The trade-off between egg size and fecundity in the bruchid beetle Callosobruchus maculatus: a quantitative genetic approach

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"In order to spend on one side, nature is forced to economise on the other side"

The law of compensation of growth. Geoffroy & Goethe. quoted by Darwin (1859)

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Summary

Title: The *trade-off between egg size and fecundity in the bruchid beetle Callosobruchus maculatus*: a quantitative genetic approach.

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Female *Callosobruchus maculatus* have fixed reproductive resources to allocate to offspring. It is predicted that the option of investing in a few large eggs or many small eggs will result in a trade-off between egg size and fecundity in this species. The aim of this study was to examine this proposed trade-off and obtain empirical evidence for its significance in the life history of *C. maculatus*. Breeding and selection experiments were used to measure the genetic correlation between egg size and fecundity as evidence for the existence of the trade-off. Selection experiments also allowed egg size to be manipulated to investigate its intrinsic effects on offspring fitness.

Chapter 1 introduces life history theory and trade-off from the distinct but complementary perspectives of optimality theory and evolutionary genetics. Empirical methods that can be used to measure trade-offs are evaluated with respect to the egg size versus fecundity trade-off and the biology of *C. maculatus*. In Chapter 2 the general experimental methods are described.

Phenotypic correlations involving egg size, fecundity and other life history traits are measured in Chapter 3. Egg size is not phenotypically correlated with fecundity but varies with maternal emergence weight and age. Correlative

effects of egg size on offspring fitness appear to be due to effects on offspring body weight and development rate.

The presence of genetic variation in egg size and fecundity are demonstrated in Chapter 4. In Chapter 5 the genetic correlation between egg size and fecundity is measured by means of a half sib breeding experiment. A negative genetic correlation of low precision was obtained. Chapter 6 provides a second estimate of the genetic correlation from an experiment in which egg size was increased and decreased by artificial selection. The correlated response in fecundity was downwards in both directions of selection which was not consistent with the proposed trade-off. The genetic correlation estimated was positive. Reasons for the unexpected response are discussed.

The effects of selection for egg size on other life history traits is examined in Chapter 7. Only development rate, fecundity and emergence weight in females had changed after selection.

Chapter 8 discusses what the quantitative experiments have revealed about the trade-off between egg size and fecundity and assesses the merits of the methodologies.

Contents

CHAPTER 1

INTRODUCTION

1. Introduction

The central tenet of life history theory is that allocation constraints, manifested as trade-offs, restrict the simultaneous optimisation of all traits. The great diversity and variation in the life histories of organisms observed today reflects the apparently endless solutions to the restrictions that are imposed by trade-offs on the evolution of all traits. A problem exists in that despite the widely accepted importance of trade-offs, empirical studies are far from unanimous in revealing their existence (Bell & Koufopanou, 1986). Without the support of experimental evidence, continuing criticism of the trade-off concept cannot be entirely ignored (Gould & Lewontin, 1979; Pease & Bull, 1988). The specific focus of this study is the proposed trade-off between egg size and fecundity in the bruchid beetle *Callosobruchus maculatus*. Quantitative genetic methods employed in this study are used to asses both the role of this trade-off in the life history of this species, and the general utility of the methods in revealing trade-offs.

In an attempt to clarify the biological concept of what trade-offs represent the following sections introduce life history theory from optimisation and genetic perspectives before addressing the specific trade-off between egg size and offspring number in the bruchid beetle *Callosobruchus maculatus*. In the latter sections of this chapter the general biology of this beetle is described.

1.1 Life-history theory and trade-offs

Life history theory is the region of evolutionary biology that is concerned with the explanation of organism lifestyles in terms of age specific values of traits

associated with reproduction and survival. Models of life history evolution have been explored since the middle of the century and were initiated by attempts to furnish adaptive explanations for observed patterns of reproductive investment (Lack, 1947; Cole, 1954; Williams, 1966). In the 1970's and 1980's a wide range of traits have been studied in relation to their contribution to life history strategies but all of these essentially play a role through their impact on either reproduction or survival. In addition to providing an understanding of the diversity of lifestyles found in nature, which range from semelparity to extreme iteroparity and from brief to greatly extended lifespans; the life history framework can be used to predict the responses of populations to environmental change and to gain some insight into past and present mechanisms of adaptive evolution.

1.1.1 The central concept of fitness

Fitness is a description of the evolutionary potential of an allele to spread through a population subject to natural selection. Survival and reproduction are the key life history variables because they are directly related to fitness in that an increase in the value of either of these traits, the other being held constant, leads to a corresponding increase in fitness. Other life history traits such as body weight at maturity and growth rate are indirectly related to fitness because their value that maximises fitness may be intermediate (Lessells, 1991). The primary influence of survival and reproduction on fitness is expressed in the Euler-Lotka equation (Lotka, 1913), which defines fitness as the Malthusian parameter (r) which is the intrinsic rate of increase of a genotype (Charlesworth, 1980; Caswell, 1989)

$$1 = \sum_{x=1}^{\infty} l_x m_x e^{-rx} \qquad [1.1]$$

This equation includes a term for survival to age x (l_x) and a term for the number of female offspring produced at that age (m_x). Sibly and Calow (1986) defined fitness more specifically as the rate of increase of a dominant gene (F) which can be substituted for r in equation [1.1] (Sibly, 1989). A more tractable definition of fitness for empirical studies is to equate it with reproductive value (RV; Fisher, 1930; Williams, 1966; Charlesworth, 1980; Goodman, 1982) which represents the sum of reproductive effort at each age x plus the chance of surviving from age x to age y (l_y/l_x) multiplied by the value of future reproduction $e^{-r(y-x+1)}$ (Lessells, 1991)

$$RV = \sum_{y=x}^{\infty} (l_y/l_x) m_y e^{-r(y-x+1)} \qquad [1.2]$$

1.1.2 The central concept of trade-offs

All the age-specific components of RV would be expected to be maximised by natural selection if an organism was to be ideal in evolutionary terms (Partridge & Harvey, 1988). However, the reason that life history theory is an issue at all is because constraints exist which prevent all traits associated with fitness from being simultaneously maximised by natural selection. The observed diversity in life histories is a direct illustration and consequence of this fact. Constraints between life history traits occur because resources such as energy or time are limited and can be utilised only once (Levin, 1968). If the value of one trait is increased then the value of another trait must decrease as a result, and therefore fitness itself may not necessarily be increased. This form of relationship arising from the existence of constraints between traits is known as a trade-off (Rose, 1983; Sibly & Calow, 1983; Bell & Koufopanou, 1986). Consequently, the trade-off concept is central to the study of life history evolution.

1.2 Perspectives on life histories

The evolution of life histories has been studied using two quite different approaches. In one, studies at a phenotypic level investigate the adaptive value of traits in terms of the selection pressures currently acting upon them. This is commonly explored using optimality modelling which can be used to predict and test the principle traits and constraints determining the observed life history patterns. The alternative approach is the study of life history evolution at the genetic level which is concerned with gene frequencies and the maintenance of genetic variation necessary for future evolutionary response to selection to be possible. Genetic models are used to make predictions about the speed and direction of gene frequency change when selection pressures alter. Both perspectives emphasise the importance of trade-offs but interpret them for different purposes which are described in the following two sections.

1.2.1 Optimality theory and trade-offs

Beyond the fundamental constraints of physical and chemical laws, constraints may also occur because of the finite contents of the gene pool, environmental factors and interactions between the two. Optimality models applicable to life history theory analyse costs and benefits of varying resource allocation between traits to predict the best outcome in terms of fitness in a given context of natural selection. Optimality models have three main components (Krebs & Kacelnik, 1991). One of these is the decision variable which is the actual life history trait under study such as longevity, age at first reproduction or offspring size. Life history traits can also represent constraints in optimisation models and which they represent depends on the question being examined. The second component is the currency which may be of two kinds. One currency type is some measure of fitness that can be used for comparing the relative merits of different

strategies, for example, energy gained per unit time in foraging models. The other form of currency is some measure of energetic value that has to be allocated between traits (Cheverton *et al*, 1985). The final component is the constraint function which determines the relationship between the decision variable and the currency. Two types of constraint may be present in optimality models (Lessells, 1991). The first is the relationship between the decision variable and fitness. An example of this is the relationship between brood size and juvenile survival in birds where survival of young birds over their first winter is the measured component of fitness. This form of constraint function is known as a fitness curve. The second kind of constraint occurs between two life history traits in the same individual, for example, brood size and egg size. This relationship arises from the problem faced by a female of allocating resources between the size and number of eggs in a clutch and as such it is also an example of a trade-off. Such a constraint function is therefore known as a trade-off curve and it is this optimisation concept of trade-offs which has been central to life history theory for many years (Gadgil & Bossert, 1970; Calow & Sibly, 1983; Rose, 1983; Bell & Koufopanou, 1986; Sibly & Calow, 1986). If a trade-off has been present in a population for many generations it is expected to result in a negative genetic correlation between the traits concerned. This is because selection will fix or eliminate plieotropic genes that affect total resource acquisition which increases or decreases the value of both traits involved in a trade-off. Conversely, genes that affect allocation between the traits (increasing one whilst decreasing the other) will be relatively neutral with respect to selection as their net effect on fitness is small. Thus over many generations allocation genes will remain at intermediate frequencies and will constitute the bulk of genetic covariation between the trade-off traits.

1.2.2 Quantitative genetics and trade-offs

Quantitative genetics is concerned with the inheritance of continuously varying polygenic characters. This is in contrast to Mendelian genetics which addresses the large effects of alleles at a small number of loci. During a century of development which started with the work of Galton (1889), quantitative genetics has shifted only recently from a close alliance with statistics (Fisher, 1918), animal and plant breeding (Lush, 1945; Falconer, 1989) and population genetics (Wright, 1952) to become properly integrated with evolutionary biology (Lande, 1976). The following description of genetic theory is given here to outline why this approach also expects to discover trade-offs between life history traits.

The basis of quantitative genetics is that phenotypic variation (V_P) in traits can be partitioned into genetic (V_G) and environmental (V_E) components.

$$V_P = V_G + V_E \qquad [1.3]$$

If interactions occur between V_G and V_E these are by convention included as part of the latter. Genetic interactions due to dominance (V_D) and epistasis (V_I) are, on the other hand, separated from additive genetic effects (V_A) as only the latter is able to respond to natural selection (Falconer, 1989, p.339) and is thus the focal component in quantitative genetic analysis.

$$V_G = V_A + V_D + V_I \qquad [1.4]$$

When a number (n) of traits are considered the evolutionary change per generation is expressed as a column vector z of polygenic traits z_1-z_n (Lande, 1979; 1988)

$$\Delta\bar{z} = \mathbf{G}\nabla \ln\overline{W} \qquad (1.5)$$

G is the additive genetic variance-covariance matrix and $\nabla \ln W$ is the selection trajectory in which the rate of increase in mean fitness is maximised. If **G** contains a negative covariance between traits z_{ia} and z_{ib} then there is a negative genetic correlation between them. This represents a constraint function

analogous to that of optimisation theory which acts to restrict the possible values that can be taken by z_{ia} and z_{ib} and any other traits in the matrix that covary with these. The effect of **G** on $\nabla \ln W$ gives rise to the phenotypically observed trade-offs between at least some of the traits z_1-z_n.

The evolution of a negative genetic correlation forming part of **G** is easy to imagine intuitively. If natural selection attempts to maximise two life history traits simultaneously in a stable environment, then the genes that favour both traits will be subjected to strong selection pressure and will rapidly become fixed in the population. Similarly, genes that have deleterious effects on both traits will be rapidly eliminated. The remaining genetic variation will consequently consist of genes that favour one trait but not the other and the net selection on these will be weak allowing them to persist indefinitely (Falconer, 1989).

1.2.3 The value of phenotypic and genetic approaches

The differing approaches of pheneticists (sensu Lessells, 1991) and geneticists do not conflict but represent complementary analytical approaches to understanding life history evolution. Which is appropriate is depends on the objectives of a particular study, and it is important to recognise that these two levels are suitable for posing different questions about life histories (Lessells, 1991). In order to understand the diversity of life histories and model the roles of individual traits, pheneticists need to measure potential trade-offs to see which are important. They can measure the trade-off either by manipulation experiments or by measuring the underlying genetic correlation. Both methods are equally valid for explaining observed life history patterns. Geneticists, on the other hand, are interested in describing genetic variation and predicting short term responses in gene frequencies caused by changes in selection pressures. How these affect trade-offs between pairs of traits can be examined only by investigation of their

genetic correlation. An important distinction is therefore that questions about future short term response to selection need to be addressed by genetic experiments but questions about current selection pressures can be examined using phenotypic or genetic experiments (Lessells, 1991). Which method is most informative with regard to long term evolutionary processes is a subject open to debate.

1.3 Empirical methods for measuring trade-offs

Methods for attempting to measure life history trade-offs can be divided into five groups. The first three involve phenotypic measurements and the last two use quantitative genetic estimates (Reznick, 1985; Partridge & Harvey, 1988; Lessells, 1991; Sibly, 1991).

1.3.1 Phenotypic correlations

The relationship between two traits is measured from the statistical correlation between directly measured paired phenotypic values of individuals in a population. Measurements can also be used from several populations to increase the range of variation surveyed. Once widely employed, this method is now regarded as being of little use for evaluating trade-offs (Sibly, 1991; Lessells, 1991) because potential intraindividual trade-offs in resources between pairs of traits are often likely to be obscured by the interindividual variation in the total resources available. In other words, although each individual may face a trade-off predicament of allocating its limiting resources between two traits, differential acquisition of resources for such allocation between individuals means that those individuals with the most resources are able to invest more in

both traits. The phenotypic correlation of the population may thus be positive (van Noordwijk & de Jong, 1986).

1.3.2 Indirect manipulations

Experimental manipulation is applied to an environmental variable and this indirectly changes the value of the trait of interest. For example, Kent (1981) increased clutch size by supplementary feeding in the ciliate protozoan *Tokophyra lemnarum*. This approach suffers from the same problem of being unable to distinguish between proximate causes of acquisition and environmental variation in allocation between individuals (Bell & Koufopanou, 1986). Thus changes in the relationship between manipulated broods of *T. lemnarum* may be due to the altered brood size itself or the effects of increased food supply on other life history traits; the two possibilities cannot be separated.

1.3.3 Direct manipulations

These involve direct experimental changing of a trait's value which does not affect any other life history or environmental variable. Thus, confounding environmental variation in allocation can be controlled for by randomly assigning individuals to different experimental groups (Partridge, 1989). This approach has been widely used in studies of clutch size in birds (Lessells, 1986; Linden & Moller, 1989). For example, Reid (1987) manipulated the allocation of parental care by randomly assigning clutches of 1-5 eggs to Glaucous winged gulls, *Larus glaucescens*, whose natural clutch size ranges from 1-3 eggs, and measured the effect on adult survival to breed the following year. Fowler & Partridge (1989) manipulated mating frequency whilst leaving fecundity unchanged by mating female *Drosophila melanogaster* with microcauterised males to demonstrate a cost of time and effort expended in mating.

1.3.4 Breeding experiments

Genetic correlations between traits can be measured using breeding experiments. The fundamental assumption of such breeding experiments is that relatives share genes but do not share environments. Genetic parameters and in particular the genetic correlation between two life history traits can be estimated by collecting phenotypic measures from a group of related individuals (or ideally clones if they are available). Information from relatives can be collected from groups of sibs or from parents and their offspring (Falconer, 1989). Rose & Charlesworth (1981a) used full sib and half sib family groups of *Drosophila melanogaster* to measure the genetic correlation between fecundity and longevity. The precision of genetic estimates obtained from such experiments is low, so large numbers of individuals have to measured making this method impractical in many species especially as meaningful results can be achieved only when measurements are taken in the ancestral environment (Service & Rose, 1985; Holloway et al, 1990; Sibly, 1991).

1.3.5 Selection experiments

These are the genetic analogue of manipulation experiments in which a selection pressure acting on a life history trait is changed directly and the observed response in another trait is measured. From the response over several generations of selection, genetic correlations can be determined. Selection experiments have, as Stearns (1989) put it, "an inherent logical advantage: they predict correlated responses to natural selection directly through correlated responses to artificial selection rather than indirectly through breeding design". By selecting for late fecundity in *D. melanogaster* Rose & Charlesworth (1981b) showed an indirect response of increased longevity suggesting that the traits are negatively genetically correlated. However, their experiment was not large

enough to quantitatively estimate this parameter. Like breeding experiments, the sample sizes required for precise estimates are large.

1.4 The trade-off between the number and size of offspring

As reproduction and survival are the principle life-history traits it is not surprising that the trade-off between them, which is known as the 'cost of reproduction' (Williams, 1966), has been widely studied (Stearns, 1989). This trade-off is concerned with the allocation of resources, usually energy, between current reproduction and the prospects for survival to reproduce in the future. These two components of reproductive value can be expressed in the form of equation [1.2]:

$$\text{current RV} = m_x e^{-r} \qquad [1.6]$$

$$\text{residual RV} = \sum_{y=x+1}^{\infty} (l_y/l_x) m_y e^{-r(y-x+1)} \qquad [1.7]$$

The focus of this study is another important reproductive trade-off which was the first to be studied (Lack, 1947). This can be considered as a subsidiary of the cost of reproduction as it is concerned with allocation within a breeding event. If resources are committed to reproduction a choice exists concerning investment in the size and number of offspring. If resources are limiting either a few large offspring or many small offspring can be produced for the same amount of investment. These are the extreme options of what in actuality is a range of choices made discrete only to the extent of offspring number. This is known as the trade-off between the number and size of offspring or more generally as the trade-off between the number and fitness of offspring (Lessells, 1991).

1.4.1 History

The trade-off between offspring number and fitness was first examined in studies of clutch size in birds. Lack (1947, 1954) proposed that selection acts on parents to produce a clutch size that maximises the number of offspring surviving to breed. The number of young from a clutch surviving to breed is however an incomplete measure of fitness in iteroparous species (Williams, 1966) and later models measured parental fitness in terms of lifetime reproductive success (Smith & Fretwell, 1974) and adult survival (Charnov & Krebs, 1974). Lack did not deal with how parental allocation is optimally subdivided between offspring whereas the model of Smith & Fretwell (1974) considers both parental and offspring fitness. They define parental fitness as the product of offspring fitness and the number of offspring produced at each breeding event as in equation [1.6]. This model works best for species that do not indulge in parental care but does not take into account any effects of current reproduction on the fitness of offspring produced in the future. Smith & Fretwell (1974) concluded that optimal investment in offspring depends on the shape of the offspring fitness curve so that egg size is independent of total maternal resources.

In the past decade extensions of Smith & Fretwell's model have focussed on particular life history types in an attempt to make more precise predictions of optimal trait values. Parker & Begon (1986) produced models for optimal egg and clutch size in adult feeding invertebrates and suggested that egg size could vary between mothers due to the effects of clutch size on larval competition (offspring survival). Begon & Parker (1986) modelled species that feed as larvae only and predicted declines in egg size and clutch size with increasing maternal age as a result of declining survival prospects of the mothers. The effects on offspring fitness of parental care were modelled by Lloyd (1987) and effort per offspring at each breeding event was combined with lifetime reproductive effort

in the three-dimensional model of Winkler & Wallin (1987). The most recent permutation of the offspring size versus number trade-off to be considered is a model where offspring in large clutches have higher survival, as a result of reduced probability of predation of individual eggs (McGinley, 1989). Experimental studies of the trade-off have mostly focussed on the issue of clutch size in birds (reviews in Lessells, 1986; Linden & Moller, 1989; Partridge, 1989; Dijkstra *et al*, 1990).

1.4.2 The trade-off in *Callosobruchus maculatus*

The life history of *Callosobruchus maculatus* has been the subject of numerous studies because of its economic importance and its suitability for laboratory experiments. Its reproductive biology in particular has been extensively examined. In contrast to field populations, where in some cases adults feed on flower pollen or nectar (Ashmead, 1894; Johnson & Kistle, 1987), *C. maculatus* kept in the laboratory stored products environment have no opportunity to feed but are able to successfully complete their life-cycle without doing so. All resources put into reproductive effort are assimilated during larval development and therefore *C. maculatus* is highly suitable for the study of the trade-off between offspring number and size because the adult beetles do not feed. All adult resources are assimilated during the larval stage and hence each female has a finite resource level to allocate to reproduction at the start of oviposition. Consequently, the size and number of eggs laid over a female's lifespan is directly related to her reproductive potential and natural selection should favour those females that optimise their allocation between offspring. As *C. maculatus* is an oviparous animal; and lifetime egg production is henceforth defined as fecundity; the trade-off is more tightly defined as being between *egg* size and fecundity.

In *C. maculatus*, fecundity and especially egg size cannot be manipulated in the way that is possible for clutch size in birds where there is post-laying investment (i.e. parental care) which is the easiest allocation component to manipulate. This means that the only methods available for studying the trade-off between egg size and number in *C. maculatus* are the quantitative genetic approaches; both of which are compatible with this species' life history. The quantitative methods are used in this study as an alternative to experimental manipulation to examine the trade-off between egg size and fecundity in its current context in the beetle's life history. Thus, the methods of evolutionary genetics (section 1.2.2) are used for the purposes of an optimisation perspective on life history evolution (section 1.2.1).

The exact value of life history parameters including fecundity, development rate and larval mortality varies with a number of environmental parameters. These include temperature and humidity (Shoof, 1941; El-Sawaf, 1956; Howe & Currie, 1964; Giga & Smith, 1983; 1987), host seed availability (Credland, 1986), larval density (Giga & Smith, 1981; Smith & Lessells, 1986; Messina, 1991), host seed variation (Nwanze & Harber, 1976; Giga & Smith, 1981), adult density (Bellows, 1982a), adult nutrition (Larson & Fisher, 1938) and mating frequency (Brauer, 1944; Ouedraogo, 1978; T. J. Tufton & P. Eady, pers. comm.). Measures of variation in egg size are less common (Wasserman & Assami, 1985; Holloway *et al*, 1987) but inter- and intraindividual variation in the study population does occur. Measurement of the genetic correlation requires the presence of genetic variation in egg size and fecundity. This has not been measured previously for egg size in *C. maculatus* but it has been demonstrated in fecundity in one population (Møller *et al*, 1989).

