decrease in the frequency of c during the summer. However, it was noted in chapter 5 (p. 227) that these changes may be unrealistic to some extent, as they could be a manifestation of the changes in the frequencies of alleles b' and b^o, caused by the method used to estimate the allelic frequencies of c^+ and c.

Some evidence has been obtained which indicates that the heterozygote c^+c is at an advantage over the homozygote recessive, cc, when larvae are bred under laboratory conditions (chapter 6E).

It has been stated that heterozygote advantage can maintain a polymorphism (chapter 6). It is not known whether the heterozygote is at an advantage to the dominant homozygote, c^+c^+, but, if it is, then the general advantage of the heterozygote over the homozygotes would lead to a balanced polymorphism if this were the only selective factor acting on gene C. However, there is evidence that other selective agencies are involved.

Experimental studies of selection by viral disease and parasite attack have indicated that mortality due to viral disease is higher in larvae which are homozygous recessive for gene C, than in either the heterozygotes or homozygote dominants (chapters 7B and 7C). It has also been suggested, that due to the difference in behaviour between cc and other larvae in the 3rd instar, the former type might be preyed upon by ground beetles and small mammals slightly more than the latter type.

On the other hand, mortality due to hymenopteran and dipteran parasites is greater in those larvae with c^+ in their genotype than in cc larvae (chapter 7C). Furthermore, the selection pressures for and against c^+ due to viral infection and insect parasite attack respectively were comparable, that due to viral disease, if anything, being slightly stronger. Moreover, if the other causes of death in the PE1 and PE4 samples (Table 7Ciii) are taken into account, then overall, c^+ seems to be at a very slight advantage in winter compared with c, the reverse being true in summer.

Finally, whilst it has been suggested that 3rd instar larvae are not preyed upon by birds to any great extent, some probably are taken. If birds habitually search for prey items

in the low herbage layers, larvae with c^+ in their genotype will be at an advantage, whilst, if they normally seek prey in the higher herbage layers cc larvae will gain an advantage. Experiments 8/11[1] and 8/12 tests A-1 (chapter 8) indicate that when the only prey available to starlings are 3rd instar larvae (present in phenotypic frequencies closely comparable to those found in natural populations), c^+ is initially at an advantage; however, later tests in these experiments indicate that the birds soon begin to search for the commoner c^+c^x larvae and cc becomes advantageous. It is probable that selection against the commonest colour type (green) also contributes to the increase in selection against c^+c and c^+c^+ larvae; however, it was noted (p. 377) that the proportions of cc green larvae taken fell, although not as sharply as those of OL and Br larvae. The suggestion that Br larvae are generally less conspicuous than green larvae in rough grassland in winter, the reverse being true in summer, must also be taken into account, but, even in 3rd instar larvae this is likely to affect the frequencies of alleles b' and b^o more than those of c^+ and c.

It has been noted that the A series tests of experiments 8/11 and 8/12 may not be very comparable to a natural situation because birds probably do not eat 3rd instar larvae to any great extent. On the other hand, if in a natural situation birds feed upon and form searching images for 4th instar larvae, they may take a number of late 3rd instar larvae as well. If this occurs,

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[1] Throughout the ensuing text, when referring to the experiments on selection by birds, the experiment number will be preceded by the figure 8.

then, as OL and Gr larvae comprise by far the greatest number of 4th instar larvae, it is probable that more 3rd instar larvae of these colours will be taken than of the other, rarer colour types. However, again this will have more effect on the frequencies of b' and b^{o} than of c^{+} and c, although cc may gain a slight advantage.

The suggestions put forward show that the determination of whether birds, preying upon 3rd instar larvae, affect the relative frequencies of c^{+} and c is very complicated. Further confusion arises when we remember that larvae of a considerable number of other Lepidoptera inhabit rough grassland, and the relative abundance of these must be considered before an accurate appraisal of the situation may be made.

It may therefore be of more use, at this point, to consider the overall importance of the various components of selection by birds, mentioned above, by considering the fluctuations in the allelic frequencies of c^{+} and c, taking the other selective forces already noted into account. This is not an ideal method of analysis, and I recognise that it would be dangerous to make any firm deductions from this type of approach. Thus, it must be realised that any points based on this type of approach can only be speculative suggestions.

It is suggested that the polymorphism with respect to gene C is maintained in balance by heterozygote advantage, and that, when investigated, the heterozygote will prove to have an advantage over the dominant homozygote $c^{+}c^{+}$. Furthermore, the level at which the frequencies of c^{+} and c are maintained is determined principally by the relative advantage of the heterozygote

over the two homozygotes, and the opposing selection pressures due to viral disease and parasitism.

The slight seasonal fluctuations in the frequencies of c^+ and c, noted in chapter 5, are not explained by any of the data in chapters 6 and 7. Indeed, the overall mortality due to viral disease and parasitism (Table 7Ciii) should produce trends opposite to those observed. It may then be that bird predation does favour Br and OL 3rd instar larvae in winter when these are less conspicuous than green larvae, and green larvae in summer when these are less conspicuous than the Br and OL forms. On the other hand, selective agencies which have not been considered may be involved. Table 9Fi summarises the selective factors investigated which it is thought may affect the frequencies of the allelomorphs of gene C.

ii. Gene B.

In chapter 3, it was shown that the alleles of gene B are incompletely dominant to one another, the heterozygote being olive while one homozygote is green, the other being brown. The allelic frequency estimates from the AF1 data (chapter 5) have shown that, while the frequencies of the two alleles are reasonably constant from year to year, they show appreciable seasonal variations, b' reaching a maximum frequency of between 0.67 and 0.69 in Sept/Oct., and dropping to a minimum frequency of between 0.62 and 0.63 in May. Furthermore, the AF2 data, when considered in conjunction with the AF1 data, indicates that the frequency changes are due to selective pressures which act both before and after the average age of the 4th instar larvae

Table 9Fi.

A summary of the selective forces which may affect the allelic frequencies of c^+ and c.

General position: Frequencies stable from year to year.
Slight seasonal variations.
c^+ maximum frequency of 0.5296 in Sept/Oct. 1976
c maximum frequency of 0.4926 in May/June 1977
(see Fig.5Aii page 206).

Selective factor	Genotype favoured	Evidence
Heterozygote advantage	c^+c over cc	see chapter 6E.
Heterozygote advantage	c^+c over c^+c^+	Speculation (see p. 285 and p. 402)
Viral disease infection	c^+c^+ and c^+c over cc	see chapters 7B and 7C.
Parasitism	cc over c^+c and c^+c^+	See chapter 7C.
Small mammal predation	c^+c^+ and c^+c over cc	Speculation (see chapter 7E).
Ground beetle predation	c^+c^+ and c^+c over cc	Speculation (see chapter 7D).
Bird predation (a)	Generally cc over c^+c^+ and c^+c	Speculation based on birds forming searching images for 4th instar colour types (see pp. 401-402).
Bird predation (b)	In summer c^+c^+ and c^+c over cc	Speculation based on green larvae being less conspicuous than Br and OL larvae in summer (see p. 378 & 401).
Bird predation (c)	In winter cc over c^+c^+ and c^+c	Speculation based on Br and OL larvae being less conspicuous than green larvae in winter (see p. 378 & 401).

in the AF2 samples.

The evidence from an investigation of heterozygous advantage with respect to the alleles of gene B suggests that the heterozygote $b'b^o$ is at a slight advantage over both of the homozygotes when bred in captivity. However, efforts to enhance this advantage, by submitting broods to unfavourable conditions, failed to do so.

Evidence from experimental studies of selection by viral disease (chapter 7, Table 7Bi) gave no indication that viral disease affects the genotypes differently. Furthermore, the results of the experiments on selection due to viral disease and parasite attacks (Tables 7Ci, ii and iii), indicate that the incidence of viral infection in 4th instar larvae is relatively constant with respect to the alleles of gene B, the heterozygote possibly being at a slight advantage in winter and a slight disadvantage in summer. The incidence of parasite attacks possibly confers a slight advantage on b^o. However, the figures from the PE samples are not very consistent and are far from conclusive.

We must now consider the ways in which bird predation may affect the frequencies of the different genotypes, and, therefore, the alleles themselves. It seems that birds may prey upon larvae of particular genotypes to different extents for two basic reasons. Firstly, birds may selectively prey upon those larvae which are more conspicuous to them, (i.e. which match the colour of their environment least well), irrespective of morph frequencies. A number of experiments (e.g. 8/10) have indicated that Br larvae are less conspicuous than Gr larvae in

winter, whilst other experiments (e.g. 8/9) have indicated that Gr larvae are less conspicuous than Br larvae in summer. The results of experiments 8/11 and 8/12 have led to the suggestion that the conspicuousness of OL larvae is intermediate to those of the two homozygotes in both winter and summer. If these points are taken in isolation, they seem to account for the seasonal fluctuations in the frequencies of b' and b^o, as preferential predation of Gr larvae in winter will cause a drop in the frequency of b', and preferential predation of Br larvae in summer will lead to a drop in the frequency of b^o. However, there are many other considerations which must be taken into account, and these will be mentioned later.

Secondly, a number of experiments, (e.g. 8/3, 8/5, 8/6, 8/11 and 8/12) have shown that birds can form seaching images for larvae of a particular colour, and as long as one form is not very much more conspicuous than another, they generally form searching images for the commoner morph. If the Gr, OL and Br 4th instar morphs of P. meticulosa were equally as conspicuous as one another throughout the year, and predators only fed on these larvae, then Br larvae would be preyed upon less than Gr and OL larvae which are much more common than Br larvae, and thus b^o would be selectively favoured.

If these two points are considered together, then, unless other selective pressures have a bearing on the situation, we would expect the phenotypic frequencies of the two homozygotes initially to equalise due apostatic selection, with fluctuations in the conspicuousness of larvae causing an increase in the frequency of b' in the summer, and an increase in the frequency

of b^{o} in winter. The frequency of the heterozygote would be determined by, firstly, the basic laws of Mendelian inheritence based on random mating, (for evidence that mate choice is random see chapter 6B); secondly, heterozygote advantage; thirdly, its relative conspicuousness compared with the two homozygotes; and fourthly, selection against it due to apostatic selection. However, as has been mentioned previously, many other factors must be taken into account when considering the effect of bird predation on phenotypic, and, therefore, allelic frequencies. These are, in the main, concerned with the frequencies of types of prey and the formation of searching images by birds in the wild.

(1) It has been suggested that birds do not form strong searching images for 3rd instar larvae. However, if birds form searching images for these forms at all, then these will most likely be for green larvae which comprise about 85% of 3rd instar larvae. Although P. meticulosa is not a rigidly brooded species, it is suggested that the majority of larvae present in a population at one time are in approximately the same stage of development, (p. 318). Thus, if the majority of larvae undergo the 3rd ecdysis over a short period of time, then, if birds do form searching images on the basis of 3rd instar larvae, non-green 4th instar larvae will be preyed upon less than Gr 4th instar larvae until the birds have adjusted their searching images to the 4th instar phenotypic frequencies. This will confer an advantage on b^{o}.

(2) In the 5th instar the majority of larvae are brown. Following the ideas outlined in point (1), the majority of birds,

which have formed searching images on the basis of 4th instar frequencies, will tend to still search for OL and Gr larvae during the period when the majority of larvae undergo the 4th larval ecdysis, and there will be a lag before birds adjust to searching for the common 5th instar Br larvae. However, when they do so, then the searching images for Br larvae will confer an advantage upon any remaining 4th instar larvae which are Gr or OL, and a disadvantage upon those which are Br. This will confer an advantage on b'. On the other hand, this situation suggests that those larvae which first change into the 5th instar will encounter selection against Gr and OL larvae. Thus b^{o} will be at an advantage in the 5th instar for a time.

(3) Although the majority of P. meticulosa at a particular time of year will be in roughly the same stage of development, it has been noted (chapter 2), that all stages may be found throughout the year. When 4th and 5th instar larvae occur at low densities, (for example in May and June, or September to November) it is unlikely that birds will form searching images for any of the forms in the wild as these would be comparatively unprofitable in comparison with other types of prey. Therefore, for part of the year, the relative frequencies of the morphs are unlikely to affect the degree of predation to which the morphs are subject. However, the relative conspicuousness of the morphs would influence the predation to which they are exposed.

(4) There is evidence that the reactions of different species of birds to larvae may vary, in that, firstly, some birds seem to habitually seek prey of one colour, and secondly, the

ability for birds to retain searching images appears to vary. Allen (1972a) has noted that blackbirds generally prey upon brown flour and lard baits in preference to green ones. There is some indication from the results of experiment 8/3 that individual starlings vary in their ability to retain searching images, and this point is also noted by Brower, (1960).

(5) As there are two basic components to the selection by birds of different genotypes, then the composition of the bird population, which preys on larvae in a particular area, may be important. If the majority of the bird predators are migrants, only staying within the area of a larval population for a short time, then the selection due to conspicuousness of larvae may be more important than frequency dependant selection involving searching image formation, the reverse being true if most birds remain within a small area for a long period. The latter case is particularly relevant in the nesting season.

(6) Finally, as was mentioned in chapter 8, if a comprehensive prediction of the effect of bird predation upon the larval colour frequencies of P. meticulosa is to be made, the effects of other species of Lepidoptera, which have larvae that are similar to those of P. meticulosa, on bird searching image formation must be taken into account. To do this the morphology, behaviour, palatability, and abundance throughout the year, of all these species would have to be investigated. Furthermore, the frequency of other prey items which are not similar to P. meticulosa larvae may affect the issue in that, if some other type of food is readily available to birds in large quantities, the birds may not form searching images for

Lepidoptera at all.

As I feel it is likely that bird predation does play a significant part in controlling the frequencies of b' and b^{o}, and the effect of bird predation on b' and b^{o} larvae can not be accurately assessed from the work done, the only point which may be made with reference to the maintenance of b' and b^{o} is that heterozygote advantage probably helps to keep the frequencies of b' and b^{o} balanced overall.

iii. Gene A.

It has been shown that gene A is biallelic. The allele a^{+} causes larvae to be brown in the 5th instar and is completely dominant to allele a which leaves 5th instar larval colour under the control of gene B (chapter 3). The allelic frequency estimates from the AF1 data (chapter 5A) have shown that, whilst the frequencies of the two alleles are relatively stable from year to year, there are appreciable seasonal variations in the frequencies, a^{+} reaching a maximum frequency of approximately 0.4 in May, and a minimum frequency of approximately 0.34 in Sept/Oct. The AF2 data (chapter 5B) viewed in conjunction with the AF1 data indicates that the frequency changes are due to selective pressures which act both before and after the average age of the 5th instar larvae in the AF2 samples.

The work described in chapters 6 and 7 provides no evidence that choice of mate, heterozygote advantage, viral disease, parasitism, carabid predation or hedgehog predation, have any appreciable effect on the frequencies of a^{+} and a.

We move then to a consideration of the ways in which bird

predation may affect the frequencies of a^+ and a. Again we must consider both the conspicuousness of larvae and the effects of searching image formation. The fact that Br larvae are less conspicuous than Gr or OL larvae in winter, the reverse being the case in summer (see pp. 368-369 and 378-385) seems again, taken in isolation, to account for the seasonal variations in the frequencies of a^+ and a. On the other hand, as the majority of 5th instar larvae are Br, apostatic selection would tend to confer an advantage on Gr and OL 5th instar larvae, and thus bestow a selective advantage on a, (and to a lesser extent b').

However, again, many other factors must be taken into account and I will list these referring at the same time to points mentioned in relation to the frequencies of b' and b^o.

(i) It is unlikely that point (1) (p. 407) has much relevance to predation of 5th instar larvae.

(ii) As implied in point (2) (p. 407), if birds which have been feeding on 4th instar larvae have formed searching images for the more common 4th instar forms (Gr and OL), then, when the majority of larvae in a population undergo the 4th larval ecdysis with the result that Br becomes the most common larval form, the Br 5th instar larvae, and thus a^+, will be at an advantage until birds adapt their searching images and begin to preferentially prey upon the Br larvae. Depending on the length of this period of adjustment, a number of 5th instar Br larvae, particularly those which undergo the 4th ecdysis earliest, may pupate before the adjustment has taken place. This may be particularly important to larvae from May or July

main emergences, as the period between the 4th ecdysis and pupation is shorter when the weather is hot.

However, it must be remembered that the evidence from experiment 8/2 showed that 5th instar larvae are taken in preference to 4th instar larvae in a choice situation, probably because they are larger. Thus the searching image adjustment period may be quite short.