The stock of *C. maculatus* used in this study has been maintained in laboratory conditions, kept constant for temperature and humidity, for many generations.

As a result their laboratory environment can be considered to be the natural environment to which the population is adapted. This overcomes the problems of studying life history dynamics in a novel experimental environment where trait responses are confounded by changes in selection pressures outside the design of the experimental schedule (Service & Rose, 1985).

Other reasons why *C. maculatus* was suitable for this study were the relative ease of maintaining laboratory stocks, the short generation time (determining the duration of experiments), the relative simplicity of counting and measuring eggs and the ease of discriminating between the sexes.

1.5 Callosobruchus maculatus as an experimental organism

1.5.1 General biology

The seed beetle *Callosobruchus maculatus* (Fabricus) is one of 1300 species in the family Bruchidae. Taxonomic accounts of the species are given by Southgate (1958) and Haines (1989). Along with several other species in the same genus, *C. maculatus* is a major economic pest of stored pulses in tropical regions of Africa, Asia and South America (see Southgate, 1978; 1979 for descriptions of the biology of the family and genus respectively).

The life cycle, as observed in the laboratory at Sheffield, is completed in less than one month. Within a few hours of mating females begin to lay their total egg complement of approximately 100 eggs over a period of 5-10 days. The eggs, which are shaped like a longitudinally bisected bird's egg, are attached to the outside of the host bean by means of a fast - setting adhesive. After four days, the conspicuous dark cephalic shield (van de Meer, 1979) of the differentiated first instar larva becomes visible inside the transparent egg shell and within a

further two days the larva hatches through the floor of the egg, burrowing through the testa into the cotyledon. The egg becomes white at this point as it is filled by frass deposited by the exiting larva. After three or four instars (Begum *et al*, 1982) the larva pupates inside a chamber excavated immediately below the testa which is visible from the outside as a translucent window from the outside. Three to four weeks after oviposition the adult beetle emerges from the pupa and cuts a circular exit hole through the testa. Newly emerged adults will copulate at once and will mate several times during their 1-3 week lifespan.

1.6 Objectives of this study

In the context of the theoretical, biological and empirical landscape described the principle aims of this study on *C. maculatus* are given below.

1. to measure phenotypic correlations involving egg size and fecundity in order to examine the phenotypic factors affecting the allocation of resources between these traits (Ch. 3).

2. to measure the genetic variation present in egg size and fecundity to see if further quantitative genetic analysis is possible (Ch. 4).

3. to measure the genetic correlation between egg size and fecundity to see how much can be said about the possible trade-off between egg size and fecundity using breeding experiments (Ch. 5).

4. to measure the genetic correlation between egg size and fecundity to see how much can be said about the possible trade-off between egg size and fecundity using selection experiments (Ch. 6).

5. to determine the relationship between egg size and offspring fitness (Ch. 7).

CHAPTER 2

GENERAL METHODS

2. General Methods

This chapter describes the methods used for measuring egg size (section 2.1) and fecundity (section 2.2) and the rationale behind them. Other widely used experimental methods are also given (section 2.3) as are details of the maintenance of the *C. maculatus* population in the laboratory (section 2.4). Operational definitions of all traits measured are given in Appendix A.

2.1 Measurement of egg size

Basic practical problems discussed below include the choice of a precisely defined quantitative parameter to represent egg size (2.2.2), the method by which to measure it (2.2.3) and the size of sample required to reliably estimate a single mean value for each female (2.2.4). Variation of egg size with time and position in oviposition sequence were also examined to investigate if their standardization could usefully reduce confounding phenotypic variation. Changes in egg size with the age of eggs is considered in section 2.2.5. Changes in egg size also occur with increasing maternal age at the time of oviposition. These are described in Chapter 3 and it is noted here that all eggs measured were sampled from the first 24h of oviposition except where otherwise stated.

2.1.1 Measurement procedure

A Kontron Videoplan Image Analysis System (Kontron 8057 Eching, Munchen, Germany) linked to a Reichert Jung Polyvar optical microscope (Reichert AG, Wien, Austria) allowed eggs still attached to beans to be measured when overhead illumination from a fibre optic lamp was used. Beans were mounted egg uppermost on trays using 'Blu-tac' and measured in batches on the microscope stage at X125 magnification.

2.1.2 Why measure egg length and breadth?

A major assumption of this study was that egg length represents an index of the maternal resources invested in each egg. A better index may have been egg weight but this could only be determined by destructive measurement (section 2.4.2) rendering it unusable for breeding and selection experiments where data was collected from two or more generations. However, egg length is strongly correlated with both dry and fresh egg weight (Fig. 2.1). No other index of egg size based on area or volumetric parameters was a better correlate of egg weight than egg length (Tab. 2.1). Egg length and breadth were correlated with each other ($r = 0.60$, $P < 0.001$, $n = 50$).

2.1.3 Why use an image analyser?

C. maculatus eggs are small (Tab. 2.2) and had to be measured under a microscope. The two options for taking measurements available were an image analysis system or an eyepiece graticule installed in a conventional microscope. Table 2.2 shows that measures of mean egg length did not differ between the two methods (paired $t_{(48)} = -0.61$, $P = 0.55$) but egg breadth was significantly smaller when measured with the image analyser (paired $t_{(48)} = 2.92$, $P < 0.01$). This arises from the mathematically abstract way in which the image analyser calculates breadth which is dependent on the area of the shape traced on the digitiser tablet. In contrast, egg length relies only on the accurate recording of the egg apex and it's opposite point so cumulative image tracing error is much less. However, the two measures of breadth are correlated with each other ($r = 0.77$, $P < 0.001$, $n = 49$).

The image analyser was considered to be the better method of measurement for the following reasons:- (1) measurement error was lower. This was shown using

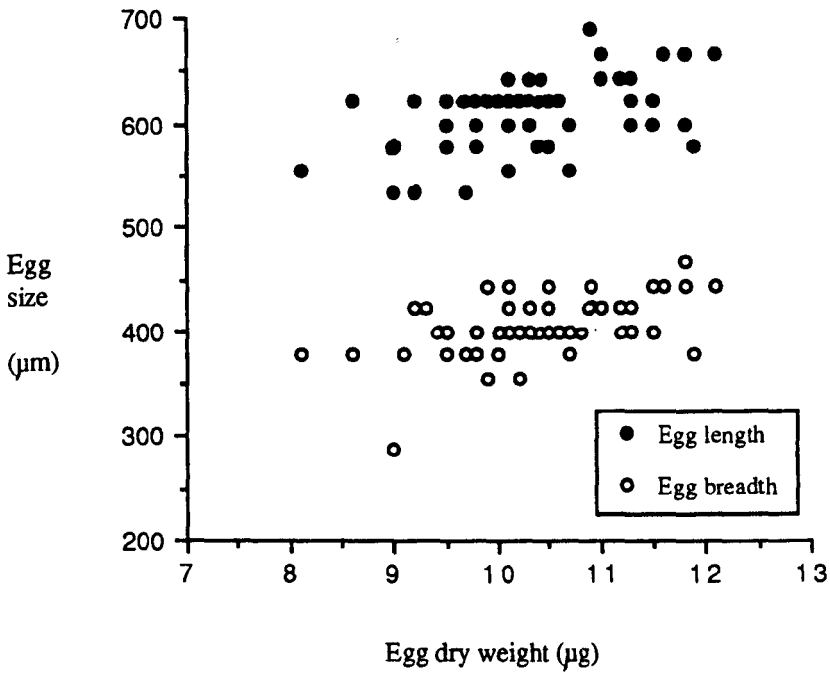


Fig 2.1 Regressions of egg length (closed circles) and egg breadth (open circles) on egg dry weight. Regressions: length = 1.95 dry weight + 4.08 ($r = 0.48$, $n = 46$, $P < 0.001$); breadth = 1.78 dry weight + 2.21 ($r = 0.52$, $n = 45$, $P < 0.001$).

Table 2.1 Comparison of measures of egg size and their correlations with egg dry weight. Single eggs from 46 different females were measured under an optical microscope within 20h of oviposition by two methods: (1) use of eyepiece graticule and (2) use of image analyser. The eggs were then dried to a constant weight at 60°C, detached from the host beans and weighed on an electrobalance. (N = 46).

Egg Character	Egg dry Weight	Egg fresh Weight
eyepiece graticule		
Length	0.48***	0.55***
Width	0.52***	0.46**
Area Index	0.58***	0.59***
Volume Index	0.58***	0.57***
image analyser		
Length	0.73***	0.70***
Breadth	0.63***	0.63***
Area	0.74***	0.73***
Volume	0.72***	0.72***
Circumference	0.74***	0.74***
Ellipsoid volume	0.72***	0.73***

** $P < 0.01$
*** $P < 0.001$

Table 2.2 Mean fresh and dry egg weights plus mean egg length and breadth measured by eyepiece graticule and image analyser.

Egg Character	Mean	s.e.	N
Fresh Weight (ug)	23.78	0.28	46
Dry Weight (ug)	10.36	0.13	46
Graticule			
Length (um)	609	6	49
Width (um)	405	4	49
Videoplan			
Length (um)	610	5	50
Width (um)	397	4	50

the repeatability statistic (r, Ch. 4) to determine the consistency of both methods to evaluate ten repeated measures on each of five eggs (image analyser; r = 0.96, s.e. = 0.03: graticule; r = 0.82, s.e. = 0.05 [r = 1.0 represents perfect consistency of measurement]); (2) egg breadth could be measured simultaneously with no additional effort to maintain a check that changes in egg length with artificial selection (Ch. 6) were not compensated by opposite changes in egg breadth; (3) the time taken to measure eggs under the microscope was less; and (4) measures of egg size correlated better with egg dry and fresh weight (Table 2.1).

2.1.4 What sample size to measure to estimate mean egg length?

Up to 60 eggs were laid by a single female in a 24 h period (pers. obs.) of which a sample must be taken to estimate mean daily egg size. The number of eggs to be sampled is a compromise between the benefit in precision accrued by measuring additional eggs versus the cost in measurement effort (i.e. time).

The repeatability statistic (r, Ch. 4) was employed to express the variance of the mean of n repeated measures of egg length ($V_{P(n)}$) as a proportion of the variance of a single measurement (V_P) using the equation given by Falconer (1989, p.143)

$$\frac{V_{Pn}}{V_P} = \frac{1 + r(n - 1)}{n} \qquad [2.1]$$

This is plotted for one to ten multiple measurements in Fig. 2.2 using r = 0.3 (estimated in Ch. 4). Seventy five percent of the total possible reduction in variance from repeated measures was gained from just four measures and 90% was gained from 10. A minimum of four measures were taken as adequate to give a good estimate of mean daily egg length.

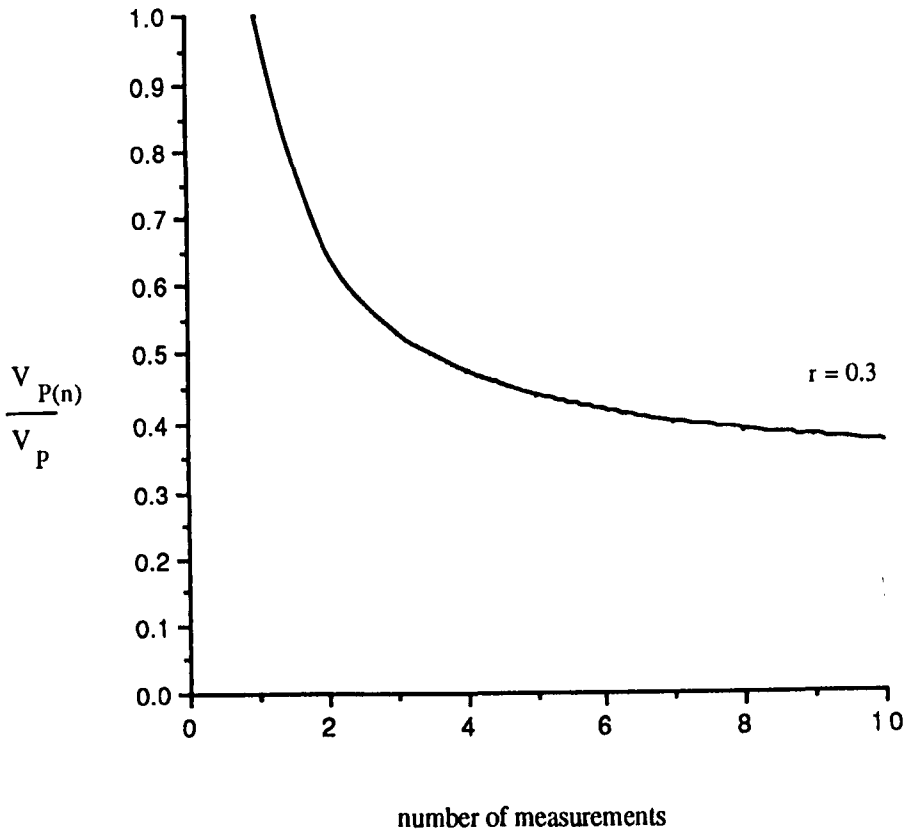


Fig. 2.2 Gain in accuracy from multiple measurements of egg length from each female. The vertical scale gives the variance of the mean of n measurements as a proportion of the variance of one measurement. The graph is plotted for a repeatability estimate of egg length of 0.3 (see equation and Falconer, 1989, p. 143).

2.1.5 The problem of increasing egg length during larval development

Egg size is not constant after oviposition. Both length and breadth of eggs increased linearly over the first five days, becoming stable after this time (Fig. 2.3). The initial daily increase in egg size was approximately 6μm for length and 5μm for width. The fifth day after oviposition corresponded with the appearance of fully developed first instar larvae (Fig. 2.4) so that egg expansion occurred at the time of larval differentiation and development within eggs. During the hatching period, egg size was approximately constant.

Five days after eggs were laid the first larvae became visible inside. The body of the first instar larva was translucent white but the head was conspicuously dark. All larvae were distinguishable by the sixth day after laying (Fig. 2.4). Hatching of eggs occurred between day 6 and day 9. Of the 48 eggs sampled, 8% did not differentiate and a further 14.5% died before or during hatching. It is important to remember that these statistics are specific to one population at one time and to the variety of black-eyed bean provided (Osuji, 1982).

2.2 Measurement of fecundity

This study was concerned with the allocation of maternal resources between progeny and therefore fecundity is taken to be the total number of eggs laid by a female during her lifetime. Undeveloped and malformed eggs are included because even if females recognise that these eggs do not contribute to their fitness, they nevertheless constitute an expenditure of maternal resources. This definition of reproductive output differs from one of reproductive potential which would include any eggs that are matured in the females oviducts and either not laid or resorbed (Wilson, 1989). Numerical differences between these two definitions, the latter being more practically difficult to determine, are likely

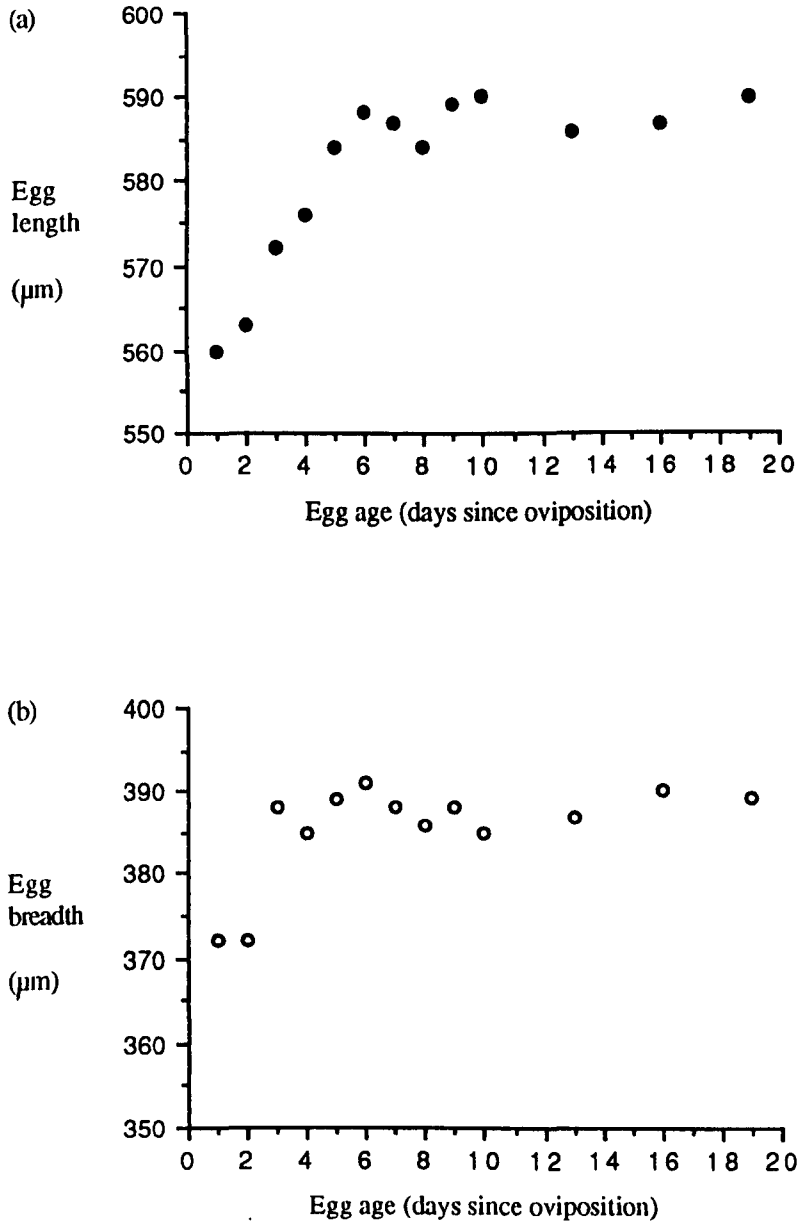


Fig. 2.3 Change in (a) egg length and (b) egg breadth, with time after oviposition. One newly oviposited egg was taken from each of 48 females and measured using an image analyser. The eggs were re-measured at 24h intervals until 10d after oviposition and then every 72h until 19d after oviposition.

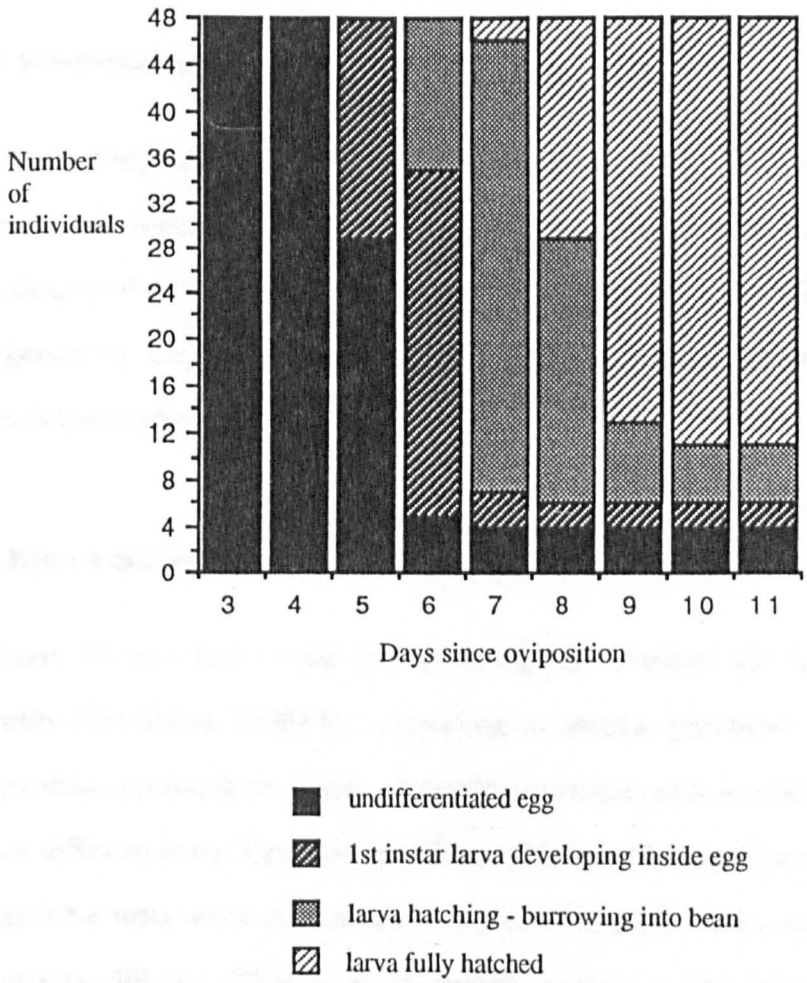


Fig. 2.4- Development and hatching chronology of first instar larvae. Larvae not fully hatched by 10th day of oviposition were dead.

to be small as long as egg fertilisation or oviposition are not restricted. R.H.C. Bonser (pers. comm.) showed that 0 - 6 eggs remained in a female's oviducts after her death. In subsequent chapters the additional term, daily fecundity, is also used to refer explicitly to total egg production of a female over a 24 hour period.

2.2.1 Measurement procedure

All beans supplied to an ovipositing female over her lifetime were inspected under a binocular microscope at X7 magnification and beans were sorted according to their egg loads. Egg load frequencies were then totalled. Counting was generally carried out more than seven days after oviposition when any undeveloped eggs could be distinguished.

2.2.2 Bean availability may constrain fecundity

Provision of too few beans for a female to oviposit on may constrain her fecundity (Credland, 1986) but providing an excess generates unnecessary work in recording egg loads on beans. Females provided with a total of 25 or 70 beans did not differ in their lifetime fecundities (Mann-Whitney U tests; Tab. 2.3) Non - parametric tests were used as the number of females measured was small. The four groups did not differ in mean female body size (Kruskal Wallis ANOVA; $X^2 = 6.90$, $P = 0.08$). Credland (1986, Campinas strain) found restricted fecundities for *C. maculatus* females provided with 40 as opposed to 100 beans and Messina (1991) found a marked difference in fecundity occurring between females given ten or 40 beans. The safest policy was to maximise bean provisioning within each experimental situation subject to the workload cost of handling larger numbers of beans. This also ensured that the frequency of egg dumping (the laying of eggs on unsuitable substrates by mated females) on the

Table 2.3 Daily and lifetime fecundities of females presented with beans for oviposition by four schedules. 46 mated females were randomly given beans on which to oviposit according to four schedules given in the table. Where appropriate, beans were replaced every 24h until the 4th day after oviposition when the last beans provided were left with the female until she died. Standard errors in brackets.

Beans Provisioned on:-	Daily fecundity Day 1	Day 2	Day 3	Day 4	Lifetime fecundity	N
70 over four days (25,15,15,15)	28 (1.2)	19 (1.6)	16 (2.3)	28 (2.9)	91 (5.6)	11
70 on day 1	-	-	-	-	95 (4.2)	12
25 over four days (10,5,5,5)	24 (1.9)	20 (0.8)	17 (2.4)	32 (2.6)	93 (5.8)	12
25 on day 1	-	-	-	-	92 (4.1)	11

walls of breeding pots was negligible (pers. obs.). Egg dumping has only been shown to be significant when mated females are deprived of beans altogether (Wilson & Hill, 1989) but it may affect fecundity scores at low levels of bean provisioning. In practice, 25 beans were given to females on their first day after mating as a minimum total.

2.3 General procedures

2.3.1 Obtaining virgin adults of known age

Emerging *C. maculatus* will copulate immediately and males will even pair with females that have not yet fully emerged from beans. To obtain newly emerged unmated adults, beans containing pupae were placed singly in the compartments (20 x 20 x 18mm) of clear polystyrene 'square repli' cell trays (Gallenkamp Ltd. Loughborough, UK). The cell trays were inspected at 12h or 24h intervals. Adult beetles emerging alone were removed using entomological forceps for soft bodied insects.

2.3.2 Removing intact eggs from beans

This was necessary for weighing eggs and to reduce bean egg loads. A sharp dissection needle was inserted into the bean testa close to the egg. This disrupted the testa and cracked the adhesive holding the egg in place. The egg was then lifted away on the tip of the dissection needle and transferred to a 5mm diameter foil cup for weighing.

2.3.3 Measuring elytral length

One elytra was peeled off each dead beetle and mounted on a glass slide. The detached elytra were measured with the image at X50 magnification.

2.3.4 Weighing eggs and adults on an electrobalance

Live adult beetles were anaesthetised by exposure to carbon dioxide for 20s which was the minimum dose sufficient to keep a beetle immobilised for the 40-60s needed to determine fresh weight. Effects of carbon dioxide on fecundity and longevity have been demonstrated in some insects (Nicholas & Sillans, 1989) but small doses probably have negligible consequences in *C. maculatus* (T.J. Tufton, pers. comm.) and could not be avoided. Anaesthetised adults were placed on a zeroed foil palette and transferred to the weighing stage of a Cahn 29 electrobalance (Cahn Instruments, Cerritos, CA, USA). The measurement was taken after 30s to allow the balance to stabilise. The residual dry body weight of adults after they had died was measured by drying them to a constant weight in an oven at 60°C, and then weighing them on the electrobalance.

2.3.5 Data processing and statistics

Data was stored and processed on the University of Sheffield's IBM 3083 mainframe computer. Statistical analysis was performed primarily using SPSS (SPSS Inc. 1989) with supplementary use of MINITAB (Minitab Inc. 1985) and SAS (SAS Institute Inc. 1985). Some of the quantitative genetic analysis was carried out using purpose written BASIC programs on a BBC microcomputer. Statistical methods were obtained from Sokal & Rohlf (1981), Snedecor & Cochran (1967) and Steel & Torrie (1980).

2.4 Maintenance of laboratory populations

2.4.1 Origin of *Callosobruchus* stocks

All experiments were carried out on a single strain of *C. maculatus* which had been cultured in approximately constant laboratory conditions for approximately 220 generations. The stock was originally collected from the field in Brazil in 1974 by B.J. Southgate and was maintained firstly at the Natural Resources Institute, Slough, UK (1974 - 1977) and then at Imperial College, Silwood Park, Ascot, UK (1977 - 1984). The strain has been cultured in the Department of Animal & Plant Sciences, Sheffield since July 1984 which represents some 50 discrete generations up to the start of experiments for this study in February 1988.

2.4.2 Laboratory culturing environment

At both Sheffield and Imperial College (Bellows, 1982a) stocks were kept in a constant temperature and humidity room at 30 ± 2 C on a 16h light: 8h dark photoperiod. Humidity was maintained at 70 ± 5% RH during the period that experiments were carried out but for some months during 1984 - 1986 and 1988 the humidity was much lower (40 - 50% RH) due to humidifier failure. The beetles were cultured on cowpeas, also known as black-eyed beans *Vignia unguiculata* (L.) Walp. obtained though a health food supplier from California, USA. Larval densities in culture were kept low to avoid the appearance of the dispersive flight morph (Caswell, 1960; Utida, 1972; Messina & Renwick, 1985) which differs from the normal flightless morph in a range of life history characters (Utida, 1954; Ouedraogo & Huignard, 1981).

2.4.3 Culturing methods

Four sub-stocks were kept in clear polystyrene culturing boxes (273 x 152 x 102mm: Stewart Plastics) and were cultured at one week intervals on a four week cycle so that newly emerged adults were available continuously. New culturing boxes were established by anaesthetising emergent adults with carbon dioxide and sieving the beetles from the beans. Approximately 150 adults were transferred to a box containing approximately 2000 beans and left to oviposit for one week after which time they were removed and discarded. The four sub-stocks were previously cultured in isolation from each other so that genetic divergence due to random drift may have occurred (Wilson, 1989). The emergence periods of boxes adjacent in the culturing sequence generally overlap by a few days so from March 1988, 10-20 individuals were added to each box from early emerged adults from the box due to be cultured in the following week. This established a degree of gene flow between the sub-stocks and they were collectively used in experiments as a single population.

CHAPTER 3

PHENOTYPIC CORRELATIONS

3. Phenotypic correlations

3.1 Introduction

Phenotypic correlations between pairs of life history traits cannot establish causal relationships for the reasons discussed in Chapter 1 (section 1.3.1). They are nevertheless able to provide a wealth of supplementary information relevant to trait variation that is more easily gathered than quantitative genetic measures.

Phenotypic correlations contribute to the preparation and interpretation of the genetic experiments in three areas:- (1) by providing quantitative trait statistics that are specific to the study population. Although values for traits such as fecundity, egg size, longevity and development time in *C. maculatus* are available in the literature precise trait values are likely to differ between populations; (2) estimates of trait means and correlations are useful in the design of further experiments. This is particularly true for maternal effects (see below); (3) heritability (Ch. 4) is a relative measure of genetic variation compared with environmental variation. Therefore knowledge of sources of environmental variation can be useful in explaining changes in heritability and ensuring that environmental variation is standardised as much as possible.

Variation in egg size is a prerequisite for examining the trade-off between egg size and fecundity. Interspecific and intraspecific comparative studies have invoked several explanations for detected egg or seed size variation in animals and plants (Temme, 1984; Tessier & Consolatti, 1989). Those potentially relevant to *C. maculatus* are: (i) adaptive explanations based on ability to respond to a variable environment (Capinera, 1979) or life-history models incorporating effects of age independent mortality (Parker & Begon, 1986; Begon & Parker, 1986; Wilson, 1989); (ii) non-adaptive explanations of which

the most popular is the resource depletion hypothesis which states that the amount expended on each egg is an unchanging function of the mother's remaining reproductive resources (Wiklund & Karlsson, 1984) as a result of physiological constraints; (iii) lack of selection pressure on egg size; i.e. the net fitness contribution is neutral (Pitelka *et al*, 1983) and variation is attributable to non-additive genetic sources, such as maternal body size. The neutral selection explanation differs from the others in that it does not predict any pattern to within female egg size variation.

The variable environment adaptive explanation is probably inappropriate to *C. maculatus* where the culturing environment has been kept relatively constant. Begon & Parker (1986) argue that the resource depletion hypothesis is implausible as the laying of large first eggs is non-adaptive if largest eggs do not give rise to the fittest adults. However, if the largest eggs do produce the fittest adults but egg size is tightly constrained by some physiological parameter linked to remaining maternal resources then this non-adaptive explanation can still account for a decline in egg size with maternal age. Models of egg size and clutch size variation presented by Begon & Parker (1986) are appropriate to *C. maculatus* as they apply to organisms where females accumulate all their resources prior to reproductive maturity. The models predict a decline in egg size with maternal age as a function of diminishing maternal survival and this occurs whether clutch size is constant or not. This is convenient as clutch size is typically defined for *C. maculatus* as the number of eggs laid on a single bean during one visit by a female (Wilson, 1989, p.89) and this can be determined only through behavioural observation. Within the constraints of the experimental design it was not possible to measure this character and the only available substitute was daily fecundity which was a crude approximation at best. Another important prediction of Begon & Parker's egg size models is that large females,

assuming that they have the greatest resources, should lay large first eggs. Unfortunately, both these predictions are shared with the resource depletion hypothesis but the presence of pattern in egg size variation with maternal age and weight would rule out any neutral selection or environmental fluctuation explanations.

Three groups of phenotypic correlations were used to examine questions concerning the covariation of egg length, fecundity and other life history traits.

3.1.1 Phenotypic correlations between adult life history traits

The phenotypic correlation between egg length and fecundity was measured to see if it did correspond with the expected trade-off (Expt. 3A). In addition, examination of correlations between these two traits and other life history traits may highlight sources of variation that produced the observed egg length - fecundity correlation. Particular emphasis was placed on body size (Expt. 3C) as its allometric effects on life history traits are widely known (Stearns, 1983; Stearns & Koella, 1986). Fecundity has repeatedly been shown to correlate with female body weight (Credland *et al*, 1986; Wilson, 1989; Messina, 1991) in *C. maculatus* but egg length and body weight have not been compared. The extent to which body size and weight are correlated was also investigated. Body weight is likely to be a better measure than size of female energetic and nutritional resources and is thus most pertinent to the allocation questions of this study. Dry weight at emergence is likely to give the best estimate of resources but this can only be measured by killing the beetle so residual dry body weight after natural death was measured as a non-destructive alternative. The usefulness of fresh emergence weight and elytral length as alternative indices of body resources depends on how closely they correspond with dry weight. These weight measures and elytra length are compared in Expt. 3B.

3.1.2 Potential fitness consequences of egg length variation

Phenotypic correlations between egg length and adult traits of the offspring that hatched from those eggs were used as circumstantial evidence for the fitness benefits *per se* of large eggs over small eggs. Again causality cannot be inferred from any correlations. For example, large females may lay large eggs that give rise to large offspring for genetic or purely non-genetic reasons and these cannot be distinguished. The within group correlations were useful for comparison with correlations between groups whose traits had been manipulated in selection experiments (Ch. 7).

3.1.3 Changes with maternal age

A potential source of within-individual variation in egg length is female maternal age at oviposition. Changes in egg length, daily fecundity and body weight were examined to see if standardisation of maternal age would improve the chances of recognising between-individual differences in quantitative genetic experiments. The consistency of the phenotypic correlation between mean daily egg size and daily fecundity was checked by measuring this correlation on each of the first five days of oviposition.

3.1.4 Aims

1) to measure the phenotypic correlation between egg size and fecundity.

2) to measure the phenotypic correlations between development rate, body size, body weight, and longevity; and between these traits and egg size and fecundity.

3) to measure the phenotypic correlations between egg size and life history traits of the offspring that hatch from them.

4) to determine the changes in suvivorship, egg size, daily fecundity and body weight that occur with increasing maternal age.

3.2 Methods

3.2.1 Correlations between egg length, fecundity and other adult traits

Experiment 3A Correlations of egg size with fecundity and other traits

Female offspring whose egg size and fecundity were to be measured were obtained by observing thirty pairs of virgin adults less than 24h old until they mated and then separating the pairs. A further 30 virgin females were mated to the same males on the subsequent two d. Mated females were placed in 20 cm^3 pots with 25 beans which were replaced with ten fresh ones at 24h intervals until 4d after mating when the fourth set of beans were left with the female until she died. Egg loads on each bean were reduced to one and egg size and development rate (1/development time) were recorded. Eight virgin female offspring from each mother were randomly chosen when they emerged and were mated when less than 24h old. Beans were given to each in an identical manner to their mothers and daily fecundity, longevity and length of eggs laid on the first and third days of oviposition were recorded. When dead, the female offspring were dried and residual dry body weight and elytral length were measured. This experiment was conducted as part of the breeding experiment (Ch. 5).

Experiment 3B Correlations between fecundity and body size

Twenty five virgin females and 25 virgin males less than 24h old were weighed and then paired and observed until they mated. The males were removed after mating and kept alone until death. The females were each placed in a 20 cm^3 pot containing 30 beans. A further 20 fresh beans were provided every 24h until all females had died. Dead females and males were dried and weighed and their elytra lengths were measured.

Experiment 3C Correlations between egg size or fecundity and body weight

Two groups of 28 virgin adult pairs were mated and the females allowed to oviposit on 25 beans each in 20 cm^3 pots for 24h before being removed and discarded. Egg loads were reduced to one and once all larvae had hatched eight eggs were randomly chosen from each pot and measured. Upon emergence of adults from the selected eggs 28 virgin adult pairs less than 24h old were chosen at random and weighed before being mated to non-sib partners. Males were separated after mating had been observed and the females were placed in 20 cm^3 pots with 25 beans. A replacement of five fresh beans were given to each female on the 2nd, 3rd and 4th days after mating. The last beans were left with the females until they died. Fecundity and egg size were recorded and longevity, development time and elytral length were measured in both sexes. This experiment was conducted during the selection program (Ch. 6). The two groups used in this experiment were from two replicate assays in the base generation of the selection experiment (Fig. 6.1).

3.2.2 Correlations between egg size and offspring traits

Experiment 3D Breeding experiment correlations

Measures of traits of adults that hatched from eggs of known size were obtained as part of the breeding experiment (Ch. 5). The methods are those described in Expt. 3A. Adult traits were measured only in female offspring and egg size was measured in both parent and offspring generations.

Experiment 3E Selection program correlations

The general assay of the base generation of the selection experiment (Ch. 6) also furnished egg sizes and measures of adult traits in the offspring that hatched from them. The methods are the same as those given in Expt. 3C. Adult traits were measured in both sexes.

3.2.3 Variation of adult traits with maternal age

Experiment 3F Adult female survivorship

Twenty two virgin females less than 1d old were mated to virgin males in 20 cm^3 pots containing 30 beans. After 24h the males were removed and discarded and the females were transferred to new pots containing 20 beans. Further sets of 20 beans were provided at 24h intervals until the females died.

Experiment 3G Correlations of egg size, fecundity and body weight with maternal age

In each of three groups, 12 virgin adult females less than 1d old were weighed and then mated to virgin males of similar age. The males were discarded and the

females were placed in 20 cm^3 pots with 25 beans. At 24h intervals the females were removed, re-weighed and returned to a new pot with 15 fresh beans until the fifth day after mating. On the sixth day the females were given five beans only left with them until death. The large number of beans provided ensured that eight eggs on different beans were obtainable for size measurement from each of the first five days of oviposition. Daily and lifetime fecundities were also recorded. This experiment was conducted during the course of the selection experiment (Ch. 6). The three groups came from the base and second generation selected lines of one replicate of the selection experiment (Fig. 6.1)

3.3 Results

3.3.1 Correlations between egg length, fecundity and other adult traits

Experiment 3A Correlations of egg size with fecundity and other traits

Fecundity had no significant phenotypic correlation with egg size on the first day of oviposition but had a small positive correlation with egg size on the third day of oviposition (Table 3.1). Fecundity is positively correlated with residual dry body weight, elytral length and development time but negatively correlated with longevity. This suggests that the most fecund females are large and develop quickly but suffer a competitive disadvantage in terms of reduced lifespan. Egg length on both the first and third day of oviposition is positively correlated with residual dry body weight and longevity. Egg length on only the third day of oviposition is correlated with development time.

Table 3.1 Correlations between adult female life history traits including egg length and fecundity. Sample sizes in brackets.

	Development rate	Fecundity	Longevity	Residual dry body weight
Fecundity	0.08* (656)			
Longevity	-0.08* (703)	-0.21*** (652)		
Residual dry wt.	-0.06 (705)	0.08* (654)	0.22*** (701)	
Elytral length	0.01 (709)	0.28*** (657)	0.25*** (704)	0.73*** (707)
Egg length 1	-0.01 (678)	0.04 (652)	0.10* (677)	0.31*** (675)
Egg length 3	0.09* (593)	0.12** (595)	0.11** (595)	0.34*** (593)

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

1 - mean egg length on first day of oviposition
3 - mean egg length on third day of oviposition

Experiment 3B Correlations between fecundity and body size

Lifetime fecundity was positively correlated with both fresh body weight and residual dry body weight (Table 3.2). Emergence weight was strongly correlated with residual dry body weight, and both weight measures correlated very strongly with elytral length in both sexes indicating that elytral length could be used as an index of weight.

Experiment 3C Correlations between egg size or fecundity and body weight

The length of eggs laid on the first day of oviposition is correlated with emergence weight (Figure 3.1) i.e. heavy females laid large first eggs. The correlation may be partly due to differences of up to 24h at which emergence weight was measured. Because egg length declines with maternal age (Expt. 3G) the females who are oldest when weighed will be both lightest and lay the smallest eggs. However, the range of emergence weights (2.97mg) is much greater than the largest recorded daily weight loss (1.24mg), so the correlation is not entirely due to variation in exact age at weighing. Fecundity on the first day of oviposition was not significantly correlated with emergence weight (Figure 3.2(a)) although a significant negative correlation exists if one female is excluded from the analysis. However, there is no justification for removing this outlier. In contrast, lifetime fecundity and emergence weight are positively correlated (Figure 3.2(b)).

Table 3.2 Correlations of fecundity and elytral length with emergence weight and residual dry body weight of females

	Emergence weight	Residual dry body weight
Females		
Fecundity (n = 22)	0.83***	0.56**
Elytral length (n = 25)	0.83***	0.76***
Males		
Elytral length (n = 25)	0.90***	0.82***
Residual dry weight (n = 22)	0.88***	

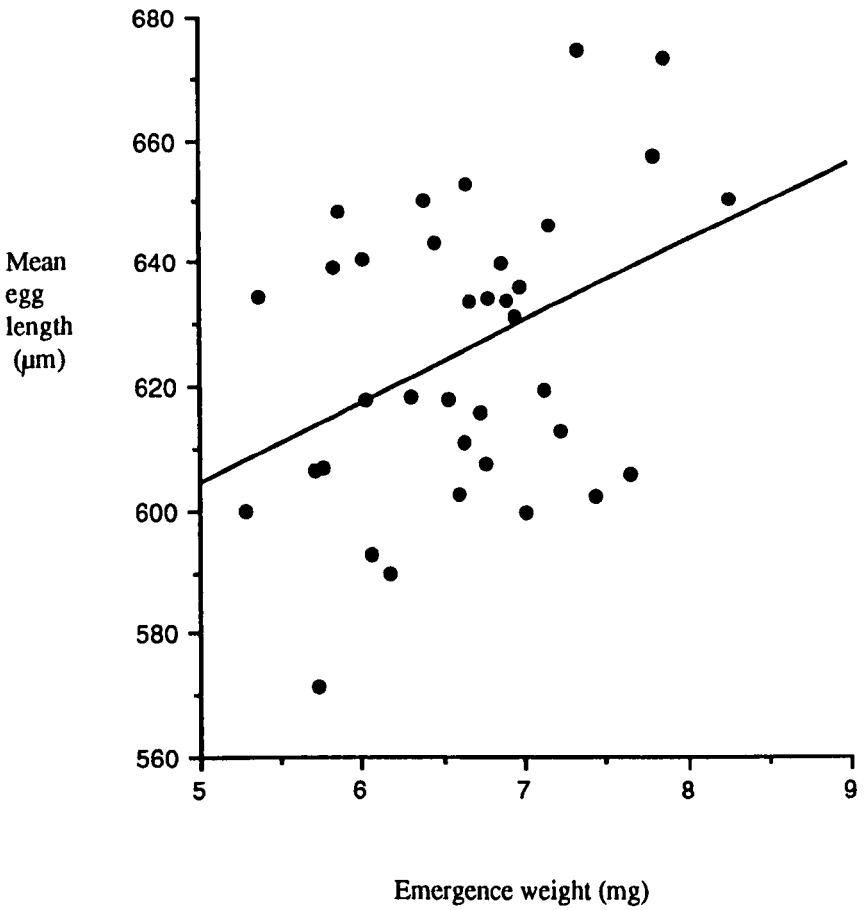


Fig. 3.1 Relationship between egg length on first day of oviposition and female emergence weight. Regression: egg length = 13.1 emergence weight + 538.7 ($r = 0.39$, $n = 36$, $P < 0.05$).