(iii), (iv), (v), (vi) The points (3), (4), (5) and (6) (pp. 408-410) are equally relevant to predation of 5th instar larvae as they are to 4th instar larvae.

iv. Genes D and E.

Analysis of the AF1 and AF2 data (chapter 5) has indicated that the phenotypic frequencies of the three yellow colour types together, remain fairly constant from year to year, and that there is little seasonal variation in the overall numbers of the yellow type larvae. It is, therefore, suggested that the frequencies of the five alleles d^+, d, e^+, e' and e^o are relatively static.

The information on the selective factors which may affect the frequencies of these alleles is very limited. There is some evidence that neither choice of mate (chapter 6B), nor death due to viral disease (chapter 7B), nor predation by carabids (chapter 7D), and hedgehogs (chapter 7E) have any effect on the frequencies of these alleles. The occurrence of heterozygote advantage has not been sought. The experiments into the effects of viral disease and parasite attacks on larvae in the field (chapter 7C) did not involve enough yellow larvae

to give results of any real significance.

The bird predation experiments (chapter 8) concentrated in the main on the Gr, Br, OL complex, but the results of experiment 8/1 indicate that starlings do not habitually prey upon PY larvae more than other colour types when these are offered on a white background in equal numbers.

I am of the opinion that the yellow larval types are more conspicuous than any of the other colour types in a rough grassland habitat, and that they are maintained in the population at a low frequency by apostatic selection.

G. Suggestions of further work, particularly with respect to selection due to bird predation.

The points mentioned, in relation to bird predation affecting the frequencies of the 4th and 5th instar Gr, OL and Br colour types, show the extreme complexity of the problem under inspection, and indicate the enormity of the task facing anyone who makes an attempt to unravel the predator-larval morph relationships.

As previously stated (p. 322) the main function of the bird predation experiments was to give indications of the direction further work might most profitably take, and my views on this subject will be outlined here.

(a) A study of all other types of prey which are eaten by those birds which feed on P. meticulosa should be undertaken, so that the relative importance of bird predation on P. meticulosa larvae may be assessed.

(b) Work should be carried out on the specificity of

searching image formation, to see how similar two prey items must be for birds, "trained" to search for one type of prey, to confuse the two. Some work has already been carried out on the specificity of bird searching images (see particularly, de Ruiter, 1952; Allen, 1972b).

(c) The effect of bird predation on a wild population (or an artificial population in which the colour types are present at natural frequencies) should be studied continuously from the start of the 3rd instar to the end of the 5th instar, looking particularly at the predation during periods when the majority of larvae ecdyse. This study should be carried out on "populations" in winter and in summer, and preferably should continue throughout a whole year, so that the effect of predation on 3rd, 4th and 5th instar larvae can be assessed even when these are rare. One of the drawbacks inherent in working on Lepidoptera larvae is that conventional marking methods can not be used as the larvae ecdyse. Therefore, it is suggested that radioactive trace elements should be used to "mark" larvae in a study of this type, (see Kettlewell, 1952, 1955a).

(d) An investigation to determine the mortality rate due to different factors, and bird, insect and small mammal predation, would be valuable in assessing the relative strengths of selection pressures due to these factors.

(e) The variation in response to larvae of individual birds, and birds of different species, which feed on P. meticulosa larvae in the field should be assessed, so that the average effect of predation of all types of birds on larval morph frequencies can be estimated.

H. Conclusions.

Lepidoptera larvae which feed on low growing herbaceous plants, and rely mainly on camouflage for defence, are often very variable, and many species appear to be genetically polymorphic, particularly in the later instars. The reason for this, I believe, is that birds eat significant numbers of these larvae, and hunt to a greater or lesser extent using searching images. Thus a lepidopterous species which has two or more larval morphs, will be at an advantage as birds would have to form more than one searching image. If this is true, then, theoretically, the more forms a species has, the greater its chances of survival will be. However, I think that two factors limit the number of forms that will confer an advantage on a species. Firstly, the forms must be distinct from one another to a degree sufficient to prevent birds confusing them. Secondly, forms which are very conspicuous will be selected against, even if they are relatively scarce.

I believe that the changes in the colours of larvae during their development are very important to the survival of some species of Lepidoptera which are preyed upon in significant numbers by birds[1].

I will use the Gr, Br, OL larval colour complex of P. meticulosa to illustrate this point. Birds, which have been

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[1] I may record that apart from P. meticulosa, I have noted larval colour changes occurring at two or more ecdyses in Deilephila elpenor L., D. porcellus L., Melanchra persicariae L., Lacanobia suasa D. and S., Ceramica pisi L., and Amphipyra tragopoginis Clerck.

preying on 4th instar larvae, will form searching images for the commonest colour types (Gr and OL), larvae of the rarer colour type (Br, and thus b^{o}) being selectively favoured. Thus, larvae which change colour to Br at the 4th larval ecdysis will initially be at an advantage over Gr and OL larvae in the 5th instar, until birds adjust their searching images to this colour when it has become commoner than Gr and OL.

The number of larvae which change colour, (and thus the allelic frequencies of a^{+} and a,) will be determined by, in the first place, the initial advantage conferred upon Br larvae in the 5th instar by changing to a colour which birds are not searching for, and secondly, the ensuing advantage conferred upon Gr and OL larvae once birds have adjusted their behaviour to seeking the commoner Br larvae. The increase in the frequency of b^{o}, due to Br being selectively favoured in the 4th instar, will be partly or wholly balanced by the advantage conferred upon Br larvae in the 5th instar once birds have started to preferentially prey upon Br larvae. Similarly the predicted decrease in the frequency of b', due to Gr and OL being at a selective disadvantage in the 4th instar when these are the most common forms, will be balanced by selection in favour of these forms when they are rarer than Br larvae in the later part of the 5th instar.

This situation may further explain the lack of linkage between loci A and B. Considering the various alternatives, if in the first place a was linked with b' and a^{+} with b^{o}, then all 4th instar OL and Br larvae would become Br in the 5th instar.

This situation then prevents any 5th instar larvae being OL, except in cases where a chiasma forms between the loci. Taken in the context of the suggestion that because birds hunt using searching images, the existence of a number of forms of larvae will be advantageous to a species as birds would have to learn more than one form, this lack of 5th instar OL larvae must be disadvantageous. Considering a theoretical situation in which a^+ is linked with b', and a with b^o, this would result in virtually all 5th instar larvae being Br, which must be even more disadvantageous on the basis of the argument given above. It may also be argued that linkage between the C locus and either the A or B loci would also be disadvantageous for similar reasons.

The system controlling colour variation in P. meticulosa is very complicated and a great deal of work must still be undertaken to provide firm evidence of the way in which this system is maintained, and to provide convincing circumstantial evidence to support hypothetical suggestions on the way in which the system evolved. It may be that, if a species could be found in which a "green-brown" larval polymorphism were controlled by a less complicated system, the selection pressures maintaining the system would be easier to resolve. However, the fact that in other species, having non-stick like green and brown larval forms, which have been studied, (e.g. D. elpenor), the system controlling larval colour variation has not been uncovered is perhaps indicative that the systems controlling this type of larval colour polymorphism may often be of a complex nature.

REFERENCES

Adamkewicz, S.L., 1969. Polymorphism in the land isopod Armadillium nasutum. Heredity, 24 : 249 - 263.

Allen, J.A., 1972a. Evidence for stabilising and apostatic selection by wild blackbirds. Nature, 237 : 348 - 349.

Allen, J.A., 1972b. Apostatic selection : the responses of wild passerines to artificial polymorphic prey. Ph. D. thesis, University of Edinburgh.

Allen, J.A., 1974a. Further evidence for apostatic selection by wild passerine birds : training experiments. Heredity, 33 : 361 - 372.

Allen, J.A., 1976. Further evidence for apostatic selection by wild passerine birds : 9:1 experiments. Heredity, 36 : 173 - 180.

Allen, J.A., and Clarke, B., 1968. Evidence for apostatic selection by wild passerines. Nature, 220 : 501 - 502.

Allison, A.C., 1954. Notes on Sickle-cell Polymorphism. Ann. Hum. Genet., 19 : 39 - 57.

Allison, A.C., 1956. The Sickle-cell and Haemoglobin C genes in some African Populations. Ann. Hum. Genet., 21 : 67 - 89.

Beck, S.D., 1950. Nutrition of the European Corn Borer, Pyrausta nubialis (Hbn). II : Some effects of diet on larval growth characteristics. Physiol. Zool., 23 : 353 - 361.

Bowden, S.R., 1958. Yellow-green larvae of Pieris napi L. Entomologist, 91 : 110 - 111.

Bowden, S.R., 1959. Yellow-green larvae of Pieris napi L. Entomologist, 92 : 107.

Bridges, C.D.B., and Quillian, T.A., 1973. Visual Pigments of Men, Moles and Hedgehogs. Vision Res., 13 : 2417 - 2421.

Brower, J. van Z., 1960. Experimental studies of mimicry. IV. The reactions of Starlings to different proportions of models and mimics. Amer. Nat., 94 : 271 - 282.

Buckmann, D., 1974. Die hormonale Steuerung der Pigmentierung und des morphologischen Farbwechsels bei den Insekten. Fortschr. Zool., 22 : (2 - 3) 1 - 22.

Cain, A.J., 1953. Visual selection by tone in Cepaea nemoralis L. J. Conch., 23 : 333 - 336.

Cain, A.J., King, J.M.B., and Sheppard, P.M., 1960. New data on the genetics of polymorphism in the snail Cepaea nemoralis L. Genetics, 45 : 393 - 411.

Cain, A.J., and Sheppard, P.M., 1954. Natural selection in Cepaea. Genetics, 39 : 89 - 116.

Camin, J.H., and Ehrlich, P.R., 1958. Natural selection in Water Snakes (Natrix sipedon L.) on Islands in Lake Erie. Evolution, 12 : 504 - 511.

Carpenter, G.D.H., and Ford, E.B., 1933. Mimicry, Methuen, London.

Carter, M.A., 1967. Selection in mixed colonies of Cepaea nemoralis and Cepaea hortensis. Heredity, 22 : 117 - 139.

Caspari, E., 1950. On the selective value of the alleles Rt and rt in Ephestia kühniella. Amer. Nat., 84 : 367 - 380.

Clarke, B., 1960. Divergent effects of natural selection on two closely related polymorhpic snails. Heredity, 14 : 423 - 443.

Clarke, B., 1962a. Balanced polymorphism and the diversity of sympatric species. In Taxonomy and Geography, ed. D. Nichols, Systematics Association, Oxford.

Clarke, B., 1962b. Natural selection in mixed populations of two polymorphic snails. Heredity, 17 : 319 - 345.

Clarke, B., 1969. The evidence for apostatic selection. Heredity, 24 : 347 - 352.

Clarke, B., and O'Donald, P., 1964. Frequency-dependant selection. Heredity, 19 : 201 - 206.

Clarke, C.A., and Sheppard, P.M., 1956. Hand-pairing of butterflies. The Lepidopterists' News, 10 : 47 - 53.

Clarke, C.A., and Sheppard, P.M., 1959a. The genetics of some mimetic forms of Papilio dardanus, Brown, and Papilio glaucus, Linn. J. Genet., 59 : 236 - 260.

Clarke, C.A., and Sheppard, P.M., 1959b. The genetics of Papilio dardanus, Brown. I. Race Cenea from South Africa. Genetics, 44 : 1347 - 1358.

Clarke, C.A., and Sheppard, P.M., 1960a. The Genetics of Papilio dardanus, Brown. II. Races Dardanus, Polytrophus, Meseres, and Tibullus. Genetics, 45 : 439 - 457.

Clarke, C.A., and Sheppard, P.M., 1960b. The evolution of dominance under disruptive selection. Heredity, 14 : 73 - 87.

Clarke, C.A., and Sheppard, P.M., 1960c. The evolution of mimicry in the butterfly Papilio dardanus. Heredity, 14 : 163 - 173.

Clarke, C.A., and Sheppard, P.M., 1960d. Super-genes and mimicry. Heredity, 14 : 175 - 185.

Clarke, C.A., and Sheppard, P.M., 1962. The genetics of the mimetic butterfly Papilio glaucus. Ecology, 43 : 159 - 161.

Cockayne, E.A., 1928. On larval variation. Proc. S. Lond. Ent. Nat. Hist. Soc. (1927 - 1928) : 55 - 67.

Corbet, G.B., and Southern, H.N., 1977. The Handbook of British Mammals. 2nd ed., Blackwell Scientific Publications. Oxford.

Croze, H.T., 1967. Dispersion in camouflaged animals and searching image in predators. D. Phil. thesis, Oxford.

Croze, H.T., 1970. Searching image in carrion crows. Z. Tierpsychol., 5 : 1 - 85.

Crowley, T.E., and Pain, T., 1970. A monographic revision of the African land snails of the genus Limicolaria Schumacher (Mollusca-Achatinidae). Mus. R. Afrique Centr., Zool., 177 : 1 - 61.

Dempster, E.R., 1955. Maintenance of genetic heterogeneity. Cold Spring Harb. symp. quant. Biol., 20 : 25 - 32.

Den Boer, M.H., 1971. A colour-polymorphism in caterpillars of Bupalus piniarius (L.) (Lepidoptera : Geometridae). Neth. J. Zool., 21 : 61 - 116.

Diver, C., 1929. Fossil records of Mendelian mutants. Nature, 124 : 183.

Dobzhansky, Th., 1947. Genetics of Natural Populations, XIX. Genetics, 35 : 288 - 302.

Dobzhansky, Th., 1958. Genetics of Natural Populations, XXVII. Evolution, 12 : 385 - 401.

Dobzhansky, Th., and Levene, H., 1948. Genetics of Natural Populations, XVII. Genetics, 33 : 537 - 547.

Dobzhansky, and Pavlovsky, O., 1960. How stable is Balance polymorphism? Proc. nat. Acad. Sci., Wash., 46 : 41 - 47.

Dowdeswell, W.H., 1961. Experimental studies on natural selection in the butterfly Maniola jurtina. Heredity, 16 : 39 - 52.

Ernst, A., 1933. Weitere untersuchungen zur phänalyse zum fertilitäts problem und zur genetik heterosyler Primeln, I. Primula viscosa. Arch. Klaus-Stift. VererbForsch., 8 : 1 - 215.

Faure, J.C., 1943. The phases of the lesser army-worm. Farming S. Afr., 18 : 69 - 78.

Federley, H., 1916. Die Vererbung des Raupendimorphismus von Chaerocampa elpenor L. Ofv. Finska Vet. Soc. Forh., A., 58 (17) : 13pp.

Feltwell, J.S.E., 1973. The metabolism of carotenoids in Pieris brassicae L. (The Large White Butterfly) in relation to its foodplant Brassica oleracea var capitata L. (The Cabbage). Ph. D. thesis, University of London, (Royal Holloway College).

Feltwell, J.S.E., and Valadon, L.R.G., 1972. Carotenoids of Pieris brassicae and of its foodplant. J. Insect Physiol., 18 : 2203 - 2215.

Feltwell, J.S.E., and Valadon, L.R.G., 1974. Carotenoid changes in Brassica oleracea var capitata with age in relation to the Large White butterfly, Pieris brassicae L. J. Agr. Sci., 83 : 19 - 26.

Fisher, R.A., 1930a. The Genetic Theory of Natural Selection, Oxford.

Fisher, R.A., 1930b. The Distribution of Gene Ratios for rare Mutations. Proc. roy. Soc. Edinb., 50 : 204 - 19.

Fisher, R.A., 1939. Selective forces in wild populations of Paratettix tetanus. Ann. Eugen., Lond., 9 : 109 - 122.

Fontaine, A.R., 1962. The colours of Ophiocemina nigra (Abilgaard), 1. Colour variations and its relation to distribution. J. mar. Biol. Ass. U.K., 42 : 1 - 8.

Ford, E.B., 1937. Problems of heredity in the Lepidoptera. Biol. Rev., 12 : 461 - 503.

Ford, E.B., 1940a. Polymorphism and Taxonomy, pp. 493 - 513, The New Systematics (ed. Julian Huxley), Clarendon Press, Oxford.

Ford, E.B., 1940b. Genetic research in the Lepidoptera. Ann. Eugen., Lond., 10 : 227 - 252.

Ford, E.B., 1953. The genetics of polymorphism in the Lepidoptera. Adv. Genet., 5 : 43 - 87.

Ford, E.B., 1955. Moths, Collins, London.