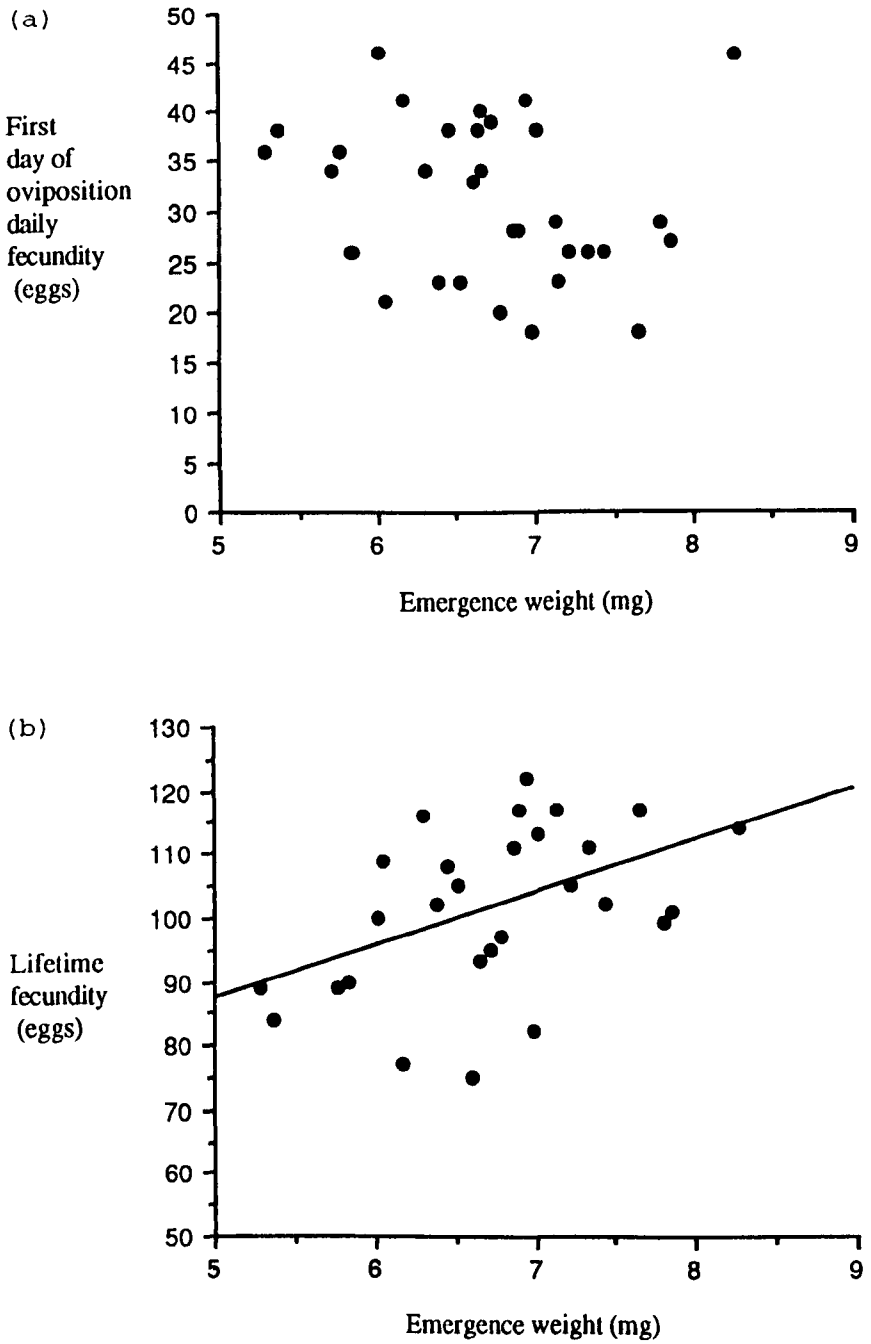


Fig. 3.2 Relationship between (a) fecundity on first day of oviposition and female emergence weight ($r = -0.19$, $n = 33$, $P < 0.28$), and (b) lifetime fecundity and female emergence weight. Regression: fecundity = 8.2 weight + 46.0 ($r = 0.55$, $n = 30$, $P < 0.01$).

3.3.2 Correlations between egg size and offspring traits

Experiment 3D Breeding experiment correlations

Residual dry body weight, elytral length and development rate of females were all positively correlated with the length of eggs from which the adults developed implying that large eggs gave rise to large adults even though they developed fastest (Table 3.3). There was no relationship between adult longevity or fecundity and the length of the egg from which the adult develops. The size of eggs that a female laid on both the first and third days of oviposition was positively correlated with the length of egg from which she herself had developed. Thus the reproductive benefit of large eggs giving rise to heavy maternal weight appears to be in the size of eggs laid by female offspring rather than in increased fecundity.

Experiment 3E Selection program correlations

Egg length and breadth were positively correlated with each other and development rate was positively correlated with egg breadth and not egg length. In males only, longevity was positively correlated with egg length ($0.1 < P < 0.05$) and breadth. In both sexes fecundity and development rate were negatively correlated with longevity. Elytral length and longevity were stongly positively correlated with emergence weight and fecundity was positively correlated with elytral length. Elytral length and emergence weight were both negatively correlated with development rate in males only although the latter correlation was close to significance in females. (Table 3.4).

Table 3.3 Pearson correlations between egg size and characters of adult female progeny that hatched from those eggs obtained from a breeding experiment.

Progeny Trait	Egg Length	Egg Breadth	N
Development rate	0.27***	0.19*	179
Fecundity	0.07	0.06	159
Longevity	0.07	-0.01	178
Dry weight	0.16*	0.05	176
Elytral length	0.19*	0.19*	178
Egg length 1	0.29***	0.22*	165
Egg length 3	0.21*	0.22*	142

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

1 mean egg length on first day of oviposition
3 mean egg length on third day of oviposition

Table 3.4 Pearson correlations between egg size and (a) female, (b) male progeny adult traits in females and males.

(a) females (n = 56)

	Egg length	Egg breadth	Fecundity	Longevity	Devel. rate	Emergence weight
Egg breadth	0.37**					
Fecundity	0.10	-0.34**				
Longevity	0.09	0.17	-0.54***			
Devel. rate	0.31*	0.31*	0.09	-0.23+		
Emergence weight	0.06	0.13	0.07	0.45**	-0.21+	
Elytral length	0.10	0.08	0.24*	0.22+	-0.11	0.87***

(b) males (n = 53)

	Egg length	Egg breadth	Fecundity	Longevity	Devel. rate	Emergence weight
Egg breadth	0.52***					
Fecundity	.	.				
Longevity	0.19+	0.36**	.			
Devel. rate	0.31*	0.38*	.	-0.22+		
Emergence weight	0.16*	0.13	.	0.58***	-0.40*	
Elytral length	0.10	-0.01	.	0.42**	-0.49***	0.84***

+ P < 0.1 * P < 0.05 ** P < 0.01 *** P < 0.001

3.3.3 Variation with maternal age

Experiment 3F Adult female survivorship

Female survivorship showed a sigmoid decline; 70% of deaths occurred between 5 and 7 d of age and all females were dead by the end of 9 d (Fig. 3.3).

Experiment 3G Correlations of egg size, fecundity and body weight with maternal age

Changes in females with maternal age were measured over the first five days of oviposition. During this period mean daily fecundities were greater than five eggs per day giving a sample size large enough to precisely estimate egg size. Egg length declined linearly with maternal age by 7μm per day (Figure 3.4(a)). Breadth of eggs declined at the same rate (ANCOVA: Test I: homogeneity of slopes $F_{(1,354)} = 0.25$, $P = 0.65$; Fig. 3.4(b)).

Daily fecundity declined at a decelerating rate with maternal age falling from a mean of 31 eggs on the first day of oviposition to 7 eggs on the fifth day (Fig. 3.5).

Egg length and daily fecundity pooled across all days of oviposition are correlated ($r = 0.34$, $n = 162$, $P < 0.001$) but this is a result of the joint decline of both with maternal age. Within days, there was no correlation between egg length and daily fecundity (Table 3.5).

Emergence weight of offspring also declined at a decelerating rate with maternal age at oviposition (Figure 3.6). Predicted daily weight loss of ovipositing females due to oviposition was estimated from the product of mean fresh egg weight and mean daily fecundity. Mean fresh egg weight was estimated from the regression

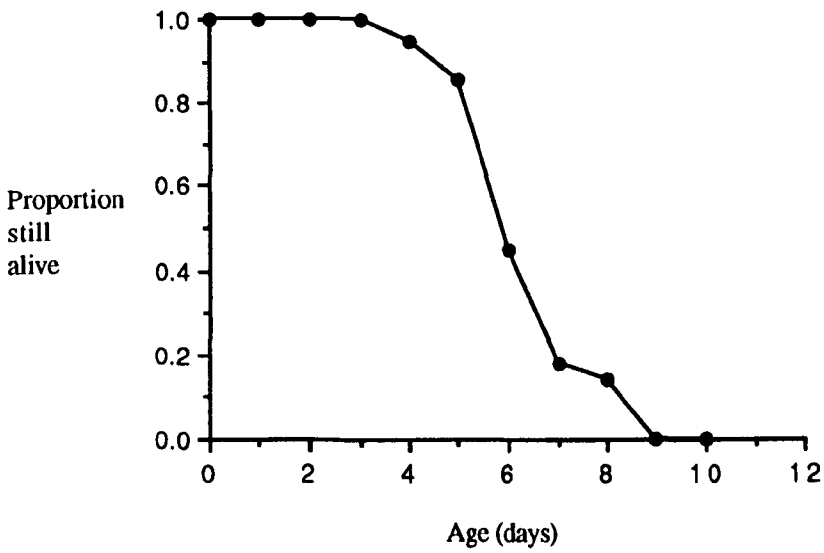


Fig. 3.3 Adult female survivorship. All females were mated once within 24h of emergence and provided with fresh beans for oviposition each day.

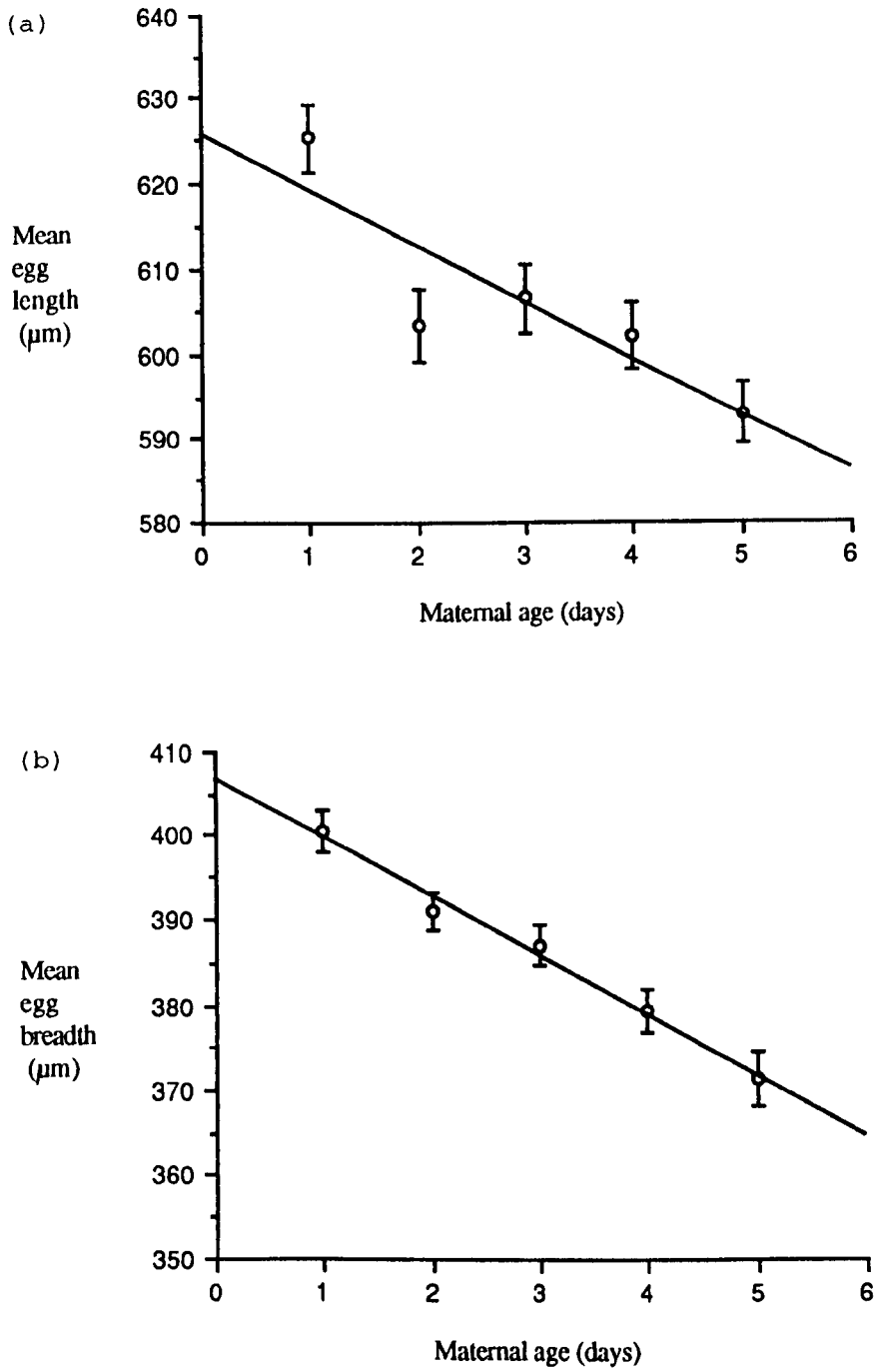


Fig. 3.4 Decline of (a) egg length with maternal age. Regression: length = -6.6 age + 625.8 ($r = -0.37$, $n = 165$, $P < 0.001$); (b) egg breadth with maternal age. Regression: breadth = -7.0 age + 406.8 ($r = -0.57$, $n = 165$, $P < 0.001$). Daily means and standard errors from 36 females are plotted.

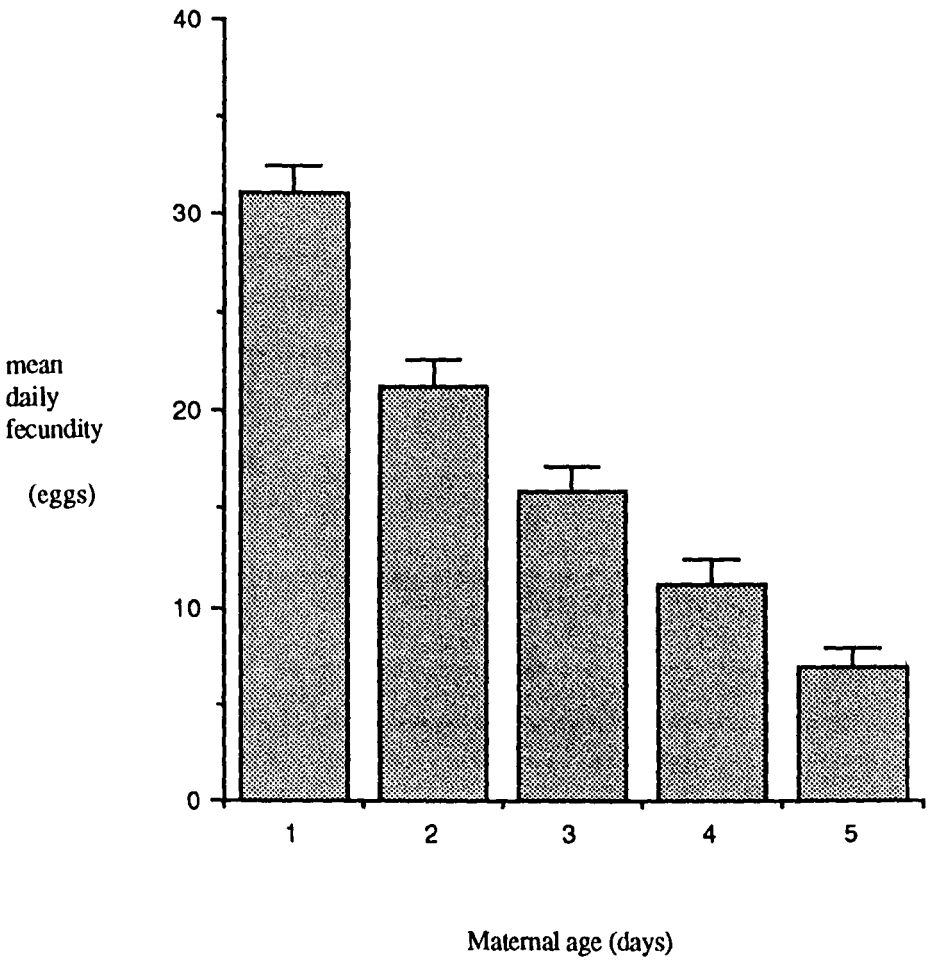


Fig. 3.5 Decline in daily fecundity with maternal age (means and standard errors from 36 females).

Table 3.5 Correlation coefficients of egg length and daily fecundity for maternal ages 1 - 5 days.

Day	r	n	P
1	-0.16	33	0.36
2	0.04	36	0.80
3	0.12	34	0.50
4	0.15	31	0.43
5	0.08	28	0.70

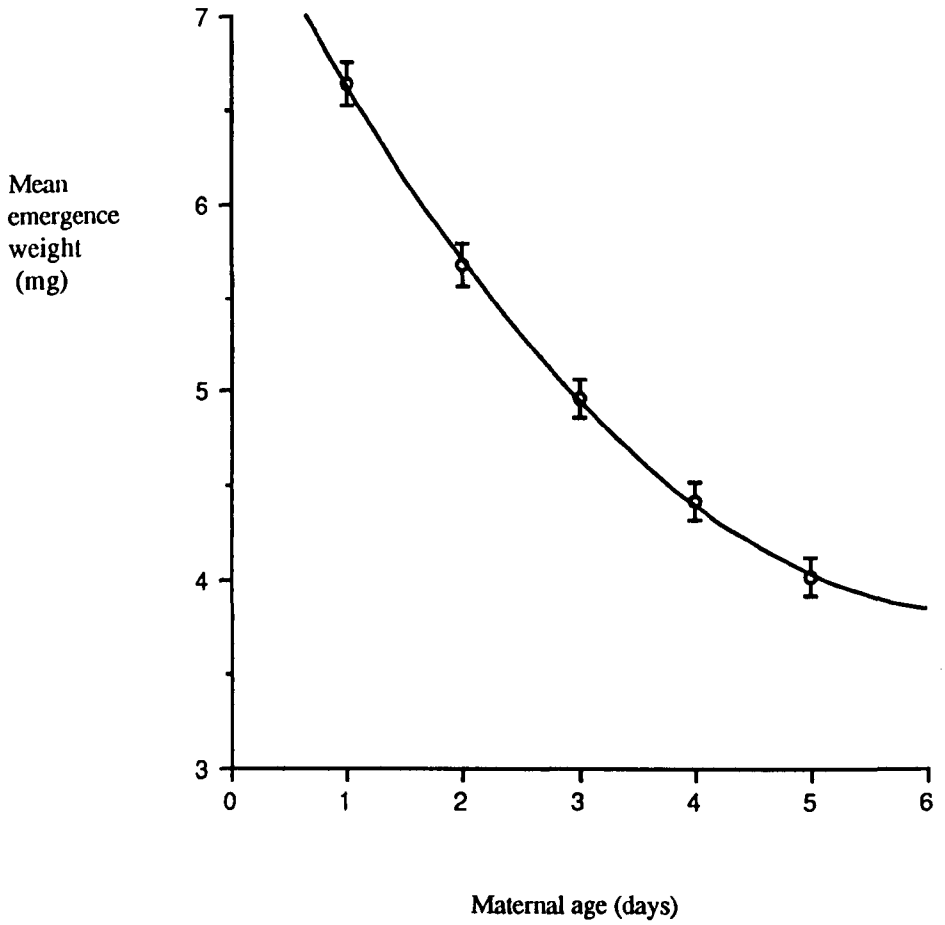


Fig. 3.6 Decline in fresh body weight with age (means and standard errors of 36 females). Regression: fresh weight = -1.2 age + 0.1 age^2 + 7.7 ($r = -0.81$, $n = 179$, $P < 0.001$).

of fresh egg weight against egg length (Figure 7.3). Predicted weight loss due to reproductive investment declined with maternal age as the number and weight of eggs produced decreased (Fig. 3.7). Actual weight loss was larger than predicted weight loss but declined at the same rate. The difference between the predicted and actual weight losses can be attributed to somatic and reproductive metabolic expenditure. This quantity is a constant proportion of current body weight as females grow older.

3.4 Discussion

3.4.1 Phenotypic correlations

The absence of a negative correlation between egg length and fecundity means that there is no phenotypic evidence for a trade-off between these traits. Indeed, there was a positive correlation between egg length on the third day of oviposition and fecundity. Both egg lengths and fecundity are positively correlated with residual dry body weight and therefore variation between individuals in body weight may be a cause of the egg length - fecundity correlation (van Noordwijk & de Jong, 1986). Correlations between egg length and other traits were clearest for egg length on the third day of oviposition. Females were mated only once and ejaculate contributions may become exhausted by the third day of oviposition. Male ejaculatory investment has been shown to increase fecundity (Brauer, 1944; Ouedraogo, 1978) and may also allow females to lay larger eggs. If males vary in the size of their contribution this might be a source of variation in first laid eggs only under the mating regime of experiments in this study, thus accounting for the poorer correlations with egg length on the first day.

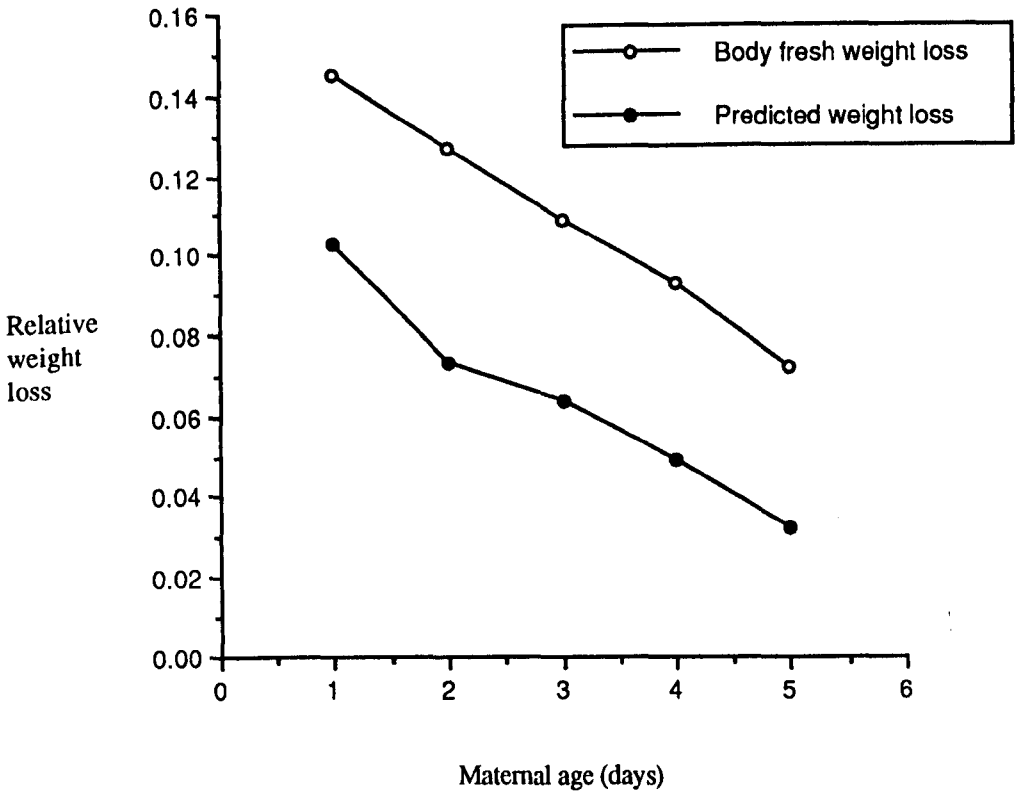


Fig. 3.7 Female fresh weight loss per day as a proportion of fresh weight at the start of each day. Actual weight loss is plotted with predicted weight loss attributable to reproductive investment. Predicted weight loss was calculated as the product of mean daily egg weight (estimated from regression with egg length) daily fecundity. (n = 12).