Ford, E.B., 1964. Ecological Genetics, Methuen, London.

Ford, E.B., 1965. Genetic Polymorphism. Faber and Faber, London.

Fox, D.L., and Pantin, C.F.A., 1941. The colours of the plumose anemone Metridium senile. Phil. Trans. R. Soc., B., 230 : 415 - 450.

Fox, H.M., and Vevers, G., 1960. The nature of Animal Colours. London : Sidgwick and Jackson.

Fǿyn, B., and Gjǿen, I., 1954. Studies on the serpulid Pomatoceros triqueter L. II. The colour pattern of the branchial crown and its inheritance. Nytt. Mag. Zool., 2 : 85 - 90.

Gerould, J.H., 1921. Blue-green caterpillars : the origin and ecology of a mutation in hemolymph color in Colias (Eurymus) philodice. J. Exp. Zool., 34 : 385 - 412.

Gerould, J.H., 1922. Olive, a mutation in Colias philodice. Anat. Rec., 23 : 95.

Gerould, J.H., 1926. Inheritance of olive-green and blue-green, variations appearing in the life-cycle of a butterfly, Colias philodice. J. Exp. Zool., 43 : 413 - 425.

Gillespie, T.H., 1932. A book of King Penguins. Jenkins, London.

Goin, C.J., 1950. Colour pattern inheritance in some frogs of the genus Eleutherodactylus. Bull. Chicago Acad. Sci., 1 : 1 - 15.

Gordon, H., and Gordon, M., 1957. Maintenance of polymorphism by potentially injurious genes in eight natural populations of the platyfish, Xiphophorus maculatus. J. Genet., 55 : 1 - 44.

Gorodenski, S.A., 1970. The genetics of three larval color forms in Chlosyne lacinia and the phenotypic frequencies of this polymorphism in natural populations. M.Sc. thesis. Arizona State University.

Gould, L.T., 1892. Experiments in 1890 and 1891 on the colour relation between lepidopterous larvae and their surroundings, together with other observations on lepidopterous larvae. Trans. Ent. Soc. Lond., 1892 : 215 - 246.

Greenwood, J.J.D., 1969. Apostatic selection and population density. Heredity, 24 : 157 - 161.

Gustafsson, A., 1946. The effect of heterozygosity of Variability and Vigour. Hereditas, 32 : 263 - 286.

Gustafsson, A., Nybom, N., and Von Wettstein, U., 1950. Chlorophyll factors and heterosis in Barley. Hereditas, 36 : 383 - 392.

Hall, B.P., Moreau, R.E., and Galbraith, I.C.J., 1966. Polymorphism and parallelism in the African bush shrikes of the genus Malaconotus. (including Chlorophoneus). Ibis, 108 : 161 - 182.

Harashima, K., Ohno, T., Sawachika, T., Hidaka, T., and Ohnishi, E., 1972. Carotenoids in orange pupae of the swallowtail Papilio xuthus. Insect Biochem., 2 : 29 - 48.

Heppner, F., 1965. Sensory mechanisms and environmental clues used by the American robin in locating earthworms. Condor, 67 : 247 - 255.

Herman, C., and Lorkovic, Z., 1962. New "spotted" gene in caterpillars of Colias croceus Fourc. Biol.Glasnik., 14 : 151 - 153.

Herter, K., 1965. Hedgehogs - a comprehensive study. Phoenix House, London.

Junge, H., 1941. Über grüne Insecktenfarbstoffe. Hoppe-Seyler's Z. Physiol. Chem., 268 : 179 - 186.

Katz, D., 1937. Animals and Men. Studies in Comparative Psychology. Longmans, Green and Co., New York.

Kayser-Wegmann, I., 1975. Untersuchungen zur Photobiologie und Endokrinologie der Farbmodificationen bei der Kohlweisslingspuppe Pieris brassicae : Zeitverlauf der Sensiblen und Kritisthen Phasen. J. Insect Physiol, 21 : 1065 - 1072.

Kettlewell, H.B.D., 1952. Use of Radioactive Tracer in the study of insect populations (Lepidoptera). Nature, 170 : 584.

Kettlewell, H.B.D., 1955a. Labelling locusts with radioactive isotopes. Nature, 175 : 821 - 822.

Kettlewell, H.B.D., 1955b. Recognition of appropriate backgrounds by the pale and black phase of Lepidoptera. Nature, 175 : 934.

Kettlewell, H.B.D., 1955c. Selection experiments on industrial melanism in the Lepidoptera. Heredity, 9 : 323 - 342.

Kettlewell, H.B.D., 1956. Further selection experiments on industrial melanism in the Lepidoptera. Heredity, 10 : 287 - 301.

Kettlewell, H.B.D., 1958. A survey of the frequencies of Biston betularia (L) (Lep.) and its melanic forms in Great Britain. Heredity, 12 : 51 - 72.

Kikkawa, H., 1953. Biochemical genetics of Bombyx Mori. Advanc. Genet., 5 : 107 - 140.

Kimura, M., 1956. A model of a genetic system which leads to closer linkage by natural selection. Evolution, 10 : 278 - 287.

Klopfer, P.H., 1961. Observational learning in birds. Behaviour, 17 : 71 - 79.

Li, C.C., 1962. On 'reflexive selection'. Science, 136 : 1055.

Long, D.B., 1953. Effects of population density on larvae of Lepidoptera. Trans. Roy. Ent. Soc. Lond., 104 : 543 - 585.

Madge, D.S., 1962. The biology of the cutworm Tryphaena pronuba L. (Lepidoptera : Noctuidae). Ent. exp. and appl., 5 : 261 - 269.

Madge, D.S., 1964a. The light reactions and feeding activity of larvae of the cutworm Tryphaena pronuba L. (Lepidoptera: Noctuidae). Part 1 Laboratory investigations. Ext. exp. and appl., 7 : 47 - 61.

Madge, D.S., 1964b. The light reactions and feeding activity of larvae of the cutworm Tryphaena pronuba L. (Lepidoptera: Noctuidae). Part II. Field investigations. Ent. exp. and appl., 7 : 105 - 114.

Mason, J.L., 1972. Ecological studies of Coleoptera of a Kentish woodland. Ph. D. thesis. University of London.

Matthée, J.J., 1945. Biochemical differences between the solitary and gregarious phases of locust and Noctuids. Bull. Ent. Res., 36 : 343 - 371.

Matthée, J.J., 1947. Phase variation in the lawn caterpillar (Spodoptera abyssinian Guen). J. Ent. Soc. S. Afr., 10 : 16 - 23.

Mather, K., 1943. Polygenic inheritance and natural selection. Biol. Rev., 18 : 32 - 64.

Mather, K., 1955. Polymorphism as an outcome of disruptive selection. Evolution, 9 : 52 - 61.

Moment, G.B., 1962. Reflexive selection : a possible answer to an old puzzle. Science, 136 : 262 - 263.

Morton Jones, F., 1932. Insect coloration and relative acceptibility of insects to birds. Trans. R. ent. Soc. Lond., 82 : 345 - 385.

Morton Jones, F., 1934. Further experiments on coloration and relative acceptibility of insects to birds. Trans. R. ent. Soc. Lond., 82 : 443 - 453.

Moss, J.E., 1933. The Natural Control of the Cabbage Caterpillars, Pieris spp. Journal of Animal Ecology, 2 : 210 - 231.

Muller, H.J., 1950. Our load of Mutations. Amer. J. hum. Genet., 2 : 111 - 176.

Muller, H.J., 1958. The mutation theory re-examined. Proc. 10th Int. Congr. Genet., 306 - 317.

Mummery, R.S., Rothschild, M., and Valadon, L.R.G., 1975. Carotenoids of two silk moths Saturnia pavonia L. and Actia luna L. Comp. Biochem. Physiol., 50B : 23 - 28.

The Munsell Book of Color. (Munsell Color Co. Inc., Baltimore, 1966).

Murton, R.K., 1971a. Why do some bird species feed in flocks? Ibis, 113 : 534 - 536.

Murton, R.K., 1971b. The significance of specific search image in the feeding behaviour of the wood-pigeon. Behaviour, 40 : 10 - 42.

Neck, R.W., 1971. Larval morph variation in Chlosyne lacinia. (Nymphalidae). J. Lep. Soc., 30 No. 2 : 91 - 94.

Oertel, H., 1910. Merkwürdige Färbung einer Raupe von Chaerocampa elpenor L. Inter. Ent. Z., 4 : 48 - 49.

Owen, D.F., 1963. Polymorphism and population density in the African land snail. (Limicolaria martensiana.) Science, 140 : 666 - 667.

Pavan, C., Dobzansky, Th., and Da Cunha, A.B., 1957. Heterosis and elimination of weak homozygotes in natural populations of three related species of Drosophila. Proc. nat. Acad. Sci., Wash., 43 : 226 - 234.

Pielowski, Z., 1959. Studies on the relationship:predator (goshawk)-prey (pigeon). Bull. Acad. pol. Sci. Cl. II Ser. Sci. biol., 7 : 401 - 403.

Pielowski, Z., 1961. Über den Unifikationseinfluss der selektiven Nahrungswahl des Habichts (Accipiter gentilis) aus Haustaben. Ekol. pol., 9 : 183 - 194.

Pough, F.H., 1964. Apostatic selection and the searching image. B.Sc. thesis, Amherst College.

Poulton, E.B., 1885. The essential nature of the colouring of phytophagous larvae (and their pupae) : with an account of some experiments upon the relation between the colour of such larvae and that of their food plant. Proc. Roy. Soc., 38 : 269 - 315.

Poulton, E.B., 1886. A further enquiry into a special colour relation between the larvae of Smerinthus ocellatus and its food plants. Proc. Roy. Soc., 40 : 135 - 173.

Poulton, E.B., 1887. An enquiry into the cause and extent of a special colour relation between certain exposed lepidopterous pupae and the surfaces which immediately surround them. Phil. Trans. Roy. Soc., B, 178 : 311 - 441.

Poulton, E.B., 1892. Further experiments upon the colour relation between certain lepidopterous larvae, pupae, cocoons and imagines, and their surroundings. Trans. Ent. Soc. Lond., 1892 : 293 - 487.

Poulton, E.B., 1893. The experimental proof that the colours of certin lepidopterous larvae are largely due to modified plant pigments derived from food. Proc. Roy. Soc., 54 : 417 - 430.

Pumphrey, R.J., 1948. The sense organs of birds. Ibis, 90 : 171 - 190.

Pumphrey, R.J., 1961. Sensory organs : Vision in Biology and Comparative Physiology of Birds, vol. II, ed. A.J. Marshall, 55 - 68, Academic Press, New York.

Robinson, R., 1971. Lepidoptera genetics. Pergamon Press, New York.

Rothschild, The Hon. M., Valadon, G. and Mummery, R., 1977. Carotenoids of the pupae of the Large White butterfly (Pieris brassicae) and the Small White butterfly (Pieris rapae). J. Zool., Lond., 181 : 323 - 339.

Ruiter, L. De., 1952. Some experiments on the camouflage of stick caterpillars. Behaviour, 4 : 222 - 232.

Sevastopulo, D.G., 1959. Yellow-green larvae of Pieris napi L. Entomologist, 92 : 17.

Sheppard, P.M., 1951. Fluctuations in the selective value of certain phenotypes in the polymorphic land snail Cepaea nemoralis (L). Heredity, 5 : 125 - 134.

Sheppard, P.M., 1952. A note on non-random mating in the moth Panaxia dominula (L). Heredity, 6 : 233 - 238.

Sheppard, P.M., 1953. Polymorphism and population studies. Symp. Soc. exp. Biol., 7 : 274 - 289.

Sheppard, P.M., 1955. Genetic variability and polymorphism: synthesis. Cold Spring Harbor Symp. Quant. Biol., 20 : 271 - 275.

Sheppard, P.M., 1958. Natural Selection and Heredity. Hutchinson, London.

Sheppard, P.M., 1959. The evolution of mimicry; a problem in ecology and genetics. Cold Spr. Harb. Symp. quant. Biol., 24 : 131 - 140.

Sheppard, P.M., 1961a. Some contributions to population genetics resulting from the study of the Lepidoptera. Advanc. Genet., 10 : 165 - 216.

Sheppard, P.M., 1961b. Recent genetical work on polymorphic mimetic Papilios. Symposia R. ent. Soc., No. 1 : 23 - 30.

Smith, D.A.S., 1971. Polymorphism and population density in Donax rugosus (Lemellibranchiata:Donacidae). J. Zool., Lond., 164 : 428 - 442.

Smith, J. Maynard, 1962. Disruptive selection, polymorphism and sympatric speciation. Nature, 195 : 60 - 62.

Snedecor, G.W., 1956. Statistical Methods. Iowa State College Press, Iowa, U.S.A.

Spiess, E.B., 1958. Chromosomal adaptive polymorphism in Drosophila persimilis, II. Evolution, 12 : 234 - 245.

South, R., 1939. The Moths of the British Isles, Series One, 3rd edition. Frederick Warne and Co. Ltd., London.

Southern, H.N., 1945. Polymorphism in Poephila gouldiae. J. Genet., 47 : 51 - 57.

Sumner, F.B., 1927. Linear and Colorimetric Measurements of Small Mammals. Journ. of Mamm., 8, No. 3 : 177 - 206.

Sumner, F.B., 1934. Does 'protective coloration' protect? Results of some experiments with fishes and birds. Proc. Natn. Acad. Sci. U.S.A., 20 : 559 - 564.

Tanaka, Y., 1953. Genetics of the silkworm, Bombyx mori. Advanc. Genet., 5 : 239 - 317.

Tazima, Y., 1964. The Genetics of the Silkworm. Logos Press, London.

Thompson, A.L., 1964. A New Dictionary of Birds. Nelson, London.

Tinbergen, L., 1946. De sperwer als roofvijand van songvogels. Ardea, 34 : 1 - 213.

Tinbergen, L., 1960. The natural control of insects in pine woods. I. Factors influencing the intensity of predation by songbirds. Arch. Neerl. Zool., 13 : 265 - 343.

Turner, E.R.A., 1961. Survival values of different methods of camouflage as shown in a model population. Proc. Zool. Soc. Lond., 136 : 273 - 283.

Turner, E.R.A., 1965. Social feeding in birds. Behaviour, 24 : 1 - 43.

Uexkull, J. von., 1934. Streifzüge durch die Uimwelten von Tieren und Menschen. Springer, Berlin.

Walls, G.L., 1942. The Vertebrate Eye and its Adaptive Radiation. Cranbrook Press, Bloomfield Hills, Michigan.

Ward, P., 1965. Feeding ecology of the black-faced dioch Quelea quelea in Nigeria. Ibis, 107 : 108 - 111.

Watson, A., and Whalley, P.E.S., 1975. The Dictionary of Butterflies and Moths. (Introduction by E. Laithwaite). George Rainbird Ltd., London.

Williamson, M.H., 1958. Selection, controlling factors and polymorphism. Am. Nat., 92 : 329 - 335.

Wright, S., 1948. On the role of directed and random changes in gene frequency in the genetics of populations. Evolution, 2 : 279 - 294.

Yalden, D.W., 1976. The Food of the Hedgehog in England. Acta Theriologica., Vol. 21, 30 : 401 - 424.

Yokoyama, T., 1959. Silkworm Genetics Illustrated. Japanese Society for the Promotion of Science, Tokyo.

Appendix Table 3Bai.

Experiments to investigate the effect of temperature on larval colour. Colour analysis of samples ET1 - 10 inclusive. First and Second instar larvae (all larvae EG colour type).

Sample	Hue type	First Instar Shade						Second Instar Shade					
		-4	-3	-2	-1	0	1	-4	-3	-2	-1	0	1
ET1	5GY					1					1		
	7.5GY		1	3	5	5	1			4	6	4	1
	10GY		1	3	3	2				3	3	3	
ET2	5GY			1	1						1	1	
	7.5GY			3	6	4	1			3	6	4	1
	10GY		1	2	3	3				3	4	2	
ET3	5GY				1							1	
	7.5GY		1	4	5	4	1			3	6	4	1
	10GY			3	3	3				2	5	3	
ET4	5GY					1					1		
	7.5GY		1	3	5	5			1	4	5	4	1
	10GY		1	3	3	3				3	3	3	
ET5	5GY					1						1	
	7.5GY		1	3	6	4	1			4	6	5	
	10GY		1	3	3	2			1	3	3	2	

Appendix Table 3Bai. (Continued)

Experiments to investigate the effect of temperature on larval colour. Colour analysis of samples ET1 - 10 inclusive. First and Second instar larvae (all larvae EG colour type).

Sample	Hue type	First Instar Shade						Second Instar Shade					
		-4	-3	-2	-1	0	1	-4	-3	-2	-1	0	1
ET6	5GY				1							1	
	7.5GY		1	4	5	3	1		1	4	5	3	1
	10GY	1		4	2	3				4	3	3	
ET7	5GY				1	1							
	7.5GY		1	4	5	4			1	4	7	4	
	10GY			3	3	3				2	4	3	
ET8	5GY			1		1						1	
	7.5GY			2	6	5	1			3	5	4	1
	10GY		1	4	2	2			1	4	3	3	
ET9	5GY				1							1	
	7.5GY		1	3	6	5	1			4	5	4	1
	10GY		1	2	3	2			1	3	4	2	
ET10	5GY					1						1	
	7.5GY		1	4	5	4	1		1	3	6	5	
	10GY			3	3	3				3	4	2	

Appendix Table 3Baii.

Experiments to investigate the effect of temperature on larval colour. Colour analysis of samples ET1 - 10 inclusive. Third instar larvae.

Sample	Hue type	Colour type	Shade -3	-2	-1	0	1	2
ET1		OL		2				
	7.5GY	3IG		3	3	2		
	10GY	3IG		5	4	5		
	2.5G	3IG			1			
ET2		Br			1			
		OL	1	2				
	7.5GY	3IG		3	5	2		
	10GY	3IG		3	4	4		
ET3		Br			1			
		OL	1	1				
	7.5GY	3IG		4	2	2		
	10GY	3IG		4	4	5		
	2.5G	3IG			1			
ET4		Br						
		OL	1	1	1			
	7.5GY	3IG		3	4	3		
	10GY	3IG	1	3	5	3		
ET5		Br		1				
		OL	1	2				
	7.5GY	3IG		3	4	2		
	10GY	3IG		3	3	6		
ET6		OL		1	1			
	7.5GY	3IG		3	4	1		
	10GY	3IG		6	5	4		
ET7		Br		1				
		OL	1	2				
	7.5GY	3IG		3	5	1		
	10GY	3IG	1	3	3	5		
ET8		Br			1			
		OL	1	1				
	7.5GY	3IG		4	3	3		
	10GY	3IG		3	5	3		
	2.5G	3IG			1			
ET9		Br		1				
	7.5Y	PY						1
		OL	1	1				
	7.5GY	3IG		3	3	2		
	10GY	3IG	1	4	5	3		
ET10		Br						
		OL		2	1			
	7.5GY	3IG		2	5	1		
	10GY	3IG	1	4	5	4		

Appendix Table 3Baiii.

Experiments to investigate the effect of temperature on larval colour. Colour analysis of samples ET1 - 10 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour type	Fourth instar						Fifth instar							
			Shade						Shade							
			-3	-2	-1	0	1	2	-4	-3	-2	-1	0	1	2	3
ET1	5YR	Br/YB								1	1	2				
	7.5YR	Br	1	1					1	4	3	1				
	10YR	Br								1	1					
	2.5Y	Br							1							
	7.5Y	PY						1								
	10Y	OL		1							1					
	2.5GY	OL	4	5	1					1	2					
	7.5GY	Gr			1	2	1					1		1		
	10GY	Gr			5	2						1	2			
ET2	5YR	Br/YB		1						1	2	1				1
	7.5YR	Br	1	1					1	4	2	1				
	10YR	Br							1	2	1					
	10Y	OL	1	1	1											
	2.5GY	OL	3	4	1					1	2	1				
	7.5GY	Gr			1	3	1					1				
	10GY	Gr		1	2	2	1					1	1	1		

Appendix Table 3Baiii. (Continued)

Experiments to investigate the effect of temperature on larval colour. Colour analysis of samples ET1 - 10 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour type	Fourth instar						Fifth instar							
			Shade						Shade							
			-3	-2	-1	0	1	2	-4	-3	-2	-1	0	1	2	3
ET3	5YR	Br	1								1	1				
	7.5YR	Br	1	1					1	4	3	1				
	10YR	Br								1	1					
	2.5Y	Br							1							
	5Y	Br/PY													1	
	10Y	OL								1						
	2.5GY	OL	4	5	2					2	1					
	5GY	OL	1													
	7.5GY	Gr			1	2						1	1	1		
	10GY	Gr			3	2	1					1	1	1		
	2.5G	Gr		1												
ET4	5YR	Br								1	2	1				
	7.5YR	Br		2						3	3	1				
	10YR	Br								2	2					
	5Y	Br/PY													1	
	7.5Y	PY						1								
	10Y	OL		1	1											
	2.5GY	OL	4	5						2	1					
	5GY	OL														
	7.5GY	Gr			2	2	2					1		1		
	10GY	Gr			3	2					1	2	1			

Appendix Table 3Baiii. (Continued)

Experiments to investigate the effect of temperature on larval colour. Colour analysis of samples ET1 - 10 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour type	Fourth instar						Fifth instar							
			Shade						Shade							
			-3	-2	-1	0	1	2	-4	-3	-2	-1	0	1	2	3
ET5	5YR	Br	1	1						2	2					
	7.5YR	Br							1	3	2	1				
	10YR	Br							1	2	2					
	7.5Y	PY						1								
	10Y	OL		1												
	2.5GY	OL	4	5	2					2	2					
	5GY	OL														
	7.5GY	Gr			1	3	1					1	1	1		
	10GY	Gr		1	3	1							1	1		
ET6	5YR	Br		1					1	1	1	1				
	7.5YR	Br	1	1						3	3	1				
	10YR	Br								2	1					
	7.5Y	PY						1								
	10Y	OL		1							1					
	2.5GY	OL	4	5	1					2	2					
	7.5GY	Gr			1	2						1	1	1		
	10GY	Gr		1	2	2	2					1	1	1		

Appendix Table 3Baiii. (Continued)

Experiments to investigate the effect of temperature on larval colour. Colour analysis of samples ET1 - 10 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour type	Fourth instar							Fifth instar							
			Shade							Shade							
			-4	-3	-2	-1	0	1	2	-4	-3	-2	-1	0	1	2	3
ET7	5YR	Br									1	2	1	1			
	7.5YR	Br		1	1						3	3	1				
	10YR	Br									2	1					
	2.5GY	OL/YG	1	3	4	2					1	2					1
	5GY	OL		1								1	1	1	1		
	7.5GY	Gr				1	2	1					1	1			
	10GY	Gr			1	3	2										
	2.5G	Gr				1	1										
ET8	5YR	Br									1	2	1				
	7.5YR	Br		2	1					1	4	3	1				
	10YR	Br									2						
	10Y	OL			1	1						1					
	2.5GY	OL		3	4	1					2	2					
	7.5GY	Gr				1	3	2					1		1		
	10GY	Gr			1	3	2						1	2			

Appendix Table 3Baiii. (Continued)

Experiments to investigate the effect of temperature on larval colour. Colour analysis of samples ET1 - 10 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour type	Fourth instar							Fifth instar							
			Shade							Shade							
			-4	-3	-2	-1	0	1	2	-4	-3	-2	-1	0	1	2	3
ET9	5YR	Br		1							2	2	1				
	7.5YR	Br			2					1	3	4	1				
	10YR	Br								1	1						
	5Y	Br								1							
	7.5Y	PY						1									
	10Y	OL		1	1							1					
	2.5GY	OL		4	5	1					2	1					
	7.5GY	Gr				1	2	1					1		1		
	10GY	Gr				3	2						1	1			
ET10	5YR	Br			1					1		1	1				
	7.5YR	Br		2						1	3	3	1				
	10YR	Br									2	1					
	2.5Y	Br								1	1						
	10Y	OL			1	1					1						
	2.5GY	OL	1	2	4	1					1	2					
	5GY	OL			1												
	7.5GY	Gr				1	2	2					1	1			
	10GY	Gr			1	3	2						1	1	1		

Appendix Table 3Bbi.

Experiments to investigate the effect of larval density on colour variation. Colour analysis of samples E01 - 32 inclusive. First and Second instar larvae. (Larvae all EG colour type).

Sample	Hue type	First instar Shade -4	-3	-2	-1	0	1	Second instar Shade -4	-3	-2	-1	0	1
E01 - 20 inclusive	5GY				1							1	
	7.5GY			3	4	3	1			3	5	3	
	10GY		1	3	2	2				3	3	2	
E021 - 24 inclusive	5GY										1		
	7.5GY			3	5	4				3	5	3	1
	10GY		1	2	3	2				3	2	2	
E025 - 26 inclusive	5GY				1	1							
	7.5GY		1	2	4	4			1	3	5	3	1
	10GY			3	2	2				2	3	2	
E027	7.5GY			2	5	4	1			3	5	3	1
	10GY		1	3	2	1	1			3	4	1	
E028	5GY				1	1							1
	7.5GY		1	5	10	6	1			6	11	6	1
	10GY		2	6	4	3				5	7	3	
E029	5GY				1	1							
	7.5GY		2	8	11	11	2		1	8	16	9	3
	10GY		2	9	7	6				8	10	5	

Appendix Table 3Bbi. (Continued)

Experiments to investigate the effect of larval density on colour variation. Colour analysis of samples E01 - 32 inclusive. First and Second instar larvae. (Larvae all EG colour type).

Sample	Hue type	First instar Shade						Second instar Shade					
		-4	-3	-2	-1	0	1	-4	-3	-2	-1	0	1
E030	5GY				1							1	1
	7.5GY		3	9	17	13	3		1	9	23	12	3
	10GY		2	13	10	8	1			11	13	6	
E031	5GY			1	1	1					1	1	1
	7.5GY		3	13	21	16	3		1	14	25	16	3
	10GY		4	16	10	10	1		1	11	17	9	
E032	5GY			2	2	2					2	1	2
	7.5GY	2	5	23	43	38	5		2	28	48	33	9
	10GY	1	7	30	20	18	2		1	23	32	18	1

Appendix Table 3Bbii.

Experiments to investigate the effect of larval density on colour variation. Colour analysis of samples E01 - 32 inclusive. Third instar larvae.

Sample	Hue type	Colour type	Shade -3	-2	-1	0	1	2
E01 - 20		OL	1	1				
inclusive	7.5GY	3IG		2	3	2		
	10GY	3IG	1	3	3	3	1	
E021 - 24		OL	1	1				
inclusive	7.5GY	3IG		2	4	1		
	10Y	3IG		4	4	3		
E025 - 26		Br		1				
inclusive		OL		2				
	7.5GY	3IG		2	3	2		
	10GY	3IG		3	4	3		
E027		Br		1				
		OL	1	2				
	7.5GY	3IG		2	3	1		
	10GY	3IG		3	3	3	1	
E028		Br			1			
		OL	1	3				
	7.5GY	3IG		4	6	3		
	10GY	3IG	1	6	7	7	1	
E029		Br			1			
		OL	2	4				
	7.5GY	3IG		6	11	4		
	10GY	3IG	1	9	11	9	2	
E030		Br		1	1			
	7.5Y	PY						1
		OL	3	5				
	7.5GY	3IG	1	7	13	6		
	10GY	3IG	1	12	15	12	2	
E031		Br		2				
	7.5Y	PY						1
		OL	3	7				
	7.5GY	3IG	1	11	16	7	1	
	10GY	3IG	3	14	17	12	5	
E032		Br		2	3			
	7.5Y	PY						1
		OL	6	15				
	7.5GY	3IG	1	20	31	14	2	
	10GY	3IG	6	27	35	28	8	
	2.5G	3IG			1			

Appendix Table 3Bbiii.

Experiments to investigate the effect of larval density and colour variation. Colour analysis of samples E01 - 32 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour Type	Fourth instar Shade								Fifth instar Shade							
			-4	-3	-2	-1	0	1	2	3	-4	-3	-2	-1	0	1	2	3
E01 - 20 inclusive	5YR	Br										1	1	1				
	7.5YR	Br		1	1						1	2	2	1				
	10YR	Br										2	1					
	5Y	Br/PY															1	
	7.5Y	PY							1									
	10Y	OL			1								1					
	2.5GY	OL		3	3	1						1	1					
	7.5GY	Gr				1	2	1						1		1		
	10GY	Gr			1	3	1							1	1			
E021-24 inclusive	5YR	Br										1	1	1				
	7.5YR	Br			2							2	2	1				
	10YR	Br										1	1					
	2.5Y	Br									1							
	5Y	Br										1						
	10Y	OL		1														
	2.5GY	OL		2	4	1						2	1	1				
	7.5GY	Gr				1	2	1						1				
	10GY	Gr				3	2	1						1	1	1		

Appendix Table 3Bbiii. (Continued)

Experiments to investigate the effect of larval density and colour variation. Colour analysis of samples E01 - 32 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour Type	Fourth instar Shade								Fifth instar Shade							
			-4	-3	-2	-1	0	1	2	3	-4	-3	-2	-1	0	1	2	3
E025-26 inclusive	5YR	Br										1	1	1				
	7.5YR	Br		2								3	3	1				
	10YR	Br									1	1	1					
	10Y	OL			1							1						
	2.5GY	OL	1	2	4	1						1	1					
	7.5GY	Gr				1	2		1					1	1	1		
	10GY	Gr				2	2	1							1			
E027	5YR	Br											2					
	7.5YR	Br		1	2						1	2	2	1				
	10YR	Br										2	1					
	5Y	Br									1							
	10Y	OL											1					
	2.5GY	OL		3	3	1						1	1	1				
	5GY	OL		1														
	7.5GY	Gr					2	1						1				
	10GY	Gr			1	3	2							1	1		1	

Appendix Table 3Bbiii. (Continued)

Experiments to investigate the effect of larval density and colour variation. Colour analysis of samples E01 - 32 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour Type	Fourth instar Shade								Fifth instar Shade							
			-4	-3	-2	-1	0	1	2	3	-4	-3	-2	-1	0	1	2	3
E028	5YR	Br										2	3	2				
	7.5YR	Br		2	3						1	5	4	2				
	10YR	Br										3	1					
	2.5Y	Br									1							
	5Y	Br/PY														1		
	7.5Y	PY							1									
	10Y	OL			1							1	1					
	2.5GY	OL	1	5	7	2						2	2	1				
	5GY	OL			1													
	7.5GY	Gr				2	4	1						1		2		
	10GY	Gr			1	5	3	1						2	2	1		
E029	5YR	Br/YB										3	3	3				1
	7.5YR	Br		3	3						2	8	7	2	1			
	10YR	Br									1	4	2					
	2.5Y	Br									1							
	7.5Y	PY							1									
	10Y	OL			2	1						1	1					
	2.5GY	OL		9	11	3						4	3	1				
	5GY	OL		1														
	7.5GY	Gr				3	6	2						2	1	2		
	10GY	Gr			2	7	3	1					1	2	3	1		
	2.5G	Gr			1		1											

Appendix Table 3Bbiii. (Continued)

Experiments to investigate the effect of larval density and colour variation. Colour analysis of samples E01 - 32 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour Type	Fourth instar Shade								Fifth instar Shade							
			-4	-3	-2	-1	0	1	2	3	-4	-3	-2	-1	0	1	2	3
E030	5YR	Br/YB		1	1							3	4	2	1			1
	7.5YR	Br		3	4						2	10	9	4				
	10YR	Br									1	5	3	1				
	2.5Y	Br									1							
	5Y	Br									1							
	7.5Y	PY							2									
	10Y	OL		1	2	1						1	1					
	2.5GY	OL/YG	1	10	15	3					1	5	5	1				1
	5GY	OL		1														
	7.5GY	Gr				4	8	3					1	3	2	2		
	10GY	Gr			3	9	6	2						3	4	2		
E031	5YR	Br/YB		1	1						1	4	6	4	1		1	
	7.5YR	Br		3	5						3	13	11	3				
	10YR	Br										6	4	1				
	2.5Y	Br									2	1						
	5Y	Br									1							
	7.5Y	PY							1									
	10Y	OL		1	3	1						1	2	1				
	2.5GY	OL	2	14	19	4					1	7	5	2				
	5GY	OL		1														
	7.5GY	Gr				4	10	4	1				1	3	2	1		
	10GY	Gr			2	11	8	2					1	4	5	2		
	2.5G	Gr			1	1												

Appendix Table 3Bbiii. (Continued)

Experiments to investigate the effect of larval density and colour variation. Colour analysis of samples E01 - 32 inclusive. Fourth and Fifth instar larvae.