Emergence weight, residual dry body weight and body size (elytral length) were all strongly correlated with each other. Large females laid large first eggs and had higher fecundities although fecundity on the first day of oviposition was not greater than that of small females.

The phenotypic evidence generally supports the assumption that the largest eggs accrue a fitness benefit to the adults that hatch from them. Egg length is positively correlated with emergence weight, development rate and egg size in the next generation. Emergence weight and development rate themselves are positively correlated with elytral length, fecundity and longevity. This general pattern of phenotypic correlations measured in the experiments in this Chapter is summarised in Fig. 3.8. One interpretation of the correlations is as follows. Large eggs develop fastest and give rise to the largest adults. The relationship between large egg size and fast development rate is surprising if emergent adults are also large. This implies that growth rate is proportionally greater than development rate in large eggs. The heaviest adults are also the largest in terms of elytral length, and they have high fecundities, long lifespans and lay larger eggs than small adults. Fast development rate is associated with reduced longevity and increased fecundity and the two latter traits are negatively correlated. In males, fast development rate results in small adult weight.

The negative correlations between the two direct correlates with egg size, emergence weight and development rate, and that between fecundity and longevity, suggest that the effects of egg size on all offspring traits cannot be simultaneously expressed. With limited resources within each egg constraints are to be expected such that, at the extreme, two types of progeny may be produced: (i) adult progeny that develop quickly and have low weight at emergence, high fecundity and short lifespan, or (ii) adults that develop slowly, have high emergence weights, low fecundity and a long lifespan. That large eggs give rise

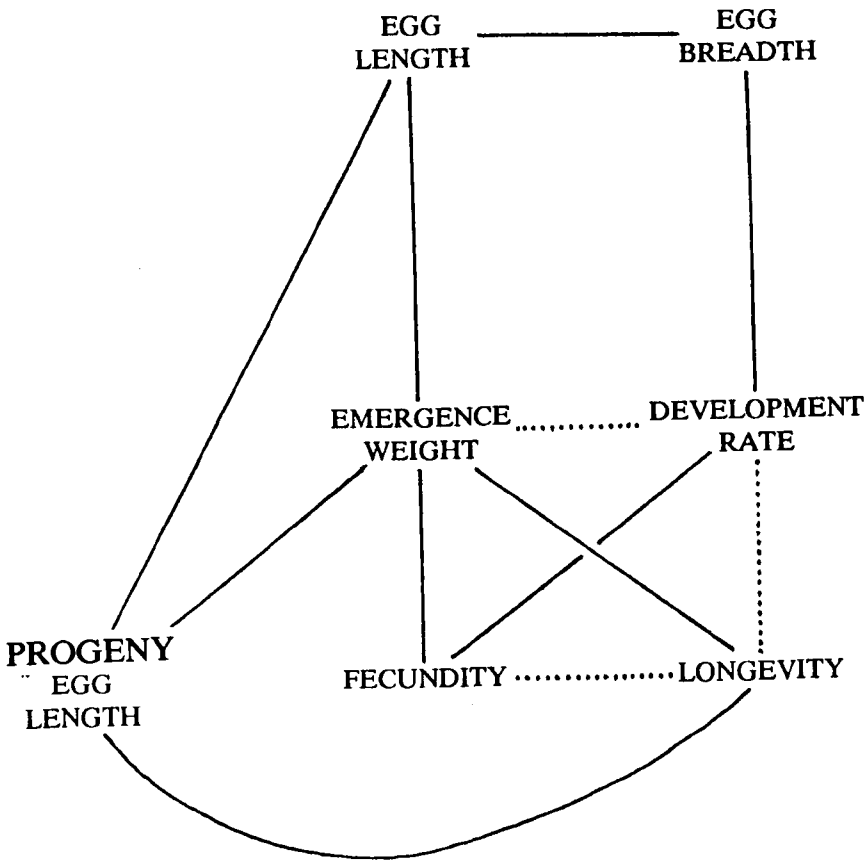


Figure 3.8 Path diagram summarising the phenotypic correlations between the size of eggs and traits of adult progeny that hatch from them.

to large adults implies that egg size delimits the size of larva that hatches and that larval growth is proportional to first instar larval size. Large egg size is also positively correlated with egg size in the next generation. It may be that large eggs can only be laid by large females because of mechanical limitations such as oviduct diameter. This intergenerational legacy of body size need not include any genetic component. If this was the case, it would constitute a maternal effect attributable to body size as is implied by the within-generation correlations. The positive correlation between longevity and emergence weight has been shown previously in *C. maculatus* (Møller *et al*, 1989; Wilson, 1989) as has that between fecundity and emergence weight (Møller *et al*, 1989; Wilson, 1989; Messina, 1991).

These consistent correlations provide indirect evidence for the beneficial consequences for at least some fitness components of adult progeny resulting from large egg size. Under the constraint of finite resources per egg, the traits which in actuality express the effects of egg size may be phenotypically plastic with environmental conditions.

3.4.2 Declines in egg size and fecundity with maternal age

Egg length and breadth declined at the same constant rate with maternal age and the eggs became more narrow relative to their length as a result. This parallel decline in egg length and breadth was shown previously in *C. maculatus* by Wasserman & Assami (1985) although they describe the decline as occurring only after four days of age. However, close examination of their results reveals that a good fit can be made by a linear regression across all days. Why the eggs change shape with increasing maternal age is unclear. Numerous studies of Lepidoptera have also shown a decline in egg weight with maternal age: for example *Tyria jacobaeae* (Richards & Myers, 1980), *Pararge aegeria* (Wiklund &

Persson, 1983), *Lastommata megera* (Wiklund & Karlsson, 1984) and *Panolis flammea* (Leather & Burnand, 1987). Phelon & Frumhoff (1991) recorded a decline in egg weight in two Hemipterid *Oncopeltus* species. All of these studies show a linear decline except Richards & Myers (1980) where the decrease is greatest during the first four days of oviposition.

Daily fecundity was also shown to decrease with maternal age but the change was at a decelerating rate. This trend was demonstrated previously in *C. maculatus* by Giga & Smith (1983) but a linear decline was shown by Bellows (1982b) and Dick & Credland (1984) and a more sigmoid curve shape was revealed by Credland & Wright (1989) and Messina (1991). Thus the form of decline in fecundity may vary with population or environmental parameters. Decelerating declines in daily fecundity have also been shown in the butterflies *Euphydryas editha* (Murphy *et al*, 1983) and *Coenonympha pamphilus* (Wiklund & Karlsson, 1987). In the Monarch butterfly *Danaus plexippus* daily fecundity also declined at a decelerating rate but only after an initial period of rapid increase (Zalucki, 1981). In another study on the same species the initial increase was absent (Svärd & Wiklund, 1988). Of all these studies, only Richards & Myers (1980) and Svärd & Wiklund (1988) demonstrated concurrent declines in egg weight and fecundity. All these Lepidopteran examples which represent the majority of invertebrate studies of egg size effects were conducted in a laboratory environment where adult feeding was restricted or prevented. Such declines may not occur under conditions experienced in the wild where access to nectar is possible (Moore & Singer, 1987). For example, in the study of Murphy *et al* (1983) the decline in fecundity was specific to one treatment where butterflies were not allowed access to food or water as adults. In other treatments the provision of water, sugars and amino acids reduced or eliminated the decline with maternal age. The life-history models of Begon & Parker (1986) which

predict a decline in both egg size and fecundity are specific to species, and perhaps circumstances, where adult feeding does not occur. Thus the potential artificial nature of the numerous Lepidopteran studies probably does not undermine their findings. *C. maculatus* adults will themselves imbibe nectar if it is provided (pers. obs.) so the decline in reproductive effort may be a plastic response to the absence of opportunities for adult nutrition.

Female weight loss per day was divided into estimated egg investment and non-invested reproductive plus somatic expenditure. Weight loss attributable to both these two activities declined with female age and the energy invested in eggs became proportionately smaller with time as expected from the declines in both egg size and fecundity. This result was also shown by Sharma & Sharma (1984). By the end of the fifth day of oviposition an estimated 27% of the mean emergence weight of females had been assimilated into eggs. This suggests that rather more than the 10% of initial energy content invested in eggs by *C. analis* (Wightman, 1978) was transferred by *C. maculatus*. Lipids are the most important source of energy in the adult beetles (Dhand & Rastogi, 1981) which are metabolised from reserves accumulated during larval feeding and which probably represent the chief energetic constraint on reproductive effort. Figure 3.7 suggests that reproductive expenditure may be a decreasing function of remaining resources which is consistent with the resource depletion hypothesis. Alternatively, Begon & Parkers' (1986) adaptive models describe reproductive effort as a function of the probability of survival to lay more eggs. As the results obtained were consistent with, but unable to differentiate between, both hypotheses, more detailed studies outside the scope of the data collected are required to distinguish between them. Large females laid the largest eggs on the first day of oviposition in accordance with the model of Begon & Parker (1986)

who themselves found little empirical evidence for this prediction. Although this may indicate adaptive variation in egg size it could simply be a result of non-adaptive body size allometry.

The largest females also had the highest lifetime fecundities but daily fecundity on the first day of oviposition was independent of maternal emergence weight. The relationship between lifetime fecundity and emergence weight has been revealed previously in *C. chinensis* by Smith & Lessells (1985) and in *C. maculatus* by Wilson (1989, p.71). Benefits for fecundity from body weight thus appear to occur as a result of higher daily fecundity during later oviposition or increased oviposition period.

CHAPTER 4

REPEATABILITY AND HERITABILITY

4. Repeatability and Heritability

4.1 Introduction

Genetic correlations, by definition, can be measured only between traits possessing detectable quantities of additive genetic variation and this potentially poses a problem for the measurement of correlations between egg size, fecundity and other life-history characters. Traits that are expected to be closely associated with fitness are predicted to contain only small amounts of additive genetic variation because they will have experienced strong selective pressures leading to the fixation of many alleles (Fisher, 1930; Falconer, 1989, Ch. 20). Although many empirical studies have found support for this prediction (Gustaffson, 1986; Mousseau & Roff, 1987), others have demonstrated substantial amounts of heritable variation in life-history traits (Istock, 1982; van Noordwijk *et al*, 1980; Heinrich & Travis, 1988)). This apparent contradiction can be accounted for in three ways: The first possible reason for the maintenance of genetic variation is that random changes in environmental conditions may occur at a sufficient rate to cause directional reversals in selection pressures through gene-environment interactions before many alleles are eliminated. Equilibrium gene frequencies are thus never achieved (Via & Lande, 1985; Lande, 1988; Stearns, 1989). *The C. maculatus* population used in this study was kept in an environment held constant for major features such as temperature and day-length, but factors such as levels of larval competition and bean quality were variable to an undetermined extent in stock cultures. Secondly, the net selective pressure on a trait may be close to zero under strong directional selection due to the cancelling out of a number of genetic correlations that oppose each other (Pease & Bull, 1988; Charlesworth, 1990). The third explanation concerns mutation-selection balance. Recent studies have

shown that mutation rates can be high enough to significantly contribute to genetic variation in traits under constant selection pressure (Lande, 1975, 1980; Crow & Simmons, 1983; Turelli, 1984; Lynch, 1985; Charlesworth, 1987). Such a large number of loci may affect a single trait that the mutation of one gene may occur frequently (Simmons *et al*, 1980). Mutation rates for characters in *C. maculatus* are not known.

4.1.1 Value of measuring heritability

In this chapter presence of additive genetic variation in egg length and fecundity is measured by the heritability estimated from a breeding experiment (Expt. 4B). Heritability expresses the additive genetic variation (or breeding value) in a trait as a proportion of the total phenotypic variation, and it represents the extent to which offspring phenotypes are determined by genes transmitted from their parents (Falconer, 1989). Heritability is determined on the assumption that similarities between related individuals are due to shared genes alone and not caused by shared environmental factors. However, in practice measures from related individuals often include certain environmental and non-additive genetic sources of variation that may bias the estimate of heritable variation upwards. Non-additive genetic variation consists of dominance and epistatic interactions between genes, and environmental variation includes maternal effects. Maternal effects in particular may be important. These are a component of shared environmental variance attributable to the phenotype of the mother of related progeny. For example, maternal effects may occur in *C. maculatus* in the form of mothers passing on a legacy of their phenotype to their offspring in terms of body size. Full-sibs may all share large body size because their mother was large and had a high total of resources which were utilised by laying large eggs. In other words, the presence of genes determining large body size does not need to be

invoked to explain the similarity in body size between offspring and parent. The magnitude of these sources of bias was estimated by further partitioning the genetic and environmental components of variance (section 4.2.3).

As a prelude to determination of heritabilities, repeated measures of egg length from different eggs within and between days of oviposition were obtained to estimate repeatability which is a measure of the consistency of the size of eggs laid by individual females (Expt 4A). Repeatability sets an upper limit to the value of heritability (Falconer, 1989). This was useful as the determination of heritability requires many more individuals to be measured than is necessary for repeatability and the latter measure was used in determining an efficient design of the heritability experiment. The repeatability was also used in a more abstract sense to quantify egg size measurement accuracy using repeated measures of the same eggs (section 2.1.3). Similarly, the heritability estimates were in turn used as estimates for the design of the selection experiment in Chapter 6.

4.1.2 Choice of relatives to estimate heritability

Several combinations of relatives can be used in breeding designs. Trait values in offspring can be measured against those of one or both their parents, or the covariation of full-sib or half-sib family groups can be analysed. The life-cycle of some organisms may exclude some of these options but all are feasible in *C. maculatus*. Causal components of variance are derived from the measurements of related individuals on the assumption that relatives share genes but do not share environments. The method chosen was the paternal half-sib design in which male parents (sires) were each mated to several females (dams) and several progeny from each dam were measured. This design furnished estimates of heritability and was also used to estimate the genetic correlation between egg length änd fecundity in Chapter 5. The most precise estimate comes from the

groups of half-sibs as this is entirely free of bias. The full-sib estimate can give greater precision (Falconer, 1989, p.183) but is more susceptible to bias, especially maternal effects as discussed above. Because fecundity is a sex - limited trait, the only offspring - parent design possible was the regression of daughters values on their mothers. This estimate is likewise particularly susceptible to bias from maternal effects.

4.1.3 Aims

1. To measure the consistency of the size of eggs laid by females over one day and their total oviposition period using the repeatability statistic.

2. To determine if egg size (measured as length on the first and third days of laying) and fecundity possess significant additive genetic variation

3. To assess the potential sources of bias in measured values of heritability by comparison of full and half-sib estimates and partitioning of the causal components of variance.

4.2 Quantitative genetics

Notation follows Falconer (1989) except where otherwise stated.

4.2.1 Definitions

Repeatability

Repeatability (r) is a statistic that can be calculated whenever repeated measures of the same character are taken from each individual in a sample population.

Repeatability represents the proportion of phenotypic variation in a character that occurs between individuals as opposed to that variation which is unique to each individual. The between - individual component comprises all the genetic variation (V_G) in a character plus general environmental variance that is shared by individuals (V_{Eg}).

$$r = (V_G + V_{Eg}) / V_P \qquad [4.1]$$

where V_P is the total phenotypic variance. Calculation of r has two main uses. One is to determine the phenotypic consistency of a temporally of spatially repeated trait within individuals. In this study for example, it was used to measure the consistency of egg sizes laid by females over their oviposition period. The phenotypic variance based on n measurements from the same individual $V_{P(n)}$ can be expressed as

$$V_{P(n)} = V_G + V_{Eg} + (1/n) V_{Es} \qquad [4.2]$$

where V_{Es} is the special environmental variance unique to each individual. This is that part of the phenotypic variance partitioned by the repeatability

$$V_{Es}/V_P = 1 - r \qquad [4.3]$$

A large value of repeatability represents high consistency of repeated trait measures. In a more abstract sense, this statistic can be used to assess the magnitude of measurement error of a particular measurement method. Repeated measures of exactly the same trait are taken to determine the gain in precision obtained from making more than one measure. The higher the repeatability, the smaller is V_{Es} (measurement error) and hence the smaller is the gain in precision of making repeated measures. Repeatability was used in this way in section 2.1.4. The second value of repeatability is that it provides an estimate of the upper limit for the heritability of the trait as explained below.

Heritability

The heritability statistic (h^2) describes the relative importance of heredity in determining the phenotypic value of a trait. It has more than one definition (Jacquard, 1983) but is used here to mean the proportion of additive genetic variance (V_A) in the total phenotypic variance (V_P) of a trait.

$$h^2 = V_A / V_P \qquad [4.4]$$

This is also known as the narrow-sense heritability. The causal components of variance are partitioned as $V_A : V_{NA} + V_{Ec} + V_{Ew}$, where V_{NA} is the non-additive genetic variance, V_{Ec} is the environmental variance common between related individuals and V_{Ew} is the environmental variance that is independent of whether individuals are related or not. This partitioning is in contrast to repeatability which partitions the phenotypic variance as $V_A + V_{NA} + V_{Eg} : V_{Es}$ where V_{Eg} and V_{Es} (defined above) are closely analogous to V_{Ec} and V_{Ew} respectively (Falconer, 1989, pp. 140 & 158). The heritability will be the same as the repeatability if V_{NA} and V_{Ec} are zero; otherwise it will be smaller.

4.2.2 Design

Heritabilities can be estimated from breeding experiments, as described in this chapter (Expt. 4B), or by means of selection experiments (Ch. 6).

4.2.2.1 Family size

Sampling variances of heritabilities are characteristically large. Reasonable precision in breeding experiments can only be obtained by employing large numbers of individuals, a problem that is exacerbated when measuring sex-limited characters. Counting and measuring eggs and recording additional life-

history characters is time consuming so experimental design needs to be efficient in terms of precision achieved for a given practical effort.

In Experiment 4B half and full sib families are used to estimate heritabilities. Because heritability and genetic correlation are calculated in analogous fashions, the optimal design for heritability is the same as that for genetic correlation estimated from the same experiment. Formulae given by Robertson (1959a) and Hill & Nicholas (1974) for sib designs were used to estimate an optimal dam family size that minimised the standard error of heritability. The formulae are expressed in terms of t, the intraclass correlation coefficient which is the sib covariance as a proportion of the total phenotypic variance (equations 4.13 & 4.15). Robertson's formulae give a solution only for a single value of heritability and total sample size so estimates of these two parameters were needed. The maximum number of individuals that could be practically managed in the experiment was estimated to be 700. This value was based on projected processing times for all daily repeated procedures during the peak period of emergence of the offspring generation. The only available estimate of heritability prior to the start of this experiment was the repeatability of 0.3 for egg length from Expt. 4A. Predicted standard errors were examined for different dam family sizes with h^2 set at 0.1, 0.2 and 0.4 (Fig. 4.1). The chosen number of dams mated to each sire was three, based on the relative gain in precision of including additional dams (Robertson, 1959a; Klein *et al*, 1973). Fig. 4.1 shows that it was safest to overestimate dam family size because the standard error of heritability increases rapidly when fewer than four progeny per dam are measured. Allowing for anticipated egg and larval mortality which could typically reduce each full-sib family by one or two, the family structure chosen was eight progeny per dam, with three dams mated to each of 30 sires, giving a total of 720 progeny.

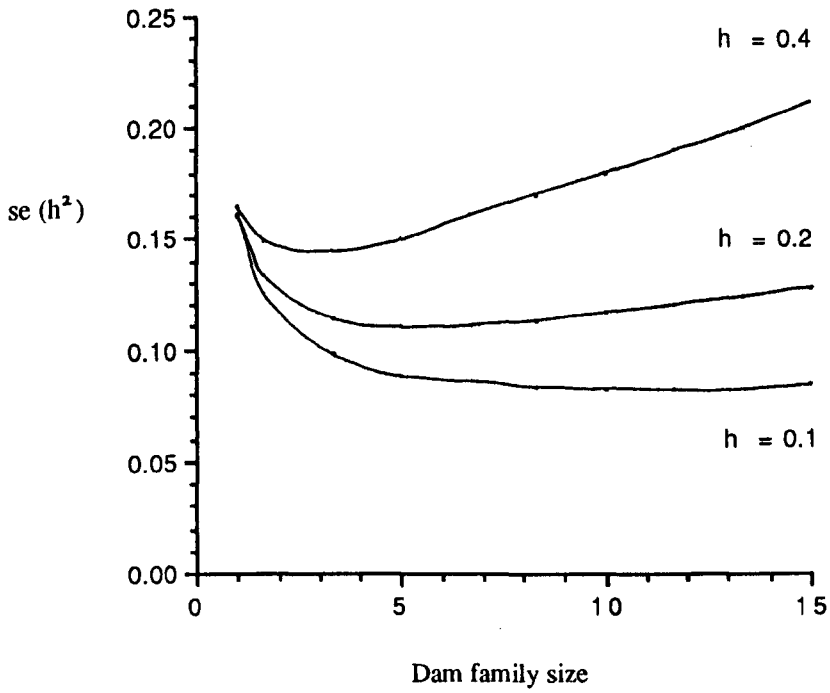


Fig. 4.1 Predicted sampling standard error of half sib heritability estimates with different dam family sizes. Graphs are shown for heritability values of 0.1, 0.2 and 0.4 with measurements from 720 progeny.

4.2.2.2 Standardisation of biological environmental parameters

Apart from the question of statistical precision, the other main issue of the experimental design was the precision with which environmental parameters were described and controlled. In particular, the position of measured eggs in the oviposition sequence, larval competition and number of matings were open to management in the experimental protocol.

The heritability life-history traits may change with maternal age. Egg length may be determined by changing proportions of genetic and environmental variation during a female's oviposition period which normally spans almost all of her adult life. The genes contributing to this trait may actually change over time. Egg lengths were sampled from the first and third days of oviposition period so that the heritabilities could be compared. Although the third day of laying is less than halfway through an average female lifespan, more than three quarters of her total egg complement have been laid by then.