Sample	Hue type	Colour Type	Fourth instar Shade								Fifth instar Shade							
			-4	-3	-2	-1	0	1	2	3	-4	-3	-2	-1	0	1	2	3
E032	5YR	Br/YB		2	2	1					2	8	11	8	2		1	
	7.5YR	Br		8	9						5	24	21	7	1			
	10YR	Br									2	12	8	1				
	2.5Y	Br									1	1						
	5Y	Br/PY									2	1					3	
	7.5Y	PY							5									
	10Y	OL		2	5	3						3	4	2				
	2.5GY	OL/YG	2	25	36	9					1	13	12	3			1	1
	5GY	OL		1	1					1								
	7.5GY	Gr			1	8	19	9	1				1	6	4	5		
	10GY	Gr		1	5	21	17	4					2	7	9	4	1	
	2.5G	Gr			1		1											

Appendix Table 3Bc/1i.

Experiments to investigate the effect of background colour on larval colour. Colour analysis of the ELA samples. First instar larvae. (All larvae EG colour type).

Hue type	Sample	shade -4	-3	-2	-1	0	1	Sample	shade -4	-3	-2	-1	0	1
5GY	1ELA1				1	2		3ELA1				2	2	
7.5GY	and		2	5	11	10	2	and		1	8	10	9	2
10GY	2ELA1		1	6	7	3		4ELA1		1	4	7	4	
5GY	1ELA2				1	1		3ELA2			1	1	2	
7.5GY	and		2	7	10	11	3	and		2	6	9	13	1
10GY	2ELA2		1	4	6	4		4ELA2		2	6	2	5	
5GY	1ELA3				1	1		3ELA3				1	2	
7.5GY	and		1	5	9	10	2	and		1	7	13	10	2
10GY	2ELA3		3	4	11	3		4ELA3		1	6	5	2	
5GY	1ELA4				2	1		3ELA4				1	1	
7.5GY	and		2	7	10	9	2	and		1	11	12	5	2
10GY	2ELA4		1	7	4	5		4ELA4		2	4	7	4	
5GY	1ELA5				2	1		3ELA5				1	3	
7.5GY	and		1	3	11	7	2	and		3	6	7	9	1
10GY	2ELA5		1	6	10	6		4ELA5			4	10	5	1
5GY	1ELA6				1	1		3ELA6				2	2	
7.5GY	and		2	8	10	8	1	and	1	2	8	6	12	1
10GY	2ELA6		1	6	6	6		4ELA6			3	5	7	1
5GY	1ELA7					2		3ELA7			1	1	1	
7.5GY	and		1	4	14	10	2	and		2	7	11	14	
10GY	2ELA7	1	1	7	3	5		4ELA7		1	6	5	1	
5GY	1ELA8				2			3ELA8				1	1	
7.5GY	and		2	5	10	12	2	and		1	4	14	10	1
10GY	2ELA8		1	7	5	4		4ELA8		2	6	2	8	
5GY	1ELA9				2			3ELA9					3	
7.5GY	and		1	11	10	5	2	and			3	10	10	1
10GY	2ELA9			6	7	6		4ELA9		1	11	6	5	
5GY	1ELA10				2	2		3ELA10				2	2	
7.5GY	and		1	7	9	14	1	and		1	7	10	8	2
10GY	2ELA10		1	1	7	4	1	4ELA10		2	5	9	2	

Appendix Table 3Bc/1ii.

Experiments to investigate the effect of background colour on larval colour. Colour analysis of the ELA samples. Second instar larvae. (All larvae EG colour type).

Hue type	Sample	shade -4	-3	-2	-1	0	1	Sample	shade -4	-3	-2	-1	0	1
5GY	1ELA1				1	1		3ELA1				1	1	
7.5GY	and		1	7	10	9	1	and			7	11	8	2
10GY	2ELA1			5	8	7		4ELA1		1	5	7	7	
5GY	1ELA2				2	1		3ELA2				1	2	
7.5GY	and		2	8	13	12	1	and		1	8	9	9	
10GY	2ELA2		2	7	1	1		4ELA2			6	8	6	
5GY	1ELA3							3ELA3				2		
7.5GY	and		1	7	16	8	3	and		1	6	13	5	
10GY	2ELA3		1	4	6	4		4ELA3		1	6	5	9	2
5GY	1ELA4					2		3ELA4						
7.5GY	and			9	11	8		and			7	12	8	1
10GY	2ELA4		1	6	7	6		4ELA4		2	7	5	8	
5GY	1ELA5				3			3ELA5						
7.5GY	and			5	15	11	2	and		1	9	10	4	1
10GY	2ELA5			7	2	5		4ELA5			6	8	10	1
5GY	1ELA6				1	1		3ELA6				1		
7.5GY	and		2	9	12	7		and	1	1	8	10	14	1
10GY	2ELA6			3	5	9	1	4ELA6		1	4	5	4	
5GY	1ELA7				1	1		3ELA7				2	1	
7.5GY	and			5	7	9	1	and		1	9	12	15	
10GY	2ELA7		1	9	10	6		4ELA7			2	5	3	
5GY	1ELA8					2		3ELA8				1	2	
7.5GY	and		1	3	15	9	1	and		1	7	9	8	
10GY	2ELA8			7	7	5		4ELA8		1	5	6	10	
5GY	1ELA9							3ELA9					1	
7.5GY	and		2	11	6	6	2	and		1	9	9	9	1
10GY	2ELA9			4	11	8		4ELA9			8	10	2	
5GY	1ELA10				1	1		3ELA10						
7.5GY	and	1		7	14	9	1	and			8	9	12	1
10GY	2ELA10			4	6	6		4ELA10			7	6	7	

Appendix Table 3Bc/1iii.

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Third instar larvae.

Hue Type	Colour Type	Sample	Shade -3	-2	-1	0	1	2	Sample	Shade -4	-3	-2	-1	0	1	2
	Br	1ELA1		1					3ELA1				1			
7.5Y	PY	and							and							1
	OL	2ELA1	1	2	1				4ELA1		2	2	1			
7.5GY	3IG			6	7	2						3	9	6		
10GY	3IG			8	10	10						7	8	9		
2.5G	3IG				2								1			
	Br	1ELA2		1	1				3ELA2			1				
	OL	and	2	1	1				and		1	3	1			
7.5GY	3IG	2ELA2		5	7	10			4ELA2			9	4	4		
10GY	3IG			7	5	8						7	8	10		
2.5G	3IG				1	1							1	1		
	Br	1ELA3		1					3ELA3			1				
7.5Y	PY	and							and							1
	OL	2ELA3	2	2					4ELA3	1	1	1	1			
7.5GY	3IG			10	3	6						8	6	3		
10GY	3IG			9	10	7					1	7	10	8		
2.5G	3IG													1		

Appendix Table 3Bc/1iii. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Third instar larvae.

Hue Type	Colour Type	Sample	Shade -3	-2	-1	0	1	2	Sample	Shade -4	-3	-2	-1	0	1	2
	Br	1ELA4			2				3ELA4			1				
7.5Y	PY	and							and							1
	OL	2ELA4	3	3					4ELA4		2	3				
7.5GY	3IG			4	12	3						7	7	4		
10GY	3IG		1	5	6	10	1					3	13	9		
	Br	1ELA5			2				3ELA5			1	1			
7.5GY	PY	and							and							1
	OL	2ELA5	2	1	1				4ELA5		1	3				
7.5GY	3IG			7	8	4						5	11	3		
10GY	3IG			8	10	7						12	4	7		
2.5G	3IG												1			
	Br	1ELA6			1				3ELA6				2			
7.5Y	PY	and						1	and							
	OL	2ELA6		3	1				4ELA6		2	4				
7.5GY	3IG			7	9	6						6	8	10		
10GY	3IG			9	6	5					1	3	9	5		

Appendix Table 3Bc/1iii. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Third instar larvae.

Hue Type	Colour Type	Sample	Shade -3	-2	-1	0	1	2	Sample	Shade -4	-3	-2	-1	0	1	2
	Br	1ELA7							3ELA7			1				
	OL	and	1	4					and		2	2	2			
7.5GY	3IG	2ELA7		6	8	5			4ELA7			7	7	2		
10GY	3IG			5	7	12	2					5	9	12	1	
	Br	1ELA8			1				3ELA8							
7.5Y	PY	and						1	and							
	OL	2ELA8	2	3					4ELA8		1	3				
7.5GY	3IG			8	7	4						7	8	6		
10GY	3IG			6	9	9						7	4	12	2	
	Br	1ELA9		1	1				3ELA9			1	2			
7.5Y	PY	and						1	and							
	OL	2ELA9	1	3	1				4ELA9		1	2	3			
7.5GY	3IG			7	10	3							2	10	4	
10GY	3IG		1	4	8	8							6	10	9	
2.5G	3IG				1											
	Br	1ELA10			1				3ELA10			1				
5Y	PY	and						1	and							
	OL	2ELA10	2	2					4ELA10			3	1			
7.5GY	3IG			9	7	2						10	4	4		
10GY	3IG		1	6	10	7	1				1	6	13	6		
2.5G	3IG				1								1			

Appendix Table 3Bc/1iv.

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Fourth instar larvae.

Hue Type	Colour Type	Sample	Shade -3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br	1ELA1								3ELA1		1						
7.5YR	Br	and	1	3						and		1	3					
7.5Y	PY	2ELA1						2		4ELA1						1	1	
10Y	OL			2	1							1	1					
2.5GY	OL		8	10	1						2	6	11	3				
7.5GY	Gr				3		3							2	6	2		
10GY	Gr				8	8							1	6	1			
2.5G	Gr													1				
5YR	Br	1ELA2	1							3ELA2		1	1					
7.5YR	Br	and	2	2						and		2	2					
7.5Y	PY	2ELA2						1		4ELA2						1		
10Y	OL			2								2	1	1				
2.5GY	OL		8	10	3							7	7	2				
5GY	OL		1									1						
7.5GY	Gr				2	5	1							3	2	1		
10GY	Gr			1	6	3	1						1	5	9			
2.5G	Gr			1										1				

Appendix Table 3Bc/1iv. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Fourth instar larvae.

Hue Type	Colour Type	Sample	Shade -3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
2.5YR	Br	1ELA3			1					3ELA3								
5YR	Br	and								and			2					
7.5YR	Br	2ELA3	2	2						4ELA3		4	3					
7.5Y	PY							1									1	
10Y	OL			2	2								1	1				
2.5GY	OL		6	11	1						1	8	5	2				
5GY	OL		1															
7.5GY	Gr				4	4	1							6	1	1		
10GY	Gr				3	7	1						1	7	5			
2.5G	Gr					1									1			
5YR	Br	1ELA4	1	1						3ELA4								
7.5YR	Br	and	2	3						and		3	3					
7.5Y	PY	2ELA4								4ELA4								
10Y	OL			1	1							1		1				
2.5GY	OL		5	14	3							8	11	1				
5GY	OL		1									1						
7.5GY	Gr				3	7	1							4	3	2		
10GY	Gr				6	1							1	5	5			
2.5G	Gr													1				

Appendix Table 3Bc/1iv. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Fourth instar larvae.

Hue Type	Colour Type	Sample	Shade -3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br	1ELA5	2							3ELA5								
7.5YR	Br	and	1	3						and		1	2					
7.5Y	PY	2ELA5					1	1		4ELA5						1	1	
10Y	OL		2	2									1					
2.5GY	OL		8	10	2							9	10	3				
7.5GY	Gr				2	3	2							3	4			
10GY	Gr				7	4							1	7	5	1		
2.5G	Gr														1			
5YR	Br	1ELA6	1							3ELA6			2					
7.5YR	Br	and	1	3						and		2	2					
7.5Y	PY	2ELA6					1			4ELA6						1		
10Y	OL		1	1									1	1				
2.5GY	OL		7	9	2						2	8	8	2				
5GY	OL/YG		1									1						1
7.5GY	Gr				5	8									6			
10GY	Gr				5	1	3						1	7	4	1		
2.5G	Gr				1													

Appendix Table 3Bc/1iv. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Fourth instar larvae.

Hue Type	Colour Type	Sample	Shade -3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br	1ELA7		1						3ELA7		1						
7.5YR	Br	and	3	2						and		3	4					
7.5Y	PY	2ELA7					1	1		4ELA7							1	
10Y	OL		1	1									1	1				
2.5GY	OL		6	7	1							7	9	1				
5GY	OL		1									2	1					
7.5GY	Gr				7	4	1							3	3	1		
10GY	Gr				10	1							1	8	2			
2.5G	Gr			1	1									1				
5YR	Br	1ELA8								3ELA8			1					
7.5YR	Br	and	1	2						and		1	2					
7.5Y	PY	2ELA8					1			4ELA8						1		
10Y	OL			1	1								3	1				
2.5GY	OL		10	11	1						1	7	9	1				
5GY	OL/YG		1						1			1						
7.5GY	Gr				3	3	1							4	3	3		
10GY	Gr				3	9							1	4	7			
2.5G	Gr					1												

Appendix Table 3Bc/1iv. (Continued)

Experiments to investigate the effect of background colour on larval colour.
Colour analysis of the ELA samples. Fourth instar larvae.

Hue Type	Colour Type	Sample	Shade -3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br	1ELA9	1	1						3ELA9								
7.5YR	Br	and	2	2						and		2	3					
7.5Y	PY	2ELA9								4ELA9							1	
10Y	OL			1										1				
2.5GY	OL		9	10	1						1	9	9	1				
5GY	OL/YG		1						1									
7.5GY	Gr				5	2	1							2	4			
10GY	Gr			1	4	4	4						1	6	4	4		
2.5G	Gr														1			
5YR	Br	1ELA10	1	1						3ELA10		1	1					
7.5YR	Br	and	1	4						and		1	3					
7.5Y	PY	2ELA10					1			4ELA10						1		
10Y	OL			1	1							1						
2.5GY	OL		8	9	2						1	12	7	1				
5GY	OL		1									1						
7.5GY	Gr				5	1								3	3	1		
10GY	Gr			1	9	2	2						1	6	5	1		

Appendix Table 3Bc/1v.

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Fifth instar larvae.

Hue Type	Colour Type	Sample	Shade -4	-3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br/YB	1ELA1		2	3	2					3ELA1		2	4	2	1			1
7.5YR	Br	and	1	6	5	3					and		5	4	4				
10YR	Br	2ELA1		3	2	1					4ELA1	1	4	2					
2.5Y	Br		1	1								1							
5Y	Br/PY		1						2									1	
10Y	OL												1						
2.5GY	OL			4	4								4	4					
7.5GY	Gr					2	2	1							1	1	2		
10GY	Gr					1	2	1							1	3	1		
2.5YR	Br	1ELA2			1						3ELA2				1				
5YR	Br	and	1	1		2					and		2	2	1				
7.5YR	Br	2ELA2	1	7	4	1					4ELA2	1	11	4	1				
10YR	Br			2	4	1							1	2					
2.5Y	Br		1	1								1	1						
5Y	Br/PY								1			1							
10Y	OL				2	1							1						
2.5GY	OL			2	4								7	2	1				
7.5GY	Gr					1	3	1							1	2	1		
10GY	Gr					2	4	2						1	1	2	1		

Appendix Table 3Bc/1v. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Fifth instar larvae.

Hue Type	Colour Type	Sample	Shade -4	-3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br	1ELA3		2	2	2					3ELA3		2	1	3	1			
7.5YR	Br	and	1	6	5	3					and	2	6	4	3	1			
10YR	Br	2ELA3	1	3	2						4ELA3	1	2	3					
2.5Y	Br			2	1								1						
5Y	Br		1																
7.5Y	PY								1									1	
10Y	OL			1	1								1	1	1				
2.5GY	OL			4	2	1						1	3	5	1				
7.5GY	Gr					1	2	2							1	2	1		
10GY	Gr					1	2	1						1	1				
5YR	Br	1ELA4	1	4	2	1					3ELA4	1	3	2	2				
7.5YR	Br	and	1	7	2	2					and		5	5	4				
10YR	Br	2ELA4	1	7	1						4ELA4	1	5	2					
2.5Y	Br			1									1	1					
10Y	OL			1	1								1						
2.5GY	OL				6	2							7	3					
7.5GY	Gr					1	4	1							1	1	1		
10GY	Gr					1	3									2	1		

Appendix Table 3Bc/1v. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Fifth instar larvae.

Hue Type	Colour Type	Sample	Shade -4	-3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
2.5YR	Br	1ELA5			1						3ELA5			1					
5YR	Br/YB	and	1	2	2	2					and	1	2		1			1	
7.5YR	Br	2ELA5	2	6	6	2					4ELA5	1	7	5					
10YR	Br			3	2	1						1	4	3					
2.5Y	Br		1									1		1					
5Y	Br/PY		1					1										1	
7.5Y	PY								1										
10Y	OL			2	1														
2.5GY	OL			3	1	1							2	7	1				
7.5GY	Gr					1	1	1							1	4	1		
10GY	Gr					2	2	1							1	2	1		
5YR	Br/YB	1ELA6		2							3ELA6		2	3	2			1	
7.5YR	Br	and	1	5							and	2	6	6	2				
10YR	Br	2ELA6	2	4							4ELA6		3	2					
2.5Y	Br		1	1								1							
5Y	PY							1											
10Y	OL			1										2					
2.5GY	OL/YG		1	2	3	1							4	4					1
7.5GY	Gr					1	1	1							1	1	2		
10GY	Gr					2	1	1							2	3			
25G	Gr						1												

Appendix Table 3Bc/1v. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Fifth instar larvae.

Hue Type	Colour Type	Sample	Shade -4	-3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br	1ELA7		2	4	2	1				3ELA7		3	3	2				
7.5YR	Br	and	1	3	5	6					and	2	6	7	2				
10YR	Br	2ELA7		2	4						4ELA7	1	3	1					
2.5Y	Br			1	2														
5Y	Br/PY		1						1	1							1		
10Y	OL												1	1					
2.5GY	OL			6	1								2	2	1				
7.5GY	Gr					2	1	1							2	2	3		
10GY	Gr					1	1	1							1	2	2		
5YR	Br	1ELA8		1	2	1					3ELA8		1	4	3				
7.5YR	Br	and	1	6	4	3					and	1	3	8	4				
10YR	Br	2ELA8	2	3	2	1					4ELA8	1	4						
2.5Y	Br			2								1	2						
5Y	Br/PY		1					1				1						1	
10Y	OL				2								1	1					
2.5GY	OL/YG			4	4				1				3	4	2				
7.5GY	Gr						2	2							1	1	1		
10GY	Gr					1	2	1							1		1		
2.5G	Gr						1												

Appendix Table 3Bc/1v. (Continued)

Experiments to investigate the effect of background colour on larval colour.

Colour analysis of the ELA samples. Fifth instar larvae.

Hue Type	Colour Type	Sample	Shade -4	-3	-2	-1	0	1	2	3	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br/YB	1ELA9	2	1	2	2	1				3ELA9		1	2	2				1
7.5YR	Br	and		8	5	6					and	2	8	6	1				
10YR	Br	2ELA9	1	3	2	1					4ELA9		3	2					
2.5Y	Br		1		1							1							
10Y	OL			1									1	1					
2.5GY	OL/YG		1	6	1	1				1			5	2	1				
7.5GY	Gr														1	4	1		
10GY	Gr					2	1								1	3			
5YR	Br	1ELA10		1	3	1					3ELA10	1	2	1	3				
7.5YR	Br	and		5	8	4					and	1	7	5	3				
10YR	Br	2ELA10	2	6							4ELA10		2	1	1				
2.5Y	Br		1	1									1						
5Y	Br/PY											1						1	
7.5Y	PY							1											
10Y	OL				1									1					
2.5GY	OL/GY		1	1	6								6	4					
5GY	Gr					1													
7.5GY	Gr					1	1	1								1	2		
10GY	Gr					1	1	1						1	2		2		
2.5G	Gr						1								1				

Appendix Table 3Bc/2i.

Experiments to investigate the effect of duration of light on larval colour. Colour analysis scores for the ELB samples. First and Second instar larvae. (All larvae EG colour type).

Hue type	Sample	Shade -4	-3	-2	-1	0	1	Sample	Shade -4	-3	-2	-1	0	1
5GY	ELB 1 and 2				2	1		ELB 3 and 4				2		
7.5GY			1	5	13	8	1			1	9	10	6	2
10GY			1	2	7	9			1		8	6	5	
5GY	ELB 11 and 12				1	1		ELB 11 and 12			1	1	1	
7.5GY			1	7	10	6	1			1	5	14	10	1
10GY			2	8	5	7	1			1	4	4	7	
5GY	ELB 13 and 14				1	2		ELB 13 and 14				1	1	
7.5GY			2	7	10	10	2			2	7	10	9	2
10GY			1	5	6	4				1	5	8	4	
5GY	ELB 15 and 16			1	1	1		ELB 15 and 16				2	1	
7.5GY			2	11	8	5	2			1	3	14	6	
10GY		1	1	7	4	5			1	1	7	6	6	1
2.5G				1							1			
5GY	ELB 17 and 18			1	3			ELB 17 and 18					1	
7.5GY			1	6	14	6	1			1	10	10	9	
10GY				4	8	6				1	4	3	10	1

Appendix Table 3Bc/2ii.

Experiments to investigate the effect of duration of light on larval colour. Colour analysis scores for the ELB samples. Third instar larvae.

Hue type	Colour type	Sample	Shade -3	-2	-1	0	1	2
7.5YR	Br	ELB 5 and 6		1	1			
7.5Y	PY							1
2.5GY	OL		2	1	1			
7.5GY	3IG			5	6	7		
10GY	3IG		1	5	10	8		
2.5G	3IG			1	1			
10YR	Br	ELB 11 and 12			1			
2.5GY	OL		1	3				
7.5GY	3IG			5	7	8		
10GY	3IG		1	7	10	6	1	
7.5YR	Br	ELB 13 and 14			2			
7.5Y	PY							1
5GY	OL		3	1				
7.5GY	3IG			10	12	6		
10GY	3IG			4	6	4		
2.5G	3IG					1		
7.5YR	Br	ELB15 and 16						
2.5GY	OL		1	4				
7.5GY	3IG			6	8	10		
10GY	3IG			8	4	7		
2.5G	3IG			1	1			
7.5YR	Br	ELB 17 and 18		1	1			
10YR	Br				1			
2.5GY	OL		3	1				
7.5GY	3IG			7	10	5		
10GY	3IG		1	5	7	8		

Appendix Table 3Bc/2iii.

Experiments to investigate the effect of duration of light on larval colour. Colour analysis scores for the ELB samples. Fourth instar larvae.

Hue type	Colour type	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br	ELB 8		1						
7.5YR	Br			1	1					
10Y	OL				1					
2.5GY	OL			3	6					
7.5GY	Gr					2	2			
10GY	Gr				1	3	3	1		
5YR	Br/YB	ELB 11 and 12		1						1
7.5YR	Br			2	3					
7.5Y	PY								1	
10Y	OL			2						
2.5GY	OL			9	9	1				
5GY	OL			1						
7.5GY	Gr					3	3	1		
10GY	Gr					5	5	2		
2.5G	Gr						1			
5YR	Br	ELB 13 and 14			1	1				
7.5YR	Br		1	1	1					
10YR	Br				1					
10Y	OL			2	1					
2.5GY	OL			6	14					
5GY	OL				1					
7.5GY	Gr					2	4	3		
10GY	Gr				1	7	2	1		
5YR	Br	ELB 15 and 16			1					
7.5YR	Br			1	1					
10YR	Br			1						
10Y	OL			1	1					
2.5GY	OL			7	9	3				
5GY	YG								1	
7.5GY	Gr				1	7	5	1		
10G	Gr					4	4	1		
2.5G	Gr						1			
5YR	Br	ELB 17 and 18		2						
7.5YR	Br			1	4					
7.5Y	PY									
10Y	OL			1	1					
2.5GY	OL		1	9	6	2				
5GY	OL			1						
7.5GY	Gr					5	3	2		
10GY	Gr					7	2			
2.5G	Gr				1	1				

Appendix Table 3Bc/2iv.

Experiments to investigate the effect of duration of light on larval colour. Colour analysis scores for the ELB samples. Fifth instar larvae.

Hue type	Colour type	Sample	Shade -4	-3	-2	-1	0	1	2	3
5YR	Br	ELB 9		1	1	1				
7.5YR	Br			2	3	1				
10YR	Br			3	1					
2.5Y	Br		1							
5Y	PY							1		
2.5GY	OL			2	2					
7.5GY	Gr					1				
10GY	Gr					1	4			
5YR	Br/YB	ELB 11 and 12		2	1	1				1
7.5YR	Br		1	6	6	1				
10YR	Br			2	3	1				
2.5Y	Br		1	1						
5Y	PY								1	
10Y	OL			1	1					
2.5GY	OL		1	2	3	1				
7.5GY	Gr					4	1			
10GY	Gr					4	1	3		
5YR	Br	ELB 13 and 14		2	4	2				
7.5YR	Br		1	3	7	2				
10YR	Br		2	3	1					
5Y	Br		1							
10Y	OL			2						
2.5GY	OL			5	3					
7.5GY	Gr					2	3	1		
10GY	Gr				1	1	3	1		
2.5YR	Br	ELB 15 and 16			1					
5YR	Br		1	2		1				
7.5YR	Br		1	8	5					
10YR	Br			3	1	1				
2.5YR	Br		1	1						
2.5GY	OL/YG		1	4	5	1				1
7.5GY	Gr					2	2	1		
10GY	Gr					3	2	2		
5YR	Br	ELB 17 and 18		2	2					
7.5YR	Br		1	7	5	2				
10YR	Br		1	4	1					
5Y	Br/PY		1						1	
10Y	OL				2					
2.5GY	OL		1	1	6	1				
7.5GY	Gr					5	1	1		
10GY	Gr					3	1			
2.5G	Gr					1				

Appendix Table 3Bc/3i.

Experiments to determine the effect of light of different wavelengths on larval colour. Colour analysis scores for the ELC samples. First and Second instars. (All larvae EG colour type).

Hue type	Sample	First instar shade -4	-3	-2	-1	0	1	Second instar shade -4	-3	-2	-1	0	1
5GY	ELC 1 and 2			2	2					1	2	1	
7.5GY			1	6	15	7			1	9	9	7	
10GY				3	8	5	1		1	7	9	3	
5GY	ELC 3 and 4			1	1	1				1	2		
7.5GY				9	8	11			1	12	13	8	1
10GY				9	6	4			1	4	4	3	
5GY	ELC 5 and 6			1	1	2					2	1	
7.5GY		1	1	3	11	10	1		1	5	14	6	2
10GY			1	7	6	5		1	1	7	3	7	
5GY	ELC 7 and 8			1								2	
7.5GY			1	10	9	10			2	6	11	8	2
10GY				5	3	9	1			5	6	7	1
2.5GY				1									
5GY	ELC 9 and 10				1						1	1	
7.5GY			1	5	14	11			1	6	15	5	1
10GY			1	6	6	4			1	3	8	8	
2.5GY				1									
5GY	ELC 11 and 12				3	1					1	1	
7.5GY				5	13	6			1	7	8	5	
10GY			1	9	3	8	1		1	8	8	9	1

Appendix Table 3Bc/3ii.

Experiments to determine the effect of light of different wavelengths on larval colour. Colour analysis scores for the ELC samples. Third instar larvae.

Hue type	Colour type	Sample	shade -4	-3	-2	-1	0	1	2
7.5YR	Br	ELC 1 and 2			1	1			
7.5Y	PY								1
2.5GY	OL			3	1				
7.5GY	3IG			1	5	11	8		
10GY	3IG			1	5	7	5		
7.5YR	Br	ELC 3 and 4			1	2			
10YR	Br				1				
2.5GY	OL			2	2				
7.5GY	3IG				9	8	5		
10GY	3IG				3	10	6		
2.5G	3IG				1				
7.5YR	Br	ELC 5 and 6			1				
2.5GY	OL		1	2	3				
7.5GY	3IG				4	6	9		
10GY	3IG			1	7	9	7		
7.5Y	PY	ELC 7 and 8							1
10Y	OL			1					
2.5GY	OL		1	3					
7.5GY	3IG			8	10	6			
10GY	3IG			5	6	8			
2.5G	3IG			1					
7.5YR	Br	ELC 9 and 10			1	1			
2.5GY	OL				2				
7.5GY	3IG				6	5	6		
10GY	3IG			1	8	9	10		
2.5G	3IG					1			
2.5GY	OL	ELC 11 and 12		1	3	1			
7.5GY	3IG				4	7	11		
10GY	3IG			1	8	5	9		

Appendix Table 3Bc/3iii.

Experiments to determine the effect of light of different wavelengths on larval colour.
Colour analysis scores for the ELC samples. 4th and 5th instar larvae.

Hue type	Colour type	Sample	Fourth instar shade								Fifth instar shade							
			-4	-3	-2	-1	0	1	2	3	-4	-3	-2	-1	0	1	2	3
5YR	Br	ELC1		1								1	1					1
7.5YR	Br	and		2	2							5	5	2				
10YR	Br	ELC2									1	3	2	2				
2.5Y	Br										1							
7.5Y	PY							1	1							1		
10Y	OL			2	1								1					
2.5GY	OL		1	9	6	1						4	5	2				
5GY	OL			1														
7.5GY	Gr					5	8	1						2	4			
10GY	Gr					2	2	3						2	3	2		
2.5G	Gr						1											
5YR	Br	ELC3										1	2	1				
7.5YR	Br	and		2							1	3	5	2				
10YR	Br	ELC4									1	4	1					
2.5Y	Br										1	2						
10Y	OL			2	2	1							1					
2.5GY	OL			7	10	2					1	2	8	2				
5GY	OL			1								1						
7.5GY	Gr					3	6	3						3	1	2		
10GY	Gr				1	7	2	1						3	1	1		

Appendix Table 3Bc/3iii. (Continued)

Experiments to determine the effect of light of different wavelengths on larval colour.
Colour analysis scores for the ELC samples. 4th and 5th instar larvae.

Hue type	Colour type	Sample	Fourth instar shade								Fifth instar shade							
			-4	-3	-2	-1	0	1	2	3	-4	-3	-2	-1	0	1	2	3
5YR	Br	ELC5		2	1						1	2	2	1				
7.5YR	Br	and		3	2						1	7	2	4				
10YR	Br	ELC6		1							1	2	2	1				
2.5Y	Br										1		1					
7.5Y	PY							1	1							1	1	
10Y	OL				2							1						
2.5GY	OL		1	4	14	1						2	4	2				
5GY	OL			1														
7.5GY	Gr					1	4							5	2			
10GY	Gr					6	2	2						2	1	1		
2.5G	Gr					1												
5YR	Br	ELC7		1							1	1	2	1				
7.5YR	Br	and		1	1						2	8	3	4				
10YR	Br	ELC8										4	2	1				
2.5Y	Br										1	1						
10Y	OL			2	2													
2.5GY	OL			6	9	2						1	3	1				
5GY	OL																	
7.5GY	Gr					1	1	2						4	4	1		
10GY	Gr				1	6	3	1						2	2	1		
2.5G	Gr						1											

Appendix Table 3Bc/3iii. (Continued)

Experiments to determine the effect of light of different wavelengths on larval colour.
Colour analysis scores for the ELC samples. 4th and 5th instar larvae.

Hue type	Colour type	Sample	Fourth instar shade								Fifth instar shade							
			-4	-3	-2	-1	0	1	2	3	-4	-3	-2	-1	0	1	2	3
5YR	Br	ELC9			1					1	1		3					1
7.5YR	Br	and		2	3						1	4	8					
10YR	Br	ELC10									2	2	1					
2.5Y	Br										1	1						
7.5Y	PY							1	1							1	1	
10Y	OL			1	1													
2.5GY	OL		1	8	11	2					1	5	4	1				
5GY	OL											1						
7.5GY	Gr						2	1						2	2	2		
10GY	Gr				1	7	4	2						1	1	3		
2.5G	Gr																	
5YR	Br	ELC11		1							1	1	3					
7.5YR	Br	and		3	2							6	8	1				
10YR	Br	ELC12										4	3	1				
2.5Y	Br											1						
10Y	OL				1													
2.5GY	OL		1	7	6							6	4					
5GY	OL				1							1						
7.5GY	Gr					4	3	4						2	3	1		
10GY	Gr				2	9	5							2	1			
2.5G	Gr					1												

Appendix Table 3Bd/1i.

Experiments to investigate the effect of foodplant colour on larval colour.

Series 1. Colour analysis scores of the EFB samples. First instar.