Larval competition and the number of matings each female obtains were both major sources of environmental variation that could have entirely obscured the chances of measuring significant genetic variation. Larval competition has an indirect (Smith & Lessells, 1986) and number of matings a direct (Wasserman & Assami, 1985; P. Eady & T. Tufton, unpubl. res.) effect on fecundity in *C. maculatus*; the same may be true for egg size. Variation in both could be readily eliminated from the design and this was done by reducing the number of larvae per bean and the number of matings to one. The cost of this action was to create differences between the general culturing environment and that experienced by the beetle population during the experiment which could mean a change in selection pressures and thus in gene frequencies. The short duration of the

experiment (three generations) meant that any effects of this change were not likely to be large.

4.2.3 Analysis

Repeatability

Statistically, repeatability is the intraclass correlation coefficient (Sokal & Rohlf, 1981) which is calculated from the between groups mean square (MS_B) and within groups mean square (MS_W) of an ANOVA of the repeated measures. Each group represents repeated measures from a single individual. Following Lessells & Boag (1987) repeatability is given by

$$r = s_B / (s_W + s_B) \qquad [4.5]$$

where s_B is the between group variance and sW is the within group variance. The variance components are calculated from the mean squares:

$$s_B = (MS_B - MS_W)/ k \qquad [4.6]$$

$$s_W = MS_W \qquad [4.7]$$

where k is a coefficient of sample size. In a balanced design (same number of individuals measured in each group) k is equal to the number of measures from each individual (m) but in an unbalanced design k is less than the mean group size (m). The value of k is calculated as

$$k = 1/(n-1) \sum m - (\sum m^2 / \sum m) \qquad [4.8]$$

where n is the number of individuals.

The standard error of r is the square root of the sampling variance of the intraclass correlation. Becker (1984) gives an approximation for balanced designs

$$s.e.(r) = \sqrt{\frac{2(1 - r)^2[1 + (k - 1)r]^2}{k(k - 1)(n - 1)}} \qquad [4.9]$$

and a separate approximate formula expressly for unbalanced designs.

Heritability

The hierarchical data produced from the sib design was analysed by nested ANOVA to obtain estimates of heritability. Pre-adult mortality meant that family sizes were not all the same and the final design was unbalanced. The ANOVA divided the phenotypic variance into observational components attributable to differences between the progeny of different sires (o_S); between the progeny of different dams mated to the same sire (o_D), and between offspring of single dams (o_W) (Falconer, 1989, p.169). The relationship between the observational components of variance and the mean squares of an unbalanced ANOVA (Searle, 1971) is given in Table 4.1(a). The observational variance components were calculated as

$$\sigma^2_W = MS_W \qquad [4.10]$$

$$\sigma^2_D = (MS_D - MS_W) / k_1 \qquad [4.11]$$

$$\sigma^2_S = (MS_S - MS_W + k_2\sigma^2_D) / k_3 \qquad [4.12]$$

where k_1, k_2 and k_3 are family size coefficients.

The sire and dam observational components contain a proportion of the additive genetic variance and different amounts of non-additive genetic and environmental variance (Table 4.1(b)). The additive genetic variance can be estimated as four times o_S or o_D but it can be seen from Table 4.1(b) that the dam estimate is most subject to upward bias, as it contains four times V_{Ec} which includes maternal effects.

Sire or half-sib estimate

$$h^2_{S/HS} = 4\sigma^2_S / \sigma^2_T = 4t_{HS} \qquad [4.13]$$

Dam estimate

$$h^2_D = 4\sigma^2_D / \sigma^2_T \qquad [4.14]$$

Table 4.1 Statistical and genetic models for estimation of heritability in an unbalanced paternal half sib breeding experiment; (a) nested analysis of variance and (b) genetic and environmental breakdown of observational components of variance. (Searle, 1971; Becker, 1984).

(a)

Source of Variation	d.f.	Mean Square	Expected Mean Square
Between sires	S - 1	MS_S	$\sigma_W^2 + k_2\sigma_D^2 + k_3\sigma_S^2$
Between dams within sires	D - S	MS_D	$\sigma_W^2 + k_1\sigma_D^2$
Between progeny within dams	N - D	MS_W	σ_W^2

(b)

Observational Component	Covariances	Causal Components V_A	V_D	V_I	V_{Ec}	V_{Ew}
Sires σ_S^2	cov_{HS}	1/4	0	5/256	0	0
Dams σ_D^2	$cov_{FS} - cov_{HS}$	1/4	1/4	31/256	1	0
Progeny σ_W^2	$\sigma_T^2 - cov_{FS}$	1/2	3/4	220/256	0	1

Sample sizes

S number of sires
D total number of dams
N total number of progeny

Coefficients of family size

k_1, k_2 no. progeny per dam
k_3 no. progeny per sire

Causal variance components

GENETIC
V_A additive genetic
V_D dominance genetic
V_I epistatic genetic

ENVIRONMENTAL
V_{Ec} common environmental
V_{Ew} within individual

If the sire and dam estimates are similar the more precise full-sib estimate can be calculated without risk of substantial bias

$$h^2_{FS} = 2(\sigma^2_S + \sigma^2_D) / \sigma^2_T \qquad = 2t_{FS} \qquad [4.15]$$

where σ^2_T = total phenotypic variance = $\sigma^2_S + \sigma^2_D + \sigma^2_W$

and t = sib intraclass correlation coefficient

Standard errors of heritability estimates from unbalanced ANOVA have not been determined but were estimated by calculating the variances of the observational variance components and substituting these into the equations for balanced designs given by Becker (1984, p.59). Calculation of the variance of the variance components is complex and is explained in full by Searle (1961, 1971).

Partitioning of variance components

A further breakdown of the genetic and environmental constituents of the measured traits was achieved by using the observational components of variance to estimate all of the causal components (Table 4.1(b); Falconer, 1989, p.170). One major assumption involved was that non-additive variation, particularly due to dominance deviations, is zero. Variance partitioning allowed this to be checked retrospectively. The description of V_A by variance partitioning can provide more information than the heritability statistic alone as the latter is simply a proportional measure and does not give any idea of absolute quantities of variance. A change in heritability can be brought about by a change in the magnitude of either the genetic or environmental variance.

4.3 Methods

4.3.1 Experiment 4A Repeatability of egg length

Twenty five virgin females and 25 virgin males less than 24h old were weighed and then paired and observed until they mated. The males were removed after mating and placed alone in $20cm^3$ pots and kept until they died. The females were each placed in a $20cm^3$ pot containing 30 beans. These were replaced with 20 fresh beans every 24h until all females had died. Daily fecundities were recorded and eggs were kept for later egg size measurement. The dry weights of dead females and males and their elytral lengths were measured. Eight days after eggs were laid five were randomly chosen from each pot and measured to obtain a mean daily egg size for each female.

4.3.2 Experiment 4B Heritability of egg length and fecundity

Larval competition was eliminated in three successive generations by rearing beetles singly in beans. In the first (grandparental) generation, many females were left to lay collectively on approximately 1600 beans for one day after which all bean egg loads were reduced to one. The breeding structure was established in the next (parental) generation. Thirty virgin adults of each sex less than 24h old were weighed and then paired and mated. On the two following days the same males were mated again to newly emerged females. All matings were observed and males were removed immediately after successful copulation. After mating, all females were placed in $20cm^3$ pots with 25 beans. Ten fresh beans were provided on the second, third and fourth days of egg laying. All beetles were kept until death, and their dry weight and elytra then measured.

Fecundities were scored before larvae hatched from eggs and a maximum of 75 beans from each female with egg loads reduced to one were placed in cell trays. Four eggs from each of the first and third days of laying were randomly selected and measured. In the final (offspring) generation all emerged adults were mated within 24h of emergence and sib matings were avoided. Mated female offspring were provided with beans as in the parental generation. Mean egg size on the first and third days of laying and fecundity were recorded as before.

4.4 Results

4.4.1 Experiment 4A Repeatability of egg length

Length and width of eggs were repeatable within days up to a maternal age of four days (Table 4.2). The repeatability values were significant but small. With falling sample size due to mortality from day 5 onwards the calculated repeatabilities became unreliable and some negative repeatabilities were generated although a real value of $r < 0$ is not possible in general. Mean daily egg length was also repeatable between days whereas breadth was not (Table 4.2). Egg breadth was not repeatable over the first five days of oviposition because it showed a marked decline with maternal age. This trend was removed in an analysis of covariance of width by female, with maternal age as the covariate, as in Table 2.5. The female MS in the ANCOVA replaced the same term from the one-way ANOVA normally used to calculate repeatability, and provided an adjusted lifetime value (Table 4.2).

Table 4.2 Daily and lifetime repeatabilities of egg size. Five eggs were measured from each female each day.

Maternal Age (days)	Repeatability (s.e.) Length		Width		N
1	0.35**	(0.11)	0.30*	(0.11)	22
2	0.24*	(0.10)	0.16	(0.10)	22
3	0.36**	(0.11)	0.39**	(0.11)	22
4	0.37**	(0.11)	0.37**	(0.11)	20
5	0.19	(0.15)	-0.03	(0.12)	15
6	0.43	(0.34)	0.74*	(0.21)	4
7	0.13	(0.37)	0.24	(0.43)	4
8	-2.13	(0.61)	-2.13	(0.61)	4
Lifetime	0.30*	(0.11)	0.02	(0.07)	22
Lifetime (adj. for maternal age)	0.29*	(0.13)	0.35**	(0.11)	22

4.4.2 Experiment 4B Heritability of egg length and fecundity

Despite the measurement of over 700 individuals, precision of heritability estimates was poor. The distributions of standard errors obtained from an unbalanced least squares analysis have not been defined and normality cannot be assumed. Even for balanced data statistical significance cannot be inferred from examination of the standard error alone. This is illustrated by a worked example in Becker (1984, p.63) for a heritability estimate of 0.42 with a standard error of 0.58 whose confidence interval does not overlap zero. Becker's approach was applied to results in this experiment using unbalanced approximations but the confidence intervals produced were greater than unity and are not described. Instead significance of heritabilities was indicated simply by using the standard error in a t-test to see if the value was different from zero.

The sire heritability estimates of egg length and breadth are significant for eggs laid on the first day of oviposition but only breadth was heritable on the third day of oviposition (Table 4.3). Heritabilities of fecundity, longevity, dry body weight and elytral length were much smaller and not significant, and the estimate for development rate was zero. The standard error of the heritability of development rate is 0.2 so that a real heritability of small magnitude cannot be excluded. However, the zero estimate means that genetic correlations involving this trait could not be calculated. In general, the dam heritability estimates were substantially larger than those based on sire means for most traits implying that common environmental (including maternal effects) and or dominance variance were present (Table 4.3). This was not the case for fecundity and longevity where sire and dam estimates were very similar and consequently combined sire + dam estimates were calculated which were substantially more precise (Table 4.3) and showed both of these traits to contain significant heritable variation.

Table 4.3 Sire and dam heritability estimates from sib analysis of 720 female progeny. Standard errors are shown in brackets. Significance levels refer to t-tests where the null hypothesis is that $h^2 = 0$.

Character	Heritability		
	Sire	Dam	Sire + Dam
Development rate	0.00 (0.22)	1.84*** (0.20)	
Fecundity	0.12 (0.10)	0.14 (0.12)	0.13* (0.06)
Longevity	0.25+ (0.13)	0.31* (0.13)	0.28*** (0.07)
Dry weight	0.20 (0.15)	0.60** (0.18)	
Elytral length	0.17 (0.16)	0.55** (0.18)	
Egg length 1	0.50* (0.22)	0.83*** (0.19)	
Egg width 1	0.59* (0.25)	0.92*** (0.19)	
Egg length 3	0.30 (0.21)	0.87*** (0.20)	
Egg width 3	0.69** (0.25)	0.70*** (0.18)	

\+ $P < 0.1$
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

1 - mean egg length on first day of oviposition
3 - mean egg length on third day of oviposition

4.4.3 Experiment 4B Causal components of variance

The causal components of egg length on the first and third days of laying can be compared directly as the units of measurement are the same (Table 4.4(a)). None of the components differed significantly between the two temporally separate traits but the standard errors were large (mean differences: V_A, t = 1.32, NS; V_{Ec}, t = -0.48, NS; V_{Ew}, t = -0.66, NS). The lower heritability estimate for egg length on the third day of laying was due to smaller V_A and greater V_{Ec} and V_{Ew} but there was no statistical evidence to suggest that genetic control of egg size changes during the first three days of laying.

Causal variance component breakdowns for fecundity and longevity are shown in Table 4.4(b). The most striking feature of both traits is that they contain minimal amounts of common environmental variance. Strictly, the estimate of V_{Ec} also contains one quarter of the dominance variance and approximately one tenth of the variance due to epistasis. Consequently if V_{Ec} is small then the assumption that V_D and V_I are negligible are valid and the causal variance component estimates are unbiased. The small amount of bias in the heritability estimates for fecundity and longevity was corroborated by similarity of the sire and dam estimates (Table 4.3). Proportions of V_{Ec} are relatively larger for egg length (first day of oviposition 8%, third day of oviposition 14%), residual dry body weight (9%) and elytral length (6%). Correspondingly, the sire and dam heritability estimates of these traits are dissimilar. This indicates that either V_{Ec} or V_{NA} are very large. For example, egg length on the first day of oviposition contains 8% V_{Ec} but this is linked to a very substantial difference of 0.33 in the sire and dam heritability estimates.

Table 4.4 Observational and causal variance componennt breakdown of (a) egg length on first and third days of oviposition, and (b) fecundity and longevity. Each breakdown is based on the measurements from 720 female progeny in a sib analysis.

(a)

Observational Component	Egg length 1 Estimate	s.e.	Egg length 3 Estimate	s.e..
σS_2^2	65.0	32.0	37.5	26.7
σD_2^2	108.1	28.4	108.9	30.6
σW_2^2	351.0	20.4	356.0	22.4
σT^2	524.0			502.4
CAUSAL COMPONENT	Egg length ($h^2 = 0.50$)		Egg length 3 ($h^2 = 0.30$)	
$V_A = 4\sigma S^2$	260.0	(50%)	150.1	(30%)
$V_{Ec} = \sigma D_2^2 - \sigma S_2^2$	43.1	(8%)	71.4	(14%)
$V_{Ew} = \sigma P^2 - 2\sigma S^2$	221.0	(42%)	280.1	(56%)
$V_P = \sigma T^2$	524.0	502.4		

(b)

Observational Component	Fecundity Estimate	s.e.	Longevity Estimate	s.e.
σS_2^2	10.2	8.5	0.25	0.14
σD_2^2	12.4	10.9	0.31	0.14
σW_2^2	328.3	19.5	3.50	0.20
σT^2	350.9		4.06	
CAUSAL COMPONENT	Fecundity ($h^2 = 0.12$)		Longevity ($h^2 = 0.25$)	
$V_A = 4\sigma S^2$	40.8	(12%)	1.00	(25%)
$V_{Ec} = \sigma D_2^2 - \sigma S_2^2$	2.2	(1%)	0.06	(1%)
$V_{Ew} = \sigma P^2 - 2\sigma S^2$	307.9	(87%)	3.00	(74%)
$V_P = \sigma T^2$	350.9		4.06	

4.5 Discussion

The length of eggs laid by females was consistent within and between the first four days of oviposition. Egg breadth was repeatable within days but not over longer oviposition intervals. Expt. 3G showed a decline in both egg length and breadth with maternal age and although this could be taken into account in the repeatability analysis it appeared preferable to measure eggs from only a single day of oviposition. Such standardisation in subsequent experiments minimised the environmental variation attributable to maternal ageing.

Heritabilities were significant for both egg length on the first day of oviposition and fecundity. The heritability of fecundity was small in line with Fisher's (1930) prediction for traits closely allied to fitness. The relatively large heritability of egg length implies it is not as closely related to fitness or that it is subject to mechanisms maintaining genetic variation. There was no evidence that the heritability of egg length on the third day of oviposition was different from than that from the first day of oviposition. These two measures are probably determined by the same genes and represent the same genetic trait suggesting that genetic control of egg size does not change with maternal age. Similarly, heritability of egg breadth on both the first and third days of oviposition were close to the heritability value of egg length on the first day of oviposition, suggesting that both egg size dimensions share common genetic determination. As only egg length on the first day of oviposition was statistically significantly different from zero it alone was used in determination of genetic correlations. Heritability estimates of egg length on the first day of oviposition were even greater than predicted from the previously estimated repeatability. By definition, heritability cannot be larger than repeatability but this discrepancy can be accounted for by either the large sampling variance or reduced environmental variance in the heritability experiment. Numerous measures of

heritability of egg size in birds are present in the literature. These almost unanimously demonstrate high heritability values (see Boag & van Noorwijk (1987) for a review) but an exception to this general rule is the northern pintail *Anas acuta* (Duncan, 1987).

Longevity was significantly heritable but development rate, residual dry body weight and elytral length were not heritable although small heritabilities cannot be ruled out due to the large standard errors of the estimates. In the only other quantitative genetic study conducted on *C. maculatus* Moller *et al* (1989) demonstrated significant heritabilities for emergence weight and longevity. Small heritabilities were also measured in fecundity and development rate but, as in this experiment, large standard errors meant that these were not significant. If the low precision is put aside, the half sib heritability estimates of fecundity and longevity in this experiment (Fecundity, $h^2 = 0.12 \pm 0.10$; Longevity, $h^2 = 0.25 \pm 0.13$) and that of Moller *et al* (Fecundity, $h^2 = 0.10 \pm 0.11$; Longevity, $h^2 = 0.30 \pm 0.13$) are strikingly similar. This may indicate that the amount of genetic variation in these two traits is maintained at similar levels in the two populations but because heritability is a proportional measure the apparent consensus of the estimates may be false.

The heritability results indicated that a genetic correlation between egg length and fecundity could be evaluated in principle although the presence of large amounts of environmental variance could make the genetic relationship difficult to distinguish. The low precision of the breeding experiment estimates of heritability implied that large standard errors were also likely for genetic correlations (Ch. 6).

CHAPTER 5

GENETIC CORRELATIONS:

BREEDING EXPERIMENT

5. Genetic correlation: Breeding experiments

5.1 Introduction

If there is a trade-off between egg size and fecundity, any genetic variation involved in the allocation of resources between these two traits will be expected to increase the value of one trait and decrease the value of the other. Genes that increase or decrease the value of both traits will have been fixed or eliminated by selection. Any trade-off present is therefore expected to be determined by antagonistic pleiotropic genes which means that egg size and fecundity will have a negative genetic correlation. Therefore, measurement of the genetic correlation can be used as evidence for the existence of a trade-off between egg size and fecundity (Rose, 1983; Reznick, 1985; Lessells, 1991).

Two quantitative genetic methods are available to measure genetic correlations. In this chapter a breeding experiment is described in which family values of egg size and fecundity are compared, based on the assumption that within family similarities are attributable to shared genes. A second method is to artificially select for one trait over several generations. The genetic correlation can be calculated from the direct response in the selected trait and the correlated response in another. This method is described in Chapter 6. The breeding experiment was able to provide estimates of additional genetic correlations involving longevity, body weight and body size. These were useful in assessing if other genetic correlations influenced the value of the correlation between egg size and fecundity. Breeding experiments uniquely provide estimates of the environmental correlations between traits. These represent the correlation of environmental and non-additive genetic deviations and provide an insight into how the interaction of genetic and environmental covariation produces the observed phenotypic correlation.

5.1.1 Aims

1. to determine if there is a negative genetic correlation between egg length and fecundity.

2. to estimate additional genetic correlations of egg size and fecundity with longevity, residual dry body weight and elytral length.

3. to compare and contrast genetic, environmental and phenotypic correlations between egg length and fecundity

5.2 Quantitative genetics

5.2.1 Definition of genetic correlation

The genetic correlation (r_A) is simply the correlation between the additive genetic deviations of two traits X and Y

$$r_A = cov_{XY} / \sqrt{\sigma_X^2 \sigma_Y^2} \qquad [5.1]$$

The degree of correlation represents the net effect of all genes affecting both traits. It can also be thought of as expressing the extent to which two measurements represent what is genetically the same character (Falconer, 1989). The genetic correlation cannot be fully compared with the phenotypic correlation without considering the environmental correlation (r_E). The environmental correlation is strictly the correlation of the environmental and non-additive genetic deviations of two traits, i.e. those causal components partitioned from V_A in the heritability expression. The phenotypic correlation (r_P) can be expressed in terms of the sum of the component correlations

weighted by the magnitude of heritable and non-heritable effects

$$r_P = h_X h_Y r_A + e_X e_Y r_E \qquad [5.2]$$

where $e^2 = 1 - h^2$. It can be seen from this equation that when the heritability of both traits is high, the genetic correlation will resemble the phenotypic correlation (Cheverud, 1988). If this not the case r_A can vary in magnitude or even sign from r_P.

Interpretation of heritabilities and genetic correlations necessitates great caution. These statistics describe characters only in the specific population and generation in which they were measured (Clark, 1987; Falconer, 1989). Any change in gene frequencies or environmental conditions will change h^2 and r_A. Conclusions concerning past or future evolutionary development, even in a controlled laboratory environment, are only likely to be valid over the short term.

5.2.2 Design

The discussion of choice of family size, position of measured eggs in the oviposition sequence, number of matings and larval competition in the experimental design in Chapter 4 (section 4.2.2) is equally relevant here. Calculation of heritability and genetic correlation were considered jointly in determining the nature of these design parameters.

5.2.3 Analysis

Genetic correlations were estimated in an analogous manner to heritabilities using a nested ANCOVA (Grossman & Gall 1968; Table 5.1(a)). Observational

Table 5.1 Statistical and genetic models for estimation of genetic correlation in an unbalanced paternal half sib breeding experiment; (a) nested analysis of covariance and (b) genetic and environmental breakdown of observational components of covariance. (Grossman & Gall, 1968; Becker, 1984).

(a)

Source of Variation	d.f.	Mean Cross Products	Expected Mean Cross Products
Sires	S - 1	MCP_S	$cov_W + k_2cov_D + k_3cov_S$
Dams	D - S	MCP_D	$cov_W + k_1cov_D$
Progeny	N - D	MCP_W	cov_W

(b)

Observational Component	Causal Components cov_A	cov_D	cov_I	cov_{Ec}	cov_{Ew}
Sires cov_S	1/4	0	5/256	0	0
Dams cov_D	1/4	1/4	31/256	0	0
Progeny cov_W	1/2	3/4	220/256	1	1

Sample sizes

S number of sires
D total number of dams
N total number of progeny

Coefficients of family size

k_1, k_2] no. progeny per dam
k_3 no. progeny per sire

Causal variance components

GENETIC
V_A additive genetic
V_D dominance genetic
V_I epistatic genetic

ENVIRONMENTAL
V_{Ec} common environmental
V_{Ew} within individual

covariance components between sires, dams and progeny were calculated from the mean cross products

$$cov_W = MCP_W \quad [5.3]$$

$$cov_D = (MCP_D - MCP_W) / k_1 \quad [5.4]$$

$$cov_S = (MCP_S - (MCP_W + k_2 cov_D)) / k_3 \quad [5.5]$$

where k_1, k_2 and k_3 are family size coefficients.

The causal interpretation of the covariances is given in Table 5.1(b). As with heritability, both the sire and dam covariances can be used to estimate the genetic correlation between two traits X and Y but the dam estimate can be biased upwards or downwards by dominance or epistasis

$$\text{Sires: } r_A = 4cov_S / 4o_X 4o_Y \quad [5.6]$$

$$\text{Dams: } r_A = 4cov_D / 4o_X 4o_Y \quad [5.7]$$

Estimates of environmental and phenotypic correlations were calculated similarly using the progeny observational covariance components (Becker, 1984). Calculation of the standard error of genetic and environmental correlations is elaborate and is detailed by Hammond & Nicholas (1972), Grossman & Norton (1974) and Becker (1984, p.123).

5.3 Methods

Measurements of egg length, fecundity and other traits were recorded from female progeny within a paternal half sib family structure (Appendix B). Eight female offspring were measured from each of 90 females belonging to 30 sire families each containing three females. The genetic correlations were calculated from the same data as the heritabilities for which the design (4.2.2) and the methods (4.3.2) are described in detail previously.

5.4 Results

All traits except longevity showed no significant trends between the first, second and third dams mated to each sire. The design of the experiment meant that to ensure matings occurred within the first day after emergence, the dams differed in development period. The dams mated to sires first, completed development on average one day earlier than those mated second which in turn had a development time one day less than those dams mated last. Successively mated dams differed in longevity (ANOVA: $F_{(2,703)} = 4.51$, $P < 0.05$) with dams mated last living longest. The significances is caused by the dams mated last which lived on average 0.5d longer than other dams (paired $t_{(459)} = 2.49$, $P < 0.05$).

Sire genetic and environmental correlation estimates between egg length and fecundity differed widely from dam or combined sire + dam estimates (Table 5.2 (a), (b) & (c)). Sire + dam estimates contain half the covariance due to common environment, dominance and epistasis contained in dam estimates (200%, 50% and 28% of the respective covariance totals). The sire estimates contain no common environment or dominance covariance, and 20% of epistatic covariance (Table 5.1(b))and hence the large difference in estimated correlations implies that at least one of these source of bias was substantial in the dam estimate between egg length and fecundity. The large discrepancy between the sire and dam correlations means that only the former can be considered to be reasonably accurate estimates despite their substantially lower precision. Therefore considering the sire correlations only, egg length was negatively genetically correlated with fecundity (Table 5.2(a)) but was not significantly different from zero. Environmental (Table 5.2(a)) and phenotypic (Table 5.2(d)) correlations between the same traits were both small and positive. Similar non - significant correlations were obtained comparing daily fecundity

Table 5.2 Genetic, environmental and phenotypic correlation estimates between egg length and fecundity traits from sib analysis of 720 female progeny; (a) sire or half sib correlations, (b) dam or full sib correlations, (c) combined sire plus dam correlations, and (d) phenotypic correlations. Standard errors are given in brackets.

	Fecundity	Daily Fecundity	Egg Breadth	Egg Length 3
(a) SIRES				
GENETIC				
Egg length 1	-0.35 (0.61)	-0.37 (0.49)	0.25 (0.37)	0.76 (0.21)
ENVIRONMENTAL				
Egg length 1	0.01 (0.06)	-0.02 (0.06)	0.73 (0.08)	0.44 (0.06)
(b) DAMS				
GENETIC				
Egg length 1	-0.68 (0.21)	.	0.67 (0.11)	0.79 (0.09)
ENVIRONMENTAL				
Egg length 1	-0.56 (0.16)	.	0.19 (0.29)	-0.07 (0.29)
(c) SIRE + DAM				
GENETIC				
Egg length 1	0.38 (0.17)	.	0.53 (0.11)	0.78 (0.07)
ENVIRONMENTAL				
Egg length 1	-0.21 (0.11)	.	0.57 (0.15)	0.29 (0.14)
(d) PHENOTYPIC				
Egg length 1	0.04 (0.05)	-0.06 (0.05)	0.54 (0.04)	0.60 (0.03)

1 - mean egg length on first day of oviposition
3 - mean egg length on third day of oviposition

and egg length. Correlations between egg length and breadth were all positive which suggested that egg length did not mis-represent egg size through opposite compensatory changes in egg breadth. Egg length on the first and third days of oviposition had a large positive genetic correlation with each other (Table 5.2(a)) suggesting that egg length on these days of oviposition and egg breadth all represented what was genetically the same trait.

Genetic correlations between egg length and longevity, residual dry body weight or elytral length were not significant. If the large standard errors of these estimates are set aside, the interesting pattern is that these genetic correlations are all negative. In contrast, environmental correlations were significant and positive.

Genetic correlations between longevity, fecundity, residual dry body weight and elytral length were not significant, except for the large genetic correlations of longevity with fecundity, and residual dry body weight with elytral length (Table 5.3). The latter result supported the assumption that there was substantial overlap in the genes expressing body size and weight.

Environmental correlations were all positive and significant between egg length, longevity, residual dry body weight and elytral length. Fecundity was only environmentally correlated with residual dry body weight. Phenotypic correlations were all positive and the only non-significant correlations were those of residual dry body weight and egg length with fecundity. The phenotypic correlations calculated from the ANOVA of the breeding design were all very similar to those determined separately by direct measurement in Expt. 3A (Table 3.1). The one exception was the correlation between fecundity and longevity which was negative in Expt. 3A and positive in this experiment.

Table 5.3 Correlation estimates between adult traits measured in a breeding experiment sib analysis of 720 female progeny. Standard errors are shown in brackets.

	Fecundity	Longevity	Residual dry weight	Elytral length
GENETIC				
Longevity	0.76 (0.27)			
Dry weight	0.05 (0.57)	0.53 (0.34)		
Elytral length	0.05 (0.49)	-0.07 (0.40)	0.90 (0.14)	
Egg length 1	-0.35 (0.61)	-0.30 (0.43)	-0.47 (0.74)	-0.10 (0.52)
Egg length 3	-0.21 (0.51)	0.18 (0.37)	0.68 (0.38)	0.68 (0.38)
ENVIRONMENTAL				
Longevity	-0.13 (0.07)			
Dry weight	0.07 (0.03)	0.11 (0.04)		
Elytral length	0.03 (0.03)	0.29 (0.04)	0.68 (0.02)	
Egg length 1	0.01 (0.06)	0.20 (0.08)	0.50 (0.06)	0.44 (0.06)
Egg length 3	0.12 (0.06)	0.03 (0.08)	0.27 (0.06)	0.22 (0.07)
PHENOTYPIC				
Longevity	0.17 (0.05)			
Dry weight	0.07 (0.04)	0.22 (0.04)		
Elytral length	0.26 (0.04)	0.23 (0.04)	0.73 (0.02)	
Egg length 1	0.04 (0.05)	0.10 (0.05)	0.31 (0.05)	0.33 (0.05)
Egg length 3	0.09 (0.05)	0.11 (0.05)	0.34 (0.04)	0.32 (0.05)

1 - mean egg length on first day of oviposition
3 - mean egg length on third day of oviposition

5.5 Discussion

A negative genetic correlation consistent with the existence of a trade-off between egg length and fecundity was suggested by the results but the sire genetic correlation estimates were inconclusive in a strict sense as a result of their large standard errors. Their precision is a function of the number of family groups measured, whereas the much smaller standard errors of environmental and phenotypic correlations are a function of to the total number of progeny measured. The same correlation was obtained if fecundity on the first day of oviposition only was substituted for lifetime fecundity, implying that both measures are correlated. The correlations between traits that are expected to be closely related to fitness were the only ones where genetic and environmental values differed in sign. Longevity and fecundity had a very large positive genetic correlation but the environmental correlation was negative. The reverse was the case between egg length and fecundity and the net phenotypic correlations in both cases were intermediate and small. Dam estimates of genetic correlation were greatly biased and were consequently of little value despite the greater precision of their estimates. It was not possible to differentiate between possible sources of bias but maternal body size effects were probably important in the light of the phenotypic correlations in Chapter 3.

One other quantitative genetic study has been conducted on *C. maculatus*. Møller *et al* (1989) estimated genetic correlations between development rate, fecundity, longevity and body weight at emergence. Although there is no reason to expect a similar genetic patterns in two separate populations, the beetles used in this study and Møller *et al*'s share the same ancestral origins. The heritabilities of fecundity and longevity are very similar in both studies but they differ in that Møller *et al* also found heritable variation in development rate. Another consistent finding is the positive genetic correlation between fecundity

and longevity which Møller *et al* explains in terms of additional positive genetic correlations of these two traits with emergence weight and development rate. In the present study there was no evidence of the positive correlation being a result of other genetic correlations but only a small number of genetic correlations were measured in total from a potentially large covariance matrix of life history traits.

Genetic correlations between residual dry body weight and elytra length; egg length and breadth; and egg length from the first and third days of oviposition supported the general expectation that each of these three pairs of traits have a substantial prportion of plieotropic genes in common.

Are the experimentally determined genetic correlations measures of the real pleiotropy in the gene pool of the study population? One issue in the interpretation of the genetic correlations is the possible distortion of estimates caused by differences between the experimental and normal culturing environments (Service & Rose, 1985). The culturing conditions were changed as little as possible to minimise altering the phenotypes that were most fit, which could have increased additive genetic variation and changed the covariances between traits (Bell & Koufopanou, 1986). Some changes were unavoidable, however, in order to control larval competition and number of matings which otherwise would have reduced the proportion of measurable genetic variation. Additionally, individuals were harvested from the peak periods of emergence in the grandparental and parental generations in order to prevent excessive spread and overlapping of generations amongst the progeny. This meant that the variance of development period was slightly reduced.

Two further assumptions implicit in the validity of the genetic correlation estimates are that there are negligible effects of inbreeding (Rose, 1984) or

linkage disequilibrium (Falconer, 1989, p.133) in the population. Inbreeding means that supposedly unrelated sire families would share more genes than would be expected in a panmictic situation. This would lead to a reduced half-sib variance and thus heritability and genetic correlation estimate. The substantial proportion of additive genetic variation found after some hundreds of generations of culturing in which individuals mated at random ensured that inbreeding was unlikely to be important. Linkage disequilibrium arises from non-random or assortative mating, or by chance (Falconer, 1989, p.133). As mating was randomised in the parental and offspring generations any disequilibrium should have been minimal.

In summary, the genetic correlation between egg length and fecundity estimated from the breeding experiment is negative and is thus consistent with the existence of a trade-off between these traits. However, the low precision of the estimate means that the result is not statistically significant although its magnitude implies that a larger experiment would reveal a significant correlation.

CHAPTER 6

GENETIC CORRELATION:

SELECTION EXPERIMENT

6. Genetic correlation: Selection program

6.1 Introduction

In the breeding experiment of Chapter 5 the genetic correlation between egg length and fecundity was estimated by calculation of the genetic covariance from unmanipulated observations in a single generation. The alternative method for empirically determining the genetic correlation is by means of short-term selection experiments (Reznick, 1985; Falconer, 1989) which by contrast gathers data from several generations. Selection experiments can be thought of as the quantitative genetic analogue of experimental manipulations in which one trait is artificially changed by selection and the response in a second trait, attributable to additive genetic covariation between the two traits, is measured. The genetic correlation is thus estimated from the intergenerational phenotypic response rather than from a relatively instantaneous single generation measure of the degree of genetic similarity between related individuals.

The main objective of carrying out a selection experiment on *C. maculatus* was not solely to measure the genetic correlation between egg length and fecundity. The correlated response in itself provides important information about the trade-off by revealing direct consequences of alteration of values in one of the traits of interest. The results are compared with the breeding experiment findings to examine the congruity of their conclusions and evaluate the merits and problems of both approaches.

If the genetic correlations estimated from the breeding experiment (Ch. 5) are assumed to be real and are only non-significant because of their large standard errors, the following predictions can be made concerning the direct effects of selection on egg length. Selection for increased egg length should lead to a

decrease in fecundity, longevity, dry body weight and elytral length. Similarly, selection for decreased egg length should lead to an increase in the same traits. As development time was not shown to be heritable, this trait was not predicted to change. If the genetic correlations between egg length and these traits are not real no responses to selection should occur.

The genetic correlation between egg size and fecundity has not previously been measured using selection experiments. The correlation has been measured in domestic poultry that were under long-term combined selection for increase fecundity, egg weight and other economically important traits but the actual evaluation was based on family means rather than the response to selection itself (Wyatt, 1954; Jerome *et al*, 1956; Hogsett & Nordskog, 1958; Jaffe, 1966; Emsley *et al*, 1977). Selection experiments have, however, been frequently used to examine other pairs of fitness related traits (Lessells, 1991, Appendices 2.1 & 2.2), especially the relationships between fecundity and longevity and early and late fecundity. In ten studies of early versus late fecundity reviewed by Bell & Koufopanou (1986) nine demonstrated a negative relationship although some of these described only the directions of response and did not quantify the correlation. For example, Rose & Charlesworth (1981b) tabulate means over three generations of selection for both early and late fecundity in *Drosophila melanogaster* but small sample size and absence of replication excluded determination of the genetic correlation. Despite the general efficacy of selection experiments in the literature correlated responses are however very sensitive to gene frequency changes which inevitably occur during selection (Bohren *et al*, 1966). Therefore, although selection experiments may give more consistent evidence for trade-offs between life-history traits than breeding experiments (Bell & Koufopanou, 1986), their results have less value in explaining the trade-off situation in the original unmanipulated population

However, if the aim of the experiment is to measure the genetic correlation in order to predict the short term response to selection, then selection experiments must clearly have the greatest predictive value.

A major practical problem concerned the actual methodology of selecting for egg length or fecundity. Selection on the basis of fecundity was impractical in terms of demands on labour and time and was not carried out for the following reasons: (i) each fecundity value involves counting approximately 100 eggs on at least 25 beans laid over several days; (ii) by the time fecundity measurement of all females could be completed many eggs could have hatched so that selected eggs could have larval competition eliminated only by reducing egg loads on each bean to one before selection; (iii) only a small number of offspring can be chosen from each female if inbreeding is to be minimised so the ratio of measurement effort to selected eggs is large; (iv) the estimated heritability of fecundity from the breeding experiment is small so that only a low selection intensity could be achieved.

In contrast, selection for egg length was much less expensive in terms of practical effort per individual measured. The relatively large heritability of egg length meant that any direct response was expected to be large and easy to measure. Only eggs laid by females on the first day of oviposition were measured for selection purposes as the results of the breeding experiment suggest that the genes controlling egg length in early and late laid eggs are mostly the same. Measurement of fecundity was confined to determination of correlated response for which only assays taken in base and final generation were required. With little additional effort, extra data were collected during the course of the selection program to examine intrinsic effects of egg size on progeny (Ch. 7).

6.1.1 Aims

1. to measure the response in egg length to selection for egg length.

2. to measure the correlated response in fecundity to selection for egg length and use this information to estimate the genetic correlation.

6.2 Quantitative genetics

6.2.1 Definitions

The descriptions of genetic terms in this section follow Falconer (1989, Chs. 11, 12 & 19) except where otherwise stated.

Realised heritability

The heritability estimated from a selection experiment is known as the realised heritability which indicates that it represents a description of achieved response. This is not necessarily an unbiased estimate of the true heritability of the base population. The realised heritability is simply the response to selection (R) divided by the selection differential (S)

$$h^2 = R / S \qquad [6.1]$$

and is normally obtained as the slope of the regression of R on S. The merit of realised heritability and any correlated responses over estimates from breeding experiments is that they represent a tangible property of the population and as such they have greater relevance as measures of the actual short - term microevolutionary response attainable.

Genetic correlation

A genetic correlation between two traits can be calculated when there is a response to direct selection in one trait and a correlated response in the other. The genetic correlation (rA) between two traits X and Y is ideally determined from the response and correlated response (CR) of both obtained from reciprocal selection experiments.

$$r_A = \left(\frac{CR_X}{R_X} \frac{CR_Y}{R_Y} \right)^{1/2} \qquad [6.2]$$

This approach avoids the need for heritability estimates but it could not be used in this study as direct selection was only conducted on egg length and not fecundity. Alternatively, the correlated response can be expressed in a form that includes the root of the heritability of the selected trait (h_X) and a term for r_A

$$CR_Y = i h_X h_Y r_A \sigma_{PY} \qquad [6.3]$$

where i = selection intensity

σPY = population phenotypic standard deviation of Y

6.2.2 Design considerations

The major parameters of the selection experiment are discussed in turn but in practise they had to be considered collectively. The use of control lines allows directional non-genetic trends occurring during the course of the experiment due to environmental or inbreeding effects to be recognised and separated from the genetic response. Efficiency in terms of the number of individuals measured may be increased by imposing divergent selection where increased and decreased trait values are selected for simultaneously in separate lines originating from the same base population. The expected response of the divergent lines is double that of a single line compared to an unselected control and statistical power is also doubled Each divergent line controls for random environmental changes in

the other but is unable to detect directional trends. The divergent line structure (Hill, 1980) was adopted in this experiment as the use of an unselected control would have been costly in terms of the number of individuals measured which did not contribute directly to the measurement of response.

Replication overcomes the effects of random genetic drift as this is averaged out when the results of replicates are combined. No loss of efficiency is involved as the variance of response in one line of size N is equal to the variance of mean response of n lines of size N/n (Hill, 1971). The main drawback of replication is that where the total number of individuals that can be measured is limited (which was an important constraint in this experiment) increased replication will lead to reduced population size and hence increased inbreeding within each replicate. As a compromise, two replicates of each selected line were used.

Like replication, the intensity of selection is limited in practical terms by the inbreeding consequences of reduced population size resulting from reducing the proportion of individuals selected. Soller and Genzi (1967) recommend that the proportion selected be in the region of 15-20% for short-term experiments but a larger compromise proportion of 35% was chosen to offset the effects of inbreeding. This represents a selection intensity of 1.058 (Falconer, 1989, appendix A). The effect on the variance of realised heritability is less if the proportion selected is larger, rather than smaller, than the optimal proportion and the variance of the realised heritability was predicted to be 120% that of the variance at the optimal selection intensity (Soller & Genzi, 1967 Table 1). Egg length as measured in the selection experiment represents a trait of the adults that hatch from them and not a trait of the mothers that laid them. Consequently, egg length is not sex-limited and the additional affects of this on selection intensity do not have to be considered.

Following Falconer (1989, Ch. 13) selection can be made on the basis of individuals, family means, individuals within families or a combined index of all methods; the latter providing the highest expected response. Individual selection gives the best expected response of the single criteria methods over a wide range of heritability values and was estimated to give an expected response of 96% of that from combined selection using the breeding experiment heritability estimate of egg length. Thus there was negligible additional benefit of using combined selection, and family or within family expected responses were both smaller (84% and 55% of combined selection respectively).

The inbreeding coefficient (F) was estimated from the effective population sizes of all possible designs considered (Robinson & Bray, 1965). With individual selection and the avoidance of sib matings the effective population size (N_e) is

$$N_e = N + 2 \qquad [6.4]$$

where N = actual population size.

The rate of inbreeding ($\triangle F$) is then

$$\triangle F = 1 / 2N_e \qquad [6.5]$$

which is the increase in the inbreeding coefficient in one generation, relative to the increase required to achieve complete inbreeding. The inbreeding coefficient represents the probability that two genes at any locus in an individual are identical by descent and is calculated as

$$F = 1 - (1 - \triangle F)t \qquad [6.6]$$

where t = number of generations of selection

and no inbreeding in the base generation is assumed. The predicted inbreeding coefficient of the chosen design was 4.9% over four generations.

The final major design parameter which had to be considered was the number of individuals that needed to be measured and selected each generation. Population size was determined in three steps: (i) an upper limit was set on the

basis of the number of beetles that could realistically be handled during the most intensive measurement periods of the experiment; (ii) the parameter $ih\sqrt{t}$ was estimated and inserted into the design efficiency graphs given by Nicholas (1980); and (iii) by trial and error adjustment of the estimates from (i) and (ii) to meet the constraints of the other criteria discussed above. Twenty pairs of beetles in each selected line was decided upon but an additional 16 individuals were selected each time to allow for mortality losses between the egg and adult stages. This excess number also meant that the spread of emergence dates of adults from selected eggs could be reduced by excluding individuals that emerged exceptionally early or late. A randomly chosen group size of eight eggs from each mated pair of beetles were measured to form a sample population of eggs of known length from which eggs were selected.

6.2.3 Analysis

The realised heritability of a selected trait in the divergent selection design was estimated as the slope of the regression of cumulative response on cumulative selection differential obtained from the pooled replicates. The standard error of this regression seriously underestimates the standard error of the realised heritability as it does not include variance between replicates and does not take into account accumulative variance due to genetic drift (Hill, 1980; Falconer, 1989, p.211) The standard error formula given below which is suitable for designs with small numbers of replicates follows Hill (1972) and involves precalculation of drift variance (o_d) and error variance (o_e) terms.

$$o_d^2 = \tfrac{1}{2}\sigma^2[h^2(1 - h^2)(1/N_m + 1/N_f) + h^4(1/M_m + 1/M_f)] \qquad [6.7]$$

$$o_e^2 = \tfrac{1}{2}\sigma^2(1-h^2)(1/M_m + 1/M_f) \qquad [6.8]$$

where

o^2 - population phenotypic variance estimated by the residual mean square of

a two-way ANOVA of egg length by line and generation

h^2 - realised heritability estimated by regression slope b

N_m/N_f - number of males/females selected each generation

M_m/M_f - number of males/females measured each generation

Selection was carried out on eggs when sex was unknown and the normal 50:50 sex ratio Wilson (1989) was assumed, i.e. $N_m = N_f = 56$ and $M_m = M_f = 160$.