Hue type	Samples	Fed on Sorrel shade -4	-3	-2	-1	0	1	Samples	Fed on Plantain shade -4	-3	-2	-1	0	1
5GY	EFB1si and 1sii				1	1		EFB1siii and 1siv		1	1			
7.5GY			2	6	12	9	1		1	4	13	8	2	
10GY			1	5	6	5			1	3	7	7		
2.5G				1							1	1		
5GY	EFB2si and 2sii					1		EFB2siii and 2siv			2	1		
7.5GY			1	7	11	9	1		1	5	14	8	3	
10GY			1	6	7	6				3	6	6	1	
5GY	EFB3si and 3sii				1	1		EFB3siii and 3siv			1			
7.5GY			1	8	11	9	2		1	5	15	7	2	
10GY			1	6	6	4			1	3	8	7		
5GY	EFB4si and 4sii				1	1		EFB4siii and 4siv	1	1	2			
7.5GY			2	7	10	10	1		1	6	13	9	1	
10GY				6	5	6				3	7	5		
2.5G				1							1			
5GY	EFB5si and 5sii				1	1		EFB5siii and 5siv			1	1		
7.5GY			2	7	11	8	2		2	6	14	6	2	
10GY			1	6	5	5				5	6	6		
2.5G				1							1			
5GY	EFB6si and 6sii					1		EFB6siii and 6siv			1			
7.5GY			1	7	10	11	2		1	5	12	10	1	
10GY			1	6	6	5			1	5	5	8	1	

Appendix Table 3Bd/1ii.

Experiments to investigate the effect of foodplant colour on larval colour.

Series 1. Colour analysis scores of the EFB samples. Second instar.

Hue type	Samples	Fed on Sorrel shade -4	-3	-2	-1	0	1	Samples	Fed on Plantain shade -4	-3	-2	-1	0	1
5GY	EFB1si and 1sii				1	1		EFB1siii and 1siv		1	1			
7.5GY			1	6	12	3	1		1	6	16	7	1	
10GY			1	11	3	5				4	6	6		
2.5G												1		
5GY	EFB2si and 2sii				1			EFB2siii and 2siv		1				
7.5GY			1	7	9	11	1		2	2	17	10	1	
10GY				7	7	6				3	6	7	1	
5GY	EFB3si and 3sii				1	1		EFB3siii and 3siv			1			
7.5GY				8	7	13	2			5	21	7		
10GY			2	7	7	2				5	4	7		
5GY	EFB4si and 4sii				1	2		EFB4siii and 4siv		2		1		
7.5GY			3	7	10	9	1		1	4	15	8	2	
10GY				5	3	8				4	6	6		
2.5G				1								1		
5GY	EFB5si and 5sii				1	1		EFB5siii and 5siv		1	2			
7.5GY			1	8	14	6	1			3	17	11	1	
10GY			1	3	9	5			1	3	4	6	1	
2.5G														
5GY	EFB6si and 6sii				1	1		EFB6siii and 6siv			2			
7.5GY			1	9	11	10	2		2	4	17	7	1	
10GY			1	5	5	4			1	2	6	8		

Appendix Table 3Bd/1iii.

Experiments to investigate the effect of foodplant colour on larval colour.

Series 1. Colour analysis series of the EFB samples. Third instar larvae.

Hue type	Colour type	Fed on Sorrel Sample	shade -3	-2	-1	0	1	Fed on Plantain Sample	shade -4	-3	-2	-1	0
5GY	3IG	EFB 1si and 1sii		1				EFB 1siii and 1siv		3	1		
7.5GY	3IG		1	7	12	7	1		1	6	12	7	2
10GY	3IG		1	5	7	6	1		1	5	8	3	1
2.5GY	3IG			1									
5GY	3IG	EFB 2si and 2sii						EFB 2siii and 2siv		2	2	1	
7.5GY	3IG		1	8	12	9	2		1	7	10	7	3
10GY	3IG		1	4	6	6				4	8	4	1
2.5GY	3IG				1								
5YR	Br	EFB 3si and 3sii		2	1			EFB 3siii and 3siv		1	3		
7.5YR	Br		3	12	3					3	9	6	
10YR	Br			3	1						1	1	
7.5GY	3IG			2	6	3				2	4	4	
10GY	3IG			3	6	5				3	7	6	

Appendix Table 3Bd/1iii. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour.

Series 1. Colour analysis series of the EFB samples. Third instar larvae.

Hue type	Colour type	Fed on Sorrel Sample	shade -3	-2	-1	0	1	Fed on Plantain Sample	shade -4	-3	-2	-1	0
5GY	3IG	EFB 4si and 4sii						EFB 4siii and 4siv		2	1	1	
7.5GY	3IG		1	8	11	11	2		1	6	9	8	2
10GY	3IG			5	6	4				6	8	5	
2.5GY	3IG				1								
5GY	3IG	EFB 5si and 5sii				1		EFB 5siii and 5siv		2	1	1	
7.5GY	3IG		1	8	10	9	1		1	6	13	9	1
10GY	3IG			6	7	6	1			4	7	5	
5GY	3IG	EFB 6si and 6sii				1		EFB 6siii and 6siv		10	7	7	
7.5GY	3IG		1	7	10	8	1		2	5	12	7	
10GY	3IG		1	8	8	5							

Appendix Table 3Bd/1iv.

Experiments to investigate the effect of foodplant colour on larval colour.

Series 1. Colour analysis series of EFB samples. Fourth instar larvae.

Hue type	Colour type	Fed on Sorrel Sample	shade -4	-3	-2	-1	0	1	2	3	Fed on Plantain Sample	shade -4	-3	-2	-1	0	1	2	3
7.5GY	Gr	EFB1si and 1sii				4	14	3			EFB1siii and				2	16	1		
10GY	Gr				1	13	11	3			1siv				18	8	4		
2.5G	Gr					1										1			
5YR	Br	EFB2si and 2sii			1						EFB2siii and			1					
7.5YR	Br		1	3	3						2siv		2	3					
10YR	Br			2									2						
10Y	OL			1	1								2						
2.5GY	OL			11	14	3							8	16	3				
7.5GY	Gr					1	3								2	5			
10GY	Gr					4	2								3	3			
5YR	Br	EFB3si and 3sii			1	1					EFB3siii and		1	2	2				
7.5YR	Br		1	15	26						3siv		11	28					
10YR	Br		1	3	1								2	3					
2.5Y	Br			1								1							
10Y	OL	EFB4si and 4sii			1						EFB4siii and								
2.5GY	OL		1	10	13	1					4siv		8	15	2				
5GY	OL			1										1					
7.5GY	Gr				2	7	1									5	3		
10GY	Gr				4	6	2								8	7	1		
2.5G	Gr				1														

Appendix Table 3Bd/1iv. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour.

Series 1. Colour analysis series of EFB samples. Fourth instar larvae.

Hue type	Colour type	Sample	Fed on Sorrel shade -4	-3	-2	-1	0	1	2	3	Sample	Fed on Plantain shade -4	-3	-2	-1	0	1	2	3
7.5Y	PY	EFB5si and 5sii							1		EFB5siii and							4	
7.5GY	Gr					2	11	5			5siv				5	14	3		
10GY	Gr				1	15	13	2						2	9	9	2		
2.5G	Gr													1	1				
7.5GY	Gr	EFB6si and 6sii				6	17	4			EFB6siii and				2	13	5		
10GY	Gr				1	10	10	1			6siv			2	5	9	3	1	
2.5G	Gr						1												

Appendix Table 3Bd/1v.

Experiments to investigate the effect of foodplant colour on larval colour. Series 1.

Colour analysis series of EFB samples. Fifth instar larvae.

Hue type	Colour type	Sample	Fed on Plantain shade -4	-3	-2	-1	0	1	2	3	Sample	Fed on Plantain shade -4	-3	-2	-1	0	1	2	3
7.5GY	Gr	EFB1si and 1sii				3	12	4			EFB1siii and				4	15	2		
10GY	Gr					16	12	3			1siv			1	17	7	3		
2.5GY	Gr												1						
5YR	Br	EFB2si and 2sii		1	2	2					EFB2siii and		3	1	2				
7.5YR	Br		1	4	10	1					2siv		17	8	2				
10YR	Br		1	6	2							1	5	3					
2.5GY	OL			2	5								2	3					
10GY	Gr					1	1								2	1			
2.5GY	Gr						1												
2.5YR	Br	EFB3si and 3sii		1	1						EFB3siii and				1				
5YR	Br		2	6	3	2					3siv	1	4	4	1				
7.5YR	Br		1	13	10							1	11	14	3	1			
10YR	Br		2	4	3								6	2					
2.5Y	Br			1								1							
5Y	Br		1																
10Y	OL	EFB4si and 4sii		2							EFB4siii and		1	1					
2.5GY	OL		1	9	14	2					4siv	1	8	16	1				
5GY	OL			1															
7.5GY	Gr					2	4	1							3	7	2		
10GY	Gr				1	7	5	1							5	4			
2.5G	Gr														1				

Appendix Table 3Bd/1v. (Continued)

Experiments to investigate the effect of foodplant colour on larval colour. Series 1.
Colour analysis series of EFB samples. Fifth instar larvae.

Hue type	Colour type	Sample	Fed on Plantain shade -4	-3	-2	-1	0	1	2	3	Sample	Fed on Plantain shade -4	-3	-2	-1	0	1	2	3
2.5YR	Br/YB	EFB5si and 5sii		1						1	EFB5siii and			1					1
5YR	Br/YB			4	3	2					5siv		2	1	1	1		1	2
7.5YR	Br		1	12	9	3						1	15	13	2				
10YR	Br		1	6	5	1						1	6	2					
2.5Y	Br		1																
2.5YR	Br	EFB6si and 6sii	2	1		1					EFB6siii and		1		1				
7.5YR	Br			9	5						6siv	1	8	7	1				
10YR	Br		1	3	1								4	2	1				
2.5Y	Br											1							
7.5GY	Gr					3	6	2							2	6	2	1	
10GY	Gr				1	8	4	1							6	5			
2.5G	Gr					1	1								1				

Appendix Table 3Bd/2i.

Experiments to investigate the effect of foodplant colour colour analysis scores for the SEF samples. All instars.

Instar	Hue type	Colour type	Samples SEF 1 - 3 inclusive Fed on Sorrel leaves picked for uniformity of colour								Samples SEF 4 - 6 inclusive Fed on Sorrel leaves picked randomly								Samples SEF 7 - 9 inclusive Fed on Plantain leaves picked for uniformity of colour								Samples SEF 10 - 12 inclusive Fed on Plantain leaves picked randomly							
			shade	-4	-3	-2	-1	0	1	2	3	shade	-4	-3	-2	-1	0	1	2	3	shade	-4	-3	-2	-1	0	1	2	3	shade	-4	-3	-2	-1	0	1	2	3
First	5GY	EG														1	1																					
	7.5GY	EG					6	26	23	1				2	10	17	15	2														1	2					
	10GY	EG					3	13	3					1	9	8	8	1					6	35	6	1					2	9	21	11	3			
	2.5G	EG															1						3	18	6						1	6	10	9				
Second	5GY	EG						1								1	2																					
	7.5GY	EG					4	28	23					2	11	18	13	2						2								1	1					
	10GY	EG					4	10	5					1	9	8	7						5	36	6						2	8	25	12	1			
	2.5G	EG														1							3	16	7							6	8	10	1			
Third	7.5YB	Br/YB					1	1							1	1			1				1	1									2					
	5Y	PY								1																												
	7.5Y	PY																	1																	1		
	10Y	OL													1																							
	2.5GY	OL				3	5	1						3	3	1							2	5								2	3	1				
	5GY	OL																														1						
	7.5GY	Gr						7	26	4					10	12	6					1	6	28	7	2				1	9	15	12	4	1		
	10GY	Gr					1	6	13	6				2	9	10	12						3	14	4	1						6	9	6	1			
	2.5G	Gr														1	1																1					
Fourth	5YR	Br/YB				1	2								1	1				1			1	1								1	1					
	7.5YR	Br				2	3								3	4							3	3								2	5					
	10YR	Br				1																		1														
	7.5Y	PY								2									1																	1	1	
	10Y	OL				1								1	1									2	1								1	2				
	2.5GY	OL		1	13	15	1						1	12	13	2						1	10	14	3						14	12	2				
	5GY	OL/YG			1									2									1				1				1						
	7.5GY	Gr						5	6	1						6	5	2							4	7	2						3	8	2		
	10GY	Gr				2	9	6	1						2	8	7	1						2	8	6	1						2	7	6	2		
	2.5G	Gr					2									1								1	1									2				
Fifth	5YR	Br/YB	1	3	4	2						2	4	3	1				1				2	2	2							3	3	1				1
	7.5YR	Br	3	10	9	2						1	9	11	3							1	10	9	2					1	9	11	2				
	10YR	Br	2	5	2	1						2	3	4	1							1	5	3	1					2	6	2	1				
	2.5Y	Br	1	1									2									1	1								1							
	5Y	Br/PY	1						1			1						1				1									1							
	7.5Y	PY								1																		1										
	10Y	OL			2	1								1									2	1								1	1				
	2.5GY	OL/YG			4	4	1					1	3	6	1								5	6	2			1		1	4	5	2				
	7.5GY	Gr					3	2	1						2	3	2							1	2	3	2						3	2	2		
	10GY	Gr					4	2	2						4	2	1								4	2	2						1	3	2	2		
	2.5G	Gr																																	1			

Appendix Table 3Bd/3i.

Results of chi-squared tests on colour scores from the third environment foodplant samples. First instar.

Samples compared	Foodplant and foodplant colour (flower petals unless stated)	Value of Chi-squared	Degrees of freedom	Probability value
TEFA1 and TEFC1	Broom/Yellow	0.809	3	0.9 - 0.8
TEFA2 and TEFC2	Broom/Yellow and red	3.619	7	0.9 - 0.8
TEFA3 and TEFC3	Rose/Dark Crimson	2.901	3	0.5 - 0.3
TEFA4 and TEFC4	Rose/Flame red	5.633	6	0.5 - 0.3
TEFA5 and TEFC5	Rose/Pink	3.310	6	0.8 - 0.7
TEFA6 and TEFC6	Rose/Pale pink	3.523	4	0.5 - 0.3
TEFA7 and TEFC7	Rose/Yellowish-pink	4.152	5	0.7 - 0.5
TEFA8 and TEFC8	Rose/Very pale pink-white	2.559	3	0.5 - 0.3
TEFA9 and TEFC9	Pansy/Navy blue	5.866	7	0.7 - 0.5
TEFA10 and TEFC10	Pansy/Pale blue and purple	3.807	6	0.8 - 0.7
TEFA11 and TEFC11	Rhodedendron/Mauve	5.808	5	0.5 - 0.3
TEFA12 and TEFC12	Dock seeds/Red and green	4.013	5	0.7 - 0.5
TEFA13 and TEFC13	Geranium leaves/White (lacking chlorophyll)	2.730	3	0.5 - 0.3
TEFA14 and TEFC14	Synthetic diet (lacking pigment)	0.965	3	0.9 - 0.8

Appendix Table 3Bd/3i. (Continued)

Results of chi-squared tests on colour scores from the third environment foodplant samples. Second instar.

Samples compared	Foodplant and foodplant colour (flower petals unless stated)	Value of Chi-squared	Degrees of freedom	Probability value
TEFA1 and TEFD1	Broom/Yellow	3.718	4	0.5 - 0.3
TEFA2 and TEFD2	Broom/Yellow and red	1.653	5	0.9 - 0.8
TEFA3 and TEFD3	Rose/Dark Crimson	3.139	4	0.7 - 0.5
TEFA4 and TEFD4	Rose/Flame red	4.058	6	0.7 - 0.5
TEFA5 and TEFD5	Rose/Pink	4.738	5	0.5 - 0.3
TEFA6 and TEFD6	Rose/Pale pink	2.434	4	0.7 - 0.5
TEFA7 and TEFD7	Rose/Yellowish-pink	8.090	5	0.2 - 0.1
TEFA8 and TEFD8	Rose/Very pale pink-white	3.486	3	0.5 - 0.3
TEFA9 and TEFD9	Pansy/Navy blue	4.88	6	0.7 - 0.5
TEFA10 and TEFD10	Pansy/Pale blue and purple	4.085	5	0.7 - 0.5
TEFA11 and TEFD11	Rhodedendron/Mauve	4.582	5	0.5 - 0.3
TEFA12 and TEFD12	Dock seeds/Red and green	6.523	5	0.3 - 0.2
TEFA13 and TEFD13	Geranium leaves/White (Lacking chlorophyll)	8.854	3	0.05 - 0.02
TEFA14 and TEFD14	Synthetic diet (lacking pigment)	2.801	3	0.5 - 0.3

Appendix Table 30i.

Colour type analysis of a number of broods bred to determine the mechanism controlling the larval colour variation in the 3rd, 4th and 5th instars, or bred for predation experiment stocks.

Brood number	Genotype of male. Alleles of genes. C	B	A	D	E	Phenotype of male	Genotype of male. Alleles of genes. C	B	A	D	E	Phenotype of female	number of ova	1st instar larvae Bl	Others	2nd instar larvae EG	Others	3rd instar larvae Gr	OL	Br	PY	YG	YB	Others	4th instar larvae Gr	OL	Br	PY	YG	YB	Others	5th instar larvae Gr	OL	Br	PY	YG	YB	Others
IB61	xx	('y)	(++)	(++ or ++)		Unknown	xx	('')	(xx)	(xx | xx)	Unknown	51	46	-	41	-	36	-	-	-	-	-	-	17	-	-	-	-	-	14	-	-	30	-	-	-	-
IB72	+-	('')	(++)	(+-)[1]	(+o)[1]	Unknown	+-	('o)	(xx)	(--)[1]	(oo)[1]	Unknown	168	155	-	150	-	128	15	-	6	-	2	-	49	55	-	24	-	10	1	-	-	101	17	-	18	-
CC44	+x	''	(++)	+-[2]	('')[2]	3Gr 4Gr 5Br	+x	''	+-	+-[2]	(oo)[2]	3Gr 4Gr 5Br	307	288	-	284	-	277	-	-	-	-	-	-	204	-	-	65	-	-	2	-	-	197	64	-	-	2
CC63	--	'o	--	(++ or ++)		3OL 4OL 5OL	--	'o	--	(xx | xx)	3OL 4OL 5OL	304	299	-	287	-	68	144	66	-	-	-	2	67	142	66	-	-	-	2	64	131	63	-	-	-	-
CC74	++	'o	'o	(++ or ++)		3Gr 4OL 5OL	xx	''	--	(xx | xx)	3Gr 4Gr 5Gr	109	104	-	81	-	68	-	-	-	-	-	-	20	36	-	-	-	-	-	17	30	-	-	-	-	-
CC91	(++)	oo	--	(++ or ++)		3Gr 4Br 5Br	+x	'o	--	(xx | xx)	3Gr 4OL 5OL	136	125	-	101	-	94	-	-	-	-	-	-	-	51	33	-	-	-	-	-	48	30	-	-	-	-	-
CC97	xx	''	++	+-	+o	3Gr 4Gr 5Br	++	oo	xx	+-	+o	3Gr 4Br 5Br	231	220	-	214	-	206	-	-	-	-	-	-	-	188	-	-	13	-	-	-	184	-	12	-	-	-
CC98	++	'o	--	(++ or ++)		3Gr 4OL 5OL	xx	''	--	(xx | xx)	3Gr 4Gr 5Gr	81	80	-	74	3	72	-	-	-	-	-	-	33	38	-	-	-	-	-	30	37	-	-	-	-	-
CC109	++	''	(++)	(+-)[2]	('')[2]	3Gr 4Gr 5Br	xx	''	(++)	+-[2]	(oo)[2]	3Gr 4Gr 5Br	111	105	2	104	-	90	-	-	-	-	-	3	69	-	-	23	-	-	-	-	-	67	23	-	-	-
CC113	++	''	--	(++ or ++)		3Gr 4Gr 5Gr	--	oo	++	(xx | xx)	3Br 4Br 5Br	214	207	-	206	-	202	-	-	-	-	-	-	-	194	-	-	-	-	-	-	-	-	186	-	-	-	3
NB349	xx	''	--	(++ or ++)		3Gr 4Gr 5Gr	xx	''	--	(xx | xx)	3Gr 4Gr 5Gr	439	427	-	421	-	419	-	-	-	-	-	-	408	-	-	-	-	-	2	404	-	-	-	-	-	-
NB321	xx	''	--	(++ or ++)		3Gr 4Gr 5Gr	xx	''	--	(xx | xx)	3Gr 4Gr 5Gr	64	53	-	49	-	48	-	-	-	-	-	-	46	-	-	-	-	-	-	42	-	-	-	-	-	3
NB329	xx	''	--	(++ or ++)		3Gr 4Gr 5Gr	xx	''	--	(xx | xx)	3Gr 4Gr 5Gr	167	149	-	146	-	138	-	-	-	-	-	1	131	-	-	-	-	-	1	127	-	-	-	-	-	-
NB037	--	oo	--	(++ or ++)		3Br 4Br 5Br	--	''	--	(xx | xx)	3Gr 4Gr 5Gr	348	329	-	324	-	-	317	-	-	-	-	-	-	313	-	-	-	-	-	-	304	-	-	-	-	-	4
NB031	--	oo	--	(++ or ++)		3Br 4Br 5Br	--	''	--	(xx | xx)	3Gr 4Gr 5Gr	169	160	-	154	-	-	153	-	-	-	-	-	-	148	-	-	-	-	-	-	145	-	-	-	-	-	1
NB33	--	oo	--	(++ or ++)		3Br 4Br 5Br	--	oo	--	(xx | xx)	3Br 4Br 5Br	236	221	2	213	-	-	-	208	-	-	-	-	-	-	-	199	-	-	-	-	-	-	189	-	-	-	-
NB334	--	oo	--	(++ or ++)		3Br 4Br 5Br	--	oo	--	(xx | xx)	3Br 4Br 5Br	172	170	-	170	-	-	-	163	-	-	-	-	-	-	-	154	-	-	-	3	-	-	150	-	-	-	2
NB341	--	oo	--	(++ or ++)		3Br 4Br 5Br	--	oo	--	(xx | xx)	3Br 4Br 5Br	359	348	-	342	-	-	-	339	-	-	-	-	-	-	-	328	-	-	-	-	-	-	326	-	-	-	1

[1] could also be $d^{+}de^{+}e^{o} \times d^{+}de^{o}e^{o}$

[2] could also be $dde^{+}e^{+} \times dde^{+}e^{o}$

Appendix Table 4Bi.

First behaviour experiment. Chi-squared and probability values resulting from the analysis of the first behaviour experiment data.

Comparison of the number of larvae of each genotype on each foodplant, using a 2 x 2 contingency test. (Day-time scores)

Sample	Chi-squared value	Probability values (D.F. = 1)
FBE1	0.167	0.7 - 0.5
FBE2	0.167	0.7 - 0.5
FBE3	0	1
FBE4	0.040	0.9 - 0.8
FBE5	0.362	0.7 - 0.5
FBE6	0.040	0.9 - 0.8
FBE7	0.162	0.7 - 0.5
FBE8	1.461	0.3 - 0.2
FBE9	0.040	0.9 - 0.8
FBE10	0.042	0.9 - 0.8
FBE11	2.080	0.2 - 0.1
FBE12	0.369	0.7 - 0.5
FBE13	0.360	0.7 - 0.5
FBE14	0.382	0.7 - 0.5
FBE15	0.040	0.9 - 0.8
FBE16	1.973	0.2 - 0.1
FBE17	0.040	0.9 - 0.8
FBE18	0.045	0.9 - 0.8
FBE19	1.026	0.5 - 0.3
FBE20	1.006	0.5 - 0.3
FBE21	0.040	0.9 - 0.8
FBE22	1.563	0.3 - 0.2

Comparison of the number of larvae of each genotype on each foodplant, using a 2 x 2 contingency test. (Night-time scores)

Sample	Chi-squared value	Probability values (D.F. = 1)
FBE1	1.461	0.3 - 0.2
FBE2	0	1
FBE3	0.041	0.9 - 0.8
FBE4	0.667	0.5 - 0.3
FBE5	0.369	0.7 - 0.5
FBE6	1.026	0.3 - 0.2
FBE7	0	1
FBE8	0.649	0.7 - 0.5
FBE9	0.362	0.7 - 0.5
FBE10	0.174	0.7 - 0.5
FBE11	0.401	0.7 - 0.5
FBE12	1.061	0.5 - 0.3
FBE13	2.667	0.2 - 0.1
FBE14	0.694	0.5 - 0.3
FBE15	0	1
FBE16	0.369	0.7 - 0.5
FBE17	1.061	0.5 - 0.3
FBE18	0	1
FBE19	0.362	0.7 - 0.5
FBE20	0.040	0.9 - 0.8
FBE21	0.162	0.7 - 0.5
FBE22	0	1

Appendix Table 4Bii.

Second behaviour experiment. Chi-squared and probability values resulting from the analysis of the second behaviour experiment data.

Comparison of the number of larvae of each genotype in each half of each box, using a 2 x 2 contingency test. (Day-time scores)			Comparison of the number of larvae of each genotype in each half of each box, using a 2 x 2 contingency test. (Night-time scores)		
Sample	Chi-squared value	Probability values (D.F. = 1)	Sample	Chi-squared value	Probability values (D.F. = 1)
SBE1	0.045	0.9 - 0.8	SBE1	0.160	0.7 - 0.5
SBE2	0.042	0.9 - 0.8	SBE2	0.040	0.9 - 0.8
SBE3	0.042	0.9 - 0.8	SBE3	1.461	0.3 - 0.2
SBE4	0.198	0.7 - 0.5	SBE4	0.360	0.7 - 0.5
SBE5	0.382	0.7 - 0.5	SBE5	0.162	0.7 - 0.5
SBE6	0.667	0.5 - 0.3	SBE6	0.045	0.9 - 0.8
SBE7	0.401	0.7 - 0.5	SBE7	0.160	0.7 - 0.5
SBE8	0.184	0.7 - 0.5	SBE8	0.040	0.9 - 0.8
SBE9	0.045	0.9 - 0.8	SBE9	1.006	0.5 - 0.3
SBE10	0.735	0.5 - 0.3	SBE10	1.960	0.2 - 0.1
SBE11	0.667	0.5 - 0.3	SBE11	0.160	0.7 - 0.5
SBE12	0.360	0.7 - 0.5	SBE12	1.000	0.5 - 0.3
SBE13	0.042	0.9 - 0.8	SBE13	0.160	0.7 - 0.5
SBE14	0.052	0.9 - 0.8	SBE14	0.174	0.7 - 0.5
SBE15	0.174	0.7 - 0.5	SBE15	0.360	0.7 - 0.5
SBE16	0.369	0.7 - 0.5	SBE16	0.649	0.5 - 0.3
SBE17	0.042	0.9 - 0.8	SBE17	0.167	0.7 - 0.5
SBE18	0.174	0.7 - 0.5	SBE18	0.040	0.9 - 0.8
SBE19	0.250	0.7 - 0.5	SBE19	0.040	0.9 - 0.8
SBE20	0.184	0.7 - 0.5	SBE20	0.362	0.7 - 0.5
SBE21	0.401	0.7 - 0.5	SBE21	0.041	0.9 - 0.8
SBE22	0.382	0.7 - 0.5	SBE22	0.040	0.9 - 0.8

Appendix Table 4Biii.

Third behaviour experiment. Chi-squared and probability values resulting from the analysis of the third behaviour experiment data.

Comparison of total numbers of larvae taken by day and night for fourth instar in each strip (i.e. TBE1 compared with TBE2).

Strip	Chi-squared value	Degrees of freedom	Probability values
A	1.860	1	0.2 - 0.1
B	15.804	1	<0.001
C	9.0	1	0.01 - 0.001
D	0.005	1	0.95 - 0.9
E	1.966	1	0.2 - 0.1

Comparison of total numbers of larvae taken by day and night for fifth instar in each strip (i.e. TBE3 compared with TBE4).

Strip	Chi-squared value	Degrees of freedom	Probability values
A	1.374	1	0.3 - 0.2
B	20.775	1	< 0.001
C	14.878	1	< 0.001
D	0.005	1	0.95 - 0.9
E	1.415	1	0.3 - 0.2

Comparison of numbers of larvae of each genotype taken in each strip of Plot 1 (i.e. TBE1 by day in the fourth instar).

Strip	Chi-squared value	Degrees of freedom	Probability values
A	2.774	3	0.5 - 0.3
B	2.217	3	0.7 - 0.5
C	1.429	3	0.7 - 0.5
D	0	3	1
E	0.829	3	0.9 - 0.8

Comparison of numbers of larvae of each genotype taken in each strip of Plot 2 (i.e. TBE2 by night in the fourth instar).

Strip	Chi-squared value	Degrees of freedom	Probability values
A	2.0	3	0.7 - 0.5
B	1.0	3	0.9 - 0.8
C	1.457	3	0.7 - 0.5
D	0.030	3	>0.99
E	1.154	3	0.8 - 0.7

Appendix Table 4Biii. (Continued)

Third behaviour experiment. Chi-squared and probability values resulting from the analysis of the third behaviour experiment data.

Comparison of numbers of larvae of each genotype taken in each strip of Plot 3 (i.e. T3E3 by day in the fifth instar).			
Strip	Chi-squared value	Degrees of freedom	Probability values
A	1.364	3	0.8 - 0.7
B	3.846	3	0.3 - 0.2
C	1.0	3	0.9 - 0.8
D	0	3	1
E	0.678	3	0.9 - 0.8

Comparison of numbers of larvae of each genotype taken in each strip of Plot 4 (i.e. TBE4 by night in the fifth instar).			
Strip	Chi-squared value	Degrees of freedom	Probability values
A	0.823	3	0.9 - 0.8
B	0.810	3	0.9 - 0.8
C	1.789	3	0.7 - 0.5
D	0.030	3	>0.99
E	0.778	3	0.9 - 0.8

Appendix Table 6Bi.

Tests with one female and three or four males

Set number	Test number	Values from comparing the number of females which mated with each male genotype present, taking a 1:1:1(:1) expected ratio			Values from comparing the number of females which mated with males of the same or different genotype, taking a 1:2 (1:3) expected ratio		
		Chi-squared	DF	Probability	Chi-squared	DF	Probability
1	1	0.737	2	0.7 - 0.5	0.419	1	0.7 - 0.5
	2	0.111	2	0.95 - 0.9	0.250	1	0.7 - 0.5
	3	0.737	2	0.7 - 0.5	0.661	1	0.5 - 0.3
2	1	0.111	2	0.95 - 0.9	0	1	1
	2	0.444	2	0.9 - 0.8	1.0	1	0.5 - 0.3
	3	1.300	2	0.7 - 0.5	0.101	1	0.8 - 0.7
3	1	0.118	2	0.95 - 0.9	0.119	1	0.8 - 0.7
	2	0.105	2	0.95 - 0.9	0.106	1	0.8 - 0.7
	3	0.333	2	0.9 - 0.8	0.250	1	0.7 - 0.5
4	1	1.222	3	0.8 - 0.7	0.333	1	0.7 - 0.5
	2	1.0	3	0.9 - 0.8	0	1	1
	3	1.2	3	0.8 - 0.7	0.133	1	0.8 - 0.7
	4	0.333	3	0.98 - 0.95	0.333	1	0.7 - 0.5

Tests with one male and three or four females

Set number	Test number	Values from comparing the number of males which mated with each female genotype present, taking a 1:1:1(:1) expected ratio			Values from comparing the number of males which mated with females of the same or different genotypes, taking a 1:2 (1:3) expected ratio		
		Chi-squared	DF	Probability	Chi-squared	DF	Probability
1	1	0.111	2	0.95 - 0.9	0.250	1	0.7 - 0.5
	2	0.700	2	0.8 - 0.7	0.024	1	0.9 - 0.8
	3	0.333	2	0.9 - 0.8	0.250	1	0.7 - 0.5
2	1	0.471	2	0.8 - 0.7	0.468	1	0.5 - 0.3
	2	0.400	2	0.9 - 0.8	0.398	1	0.7 - 0.5
	3	0.737	2	0.7 - 0.5	0.419	1	0.7 - 0.5
3	1	0.105	2	0.95 - 0.9	0.258	1	0.7 - 0.5
	2	0.823	2	0.7 - 0.5	0.738	1	0.5 - 0.3
	3	0	2	1	0	1	1
4	1	0.333	3	0.98 - 0.95	0.037	1	0.9 - 0.8
	2	1.571	3	0.7 - 0.5	0.429	1	0.7 - 0.5
	3	0.4	3	0.95 - 0.9	0.133	1	0.8 - 0.7
	4	1.222	3	0.8 - 0.7	0.333	1	0.7 - 0.5