The variance of realised heritability was calculated as

$$V_{(b)} = \frac{6}{s^2 t(t+1)(2t+1)} \left[\frac{2t^2 + 2t + 1}{5} \sigma_d^2 + \sigma_e^2 + \frac{3t(t+1)}{2(2t+1)} h^2 \sigma_e^2 \right] \qquad [6.9]$$

where

t - number of generations of selection

s - actual selection differential estimated as mean of all lines

and $se_{(b)} = \sqrt{V_{(b)}}$ (Sokal & Rohlf, 1981)

The genetic correlation can be expressed in terms of one correlated response and both heritabilities by rearrangement of equation [6.3].

$$r_A = CR_Y / i h_X h_Y \sigma_{PY} \qquad [6.10]$$

where the terms appropriate for divergent selection and their empirical estimates are:-

CR_Y - correlated response in Y to selection in X

$= (\bar{y}_U - \bar{y}_D / \sigma_{PY})/t$

$\bar{y}_U$ - mean phenotypic value of Y in final UP generation

$\bar{y}_D$ - mean in the final DOWN line

σ_{PY} - population phenotypic standard deviation

t - number of generations

i - intensity of selection = S_X / σ_{PX}

S_X - mean selection differential in X

σ_{PX} - population phenotypic standard deviation of X

h_X / h_Y - square roots of heritabilities of X / Y

The heritability of egg length could be estimated by either the realised heritability or the breeding experiment estimate from Chapter 5. For the heritability of fecundity, only the breeding experiment estimate from Chapter 5 was available.

A detailed approximation of the standard error of the genetic correlation (o(rA)) is given by Hill (1971) but more manageable formulae are available (Reeve, 1955; Robertson, 1959b; Sheridan & Barker, 1974; Falconer, 1989, p.317). Standard errors from the formula of Falconer (1989) were the most conservative (largest) and most simple to calculate and were adopted here.

$$\sigma(rA) = \frac{1 - r_A^2}{2}\left(\frac{\sigma_{h_x}^2 \sigma_{h_y}^2}{h_X^2 h_Y^2}\right)^{1/2} \qquad [6.11]$$

where

$\sigma h / \sigma h$ - standard error of heritability of X / Y.

6.3 Methods

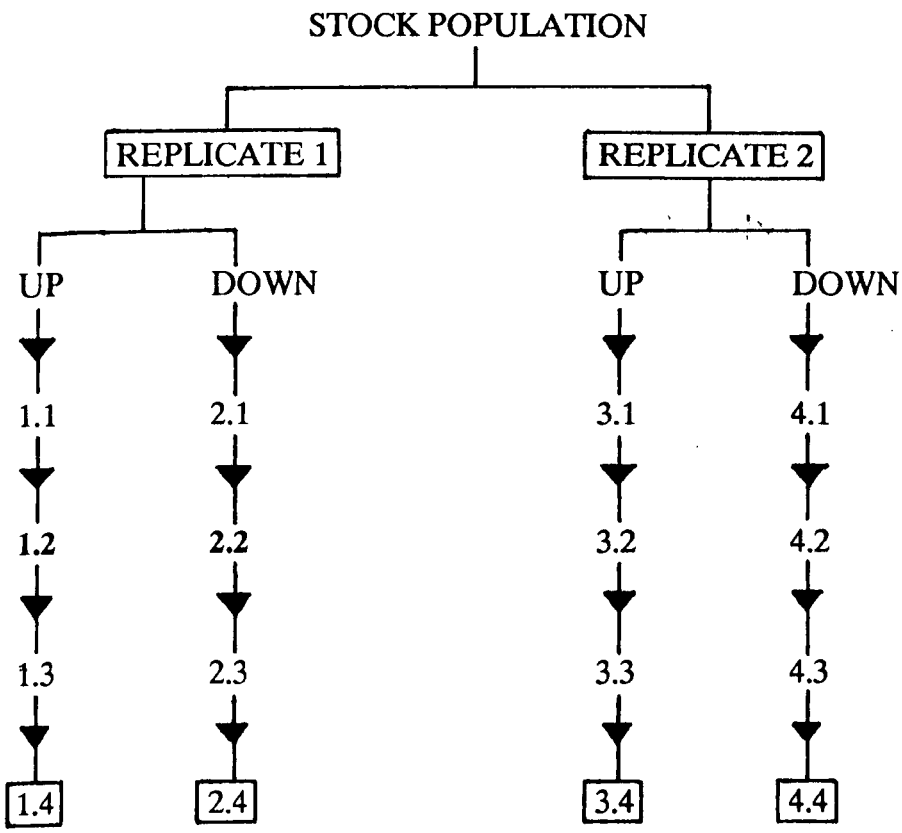
6.3.1 Selection procedure

The general structure of the breeding program for selection of egg length is schematised in Fig. 6.1. The base generation was established from beetle stocks

cultured at low density with minimal larval competition. Two replicate sub-populations were set up on the peak day of adult emergence. In each, 28 pairs of virgin adults < 24h old were mated to initiate the selection lines. Mated females were placed in $20cm^3$ pots with 25 beans for 24h and then discarded. Where necessary, egg loads on each bean were then reduced to one prior to the onset of hatching. When hatching was complete, eight eggs were randomly chosen from each of 20 of the females and measured. The 160 measured eggs were then ranked and the largest and smallest 56 (35%) were selected and transferred to cell trays to establish 'UP' and 'DOWN' lines respectively within each replicate. From the 56 eggs selected a minimum of 20 pairs of successfully emerged adults were used to propagate the next generation. This procedure was repeated for four generations in total. The only difference between successive generations was the increase in range of the emergence period necessary to collect sufficient adults to breed the next generation. This unavoidably resulted in mating and oviposition occurring over several days although individual females were less than 24h old when mated and their partners were up to 48h old. Once the UP and DOWN lines were established, selection was carried out only in one direction within each line.

6.3.2 Assays of populations after selection

Some females were randomly assigned for the collection of additional data upon emergence. These were treated on the first day of oviposition in the same way as all other females in the selection program except that they were weighed before being mated. In the base and fourth generations in both replicates, such mated females and males were retained after the first day of oviposition for assays of life-history traits in all four lines (Figure 6.1). Females were given five fresh beans on the second, third and forth days of laying to record unconstrained daily



selection event

boxed — some or all beetles included in general assay of traits

bold — some females also used to measure maternal age effects

Figure 6.1 Schematic plan of selection experiment design. Two replicates from the same stock population were each subjected to divergent selection for four generations.

fecundity. The correlated responses in longevity, development time, emergence weight and elytral length were also measured in both sexes and are examined in Chapter 7. Females from the base and second generations in one replicate were also retained after the first day of laying and used in Expt. 3G

In the fourth generation, additional measures were taken from eggs to compare egg fresh and dry weights which are described in Chapter 7.

6.4 Results

6.4.1 Direct response and realised heritability

All lines showed absolute responses to selection for egg length over the course of the experiment but the two replicates differed in the size of cumulative response (Fig. 6.2). Examination of the divergence of reciprocal lines within replicates removed shared environmental variation between generations and revealed that the difference in total response between the replicates occurred after the first selection event. Divergence was initially negative in the second replicate but in subsequent generations the rate of divergence was constant and equal in both replicates. There was no evidence of asymmetry of response between the UP and DOWN lines assuming that the unselected mean egg length did not change (fourth generation mean deviations from base generation: pooled $t_{(534)} = -1.56$, $P = 0.12$). Combination of the data from both replicates removed further sources of variation and the final selected generations were significantly different from the base generation in egg length (DOWN vs. BASE, $t_{(216)} = -15.1$, $P < 0.001$; BASE vs. UP, $t_{(215)} = -12.25$, $P < 0.001$). A consistent increase in the mean cumulative response to selection was evident across generations (Fig. 6.3). The slope of the regression line fitted to Fig. 6.3 estimated the realised

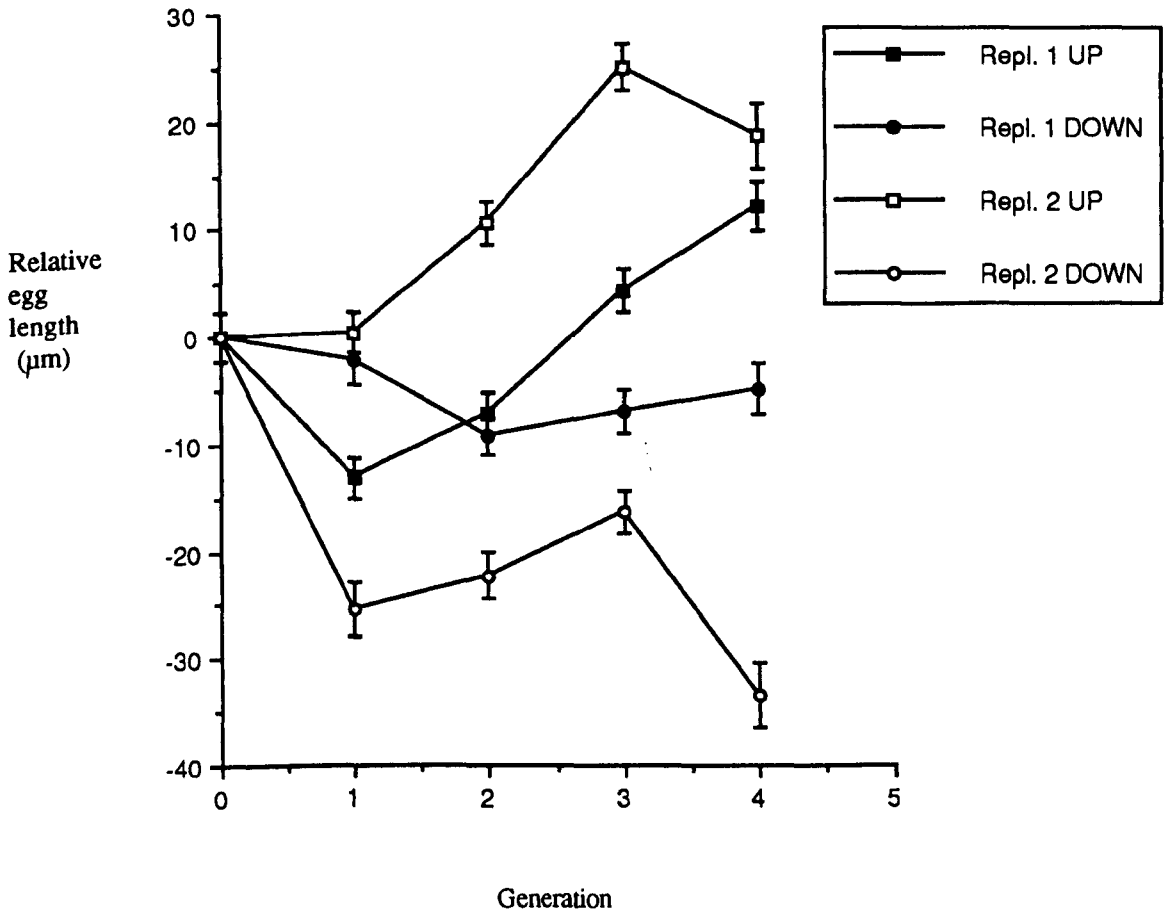


Fig. 6.2 Responses to divergent selection for egg length in two replicates. Response is shown as change from the base generation mean (error bars are standard errors of generation means).

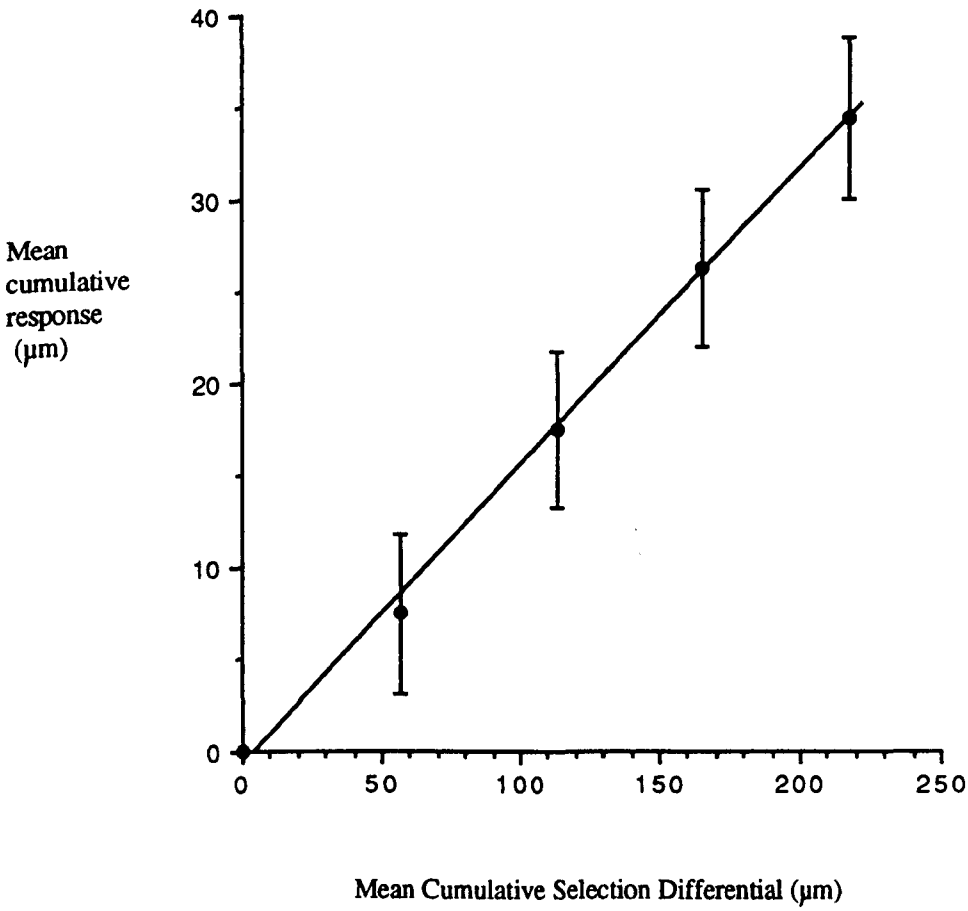


Fig. 6.3 Regression of mean cumulative response with mean cumulative selection differential for egg length over five generations. The slope of the fitted line represents the realised heritability (standard errors are shown).
The two replicates were combined by adding the divergence values in each generation. Standard errors were calculated by adding indivdual line variances using the formula $se(a+b) = Var(a) + Var(b) / n$.

heritability of egg length.

$$h^2 = 0.169 \pm 0.034$$

This value is much smaller than the estimate obtained from the breeding experiment in Chapter 5 ($h^2 = 0.50 \pm 0.22$) but is greater than zero ($t_{(3)} = 4.97$, $P < 0.05$).

6.4.2 Correlated response in fecundity and genetic correlation

In both UP and DOWN lines the correlated response in mean fecundity was downwards (Figure 6.4(a) & (b)) and the decrease was greatest in the DOWN line. The final UP and DOWN line fecundities were not quite significantly different from each other (pooled $t_{(72)} = 1.70$, $P = 0.09$). The correlated divergent response in fecundity is -3 eggs per generation. Using the heritability estimates from the breeding experiment (Ch. 5) the genetic correlation and its standard error were estimated as

$$r_A = 0.233 \pm 0.315$$

If the very different realised heritability of egg length estimate given above is substituted the genetic correlation is not very different;

$$r_A = 0.383 \pm 0.246$$

These values are opposite in sign to the breeding experiment estimate ($r_A = -0.35 \pm 0.61$) but the estimates are not significantly different from each other due to their large standard errors, caused largely by the low precision of the breeding experiment heritability estimates.

Eggs laid on the fourth day of oviposition or later in the final UP population were larger in terms of egg length than DOWN population eggs (pooled $t_{(98)} = 6.69$, $P < 0.001$) and were also more variable (Variance ratio: $F_{(50,50)} = 1.85$, $P < 0.05$). The divergence of late laid egg length in the selected lines (34.6μm) is the same as that for eggs on the first day of oviposition (34.5μm). Individual

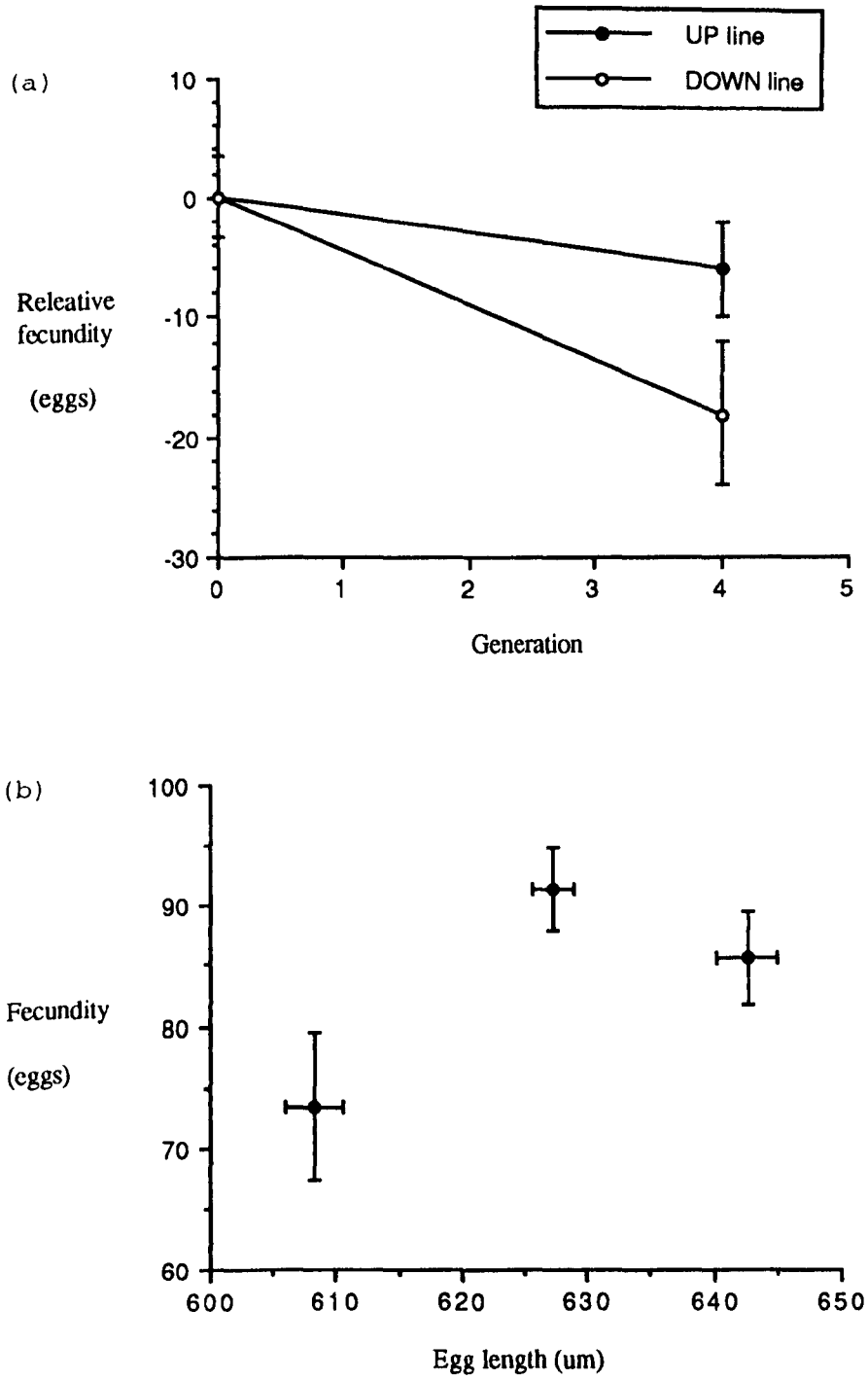


Fig. 6.4 Correlated response of fecundity to selection for egg length; (a) response to divergent selection over four generations. Fecundity is expressed as change from the base generation mean value; (b) relationship between selected egg length and fecundity. Points are the means of the base and two final selected generations (error bars are standard errors).

females within each population laid consistently different sized eggs from each other over the later days of oviposition (Nested ANOVA: $F_{(18,80)} = 7.74$, $P < 0.001$) indicating that the repeatability of egg length persisted after selection.

6.5 Discussion

6.5.1 Egg size response

The divergent response to selection for egg length was 8.64μm per generation which was approximately one third of that predicted from insertion of the breeding experiment estimates into equation [6.1] (24.23μm per generation). The predicted response was matched only in the first generation of the second replicate (R = 25.70μm) but this decreased thereafter. In the other replicate, the total response was smaller as a result of a negative response to upwards selection in the first generation only.

Other studies have demonstrated large differences in response between replicates (Falconer, 1973; Yoo, 1980) which could be caused by one of three stochastic processes; environmental change, inbreeding or genetic drift. These random effects operate independently within the lines of different replicates and cause indirect (environmental change) or direct (inbreeding and genetic drift) changes in population gene frequencies resulting in divergence of replicate lines. The magnitude of these effects is exacerbated by small population size.

Combination of replicates averaged out the effects of the random divergence of lines but did not explain the apparent discrepancy between the heritability estimates of the selection and breeding experiments (large standard errors meant that estimates were not significant statistically). The large variance associated with the selection estimate may indicate that the apparent difference is due only

to sampling error. Alternatively, there are two main explanations for why the realised heritability may deviate from the true population value. Firstly, the assumption of symmetry of response may be incorrect. The magnitude of response relative to the base population mean was equal in lines selected upwards and downwards but if underacted environmental trends were present causing a change in unselected egg length during the course of the experiment this would mean that the observed responses were in fact asymmetrical. This could have been detected only by the parallel measurement of an unselected, outbred control (Gromko *et al*, 1991) which would have been costly in terms of experimental resources. Asymmetrical responses to selection have been widely found in artificial selection experiments with reduced response normally in the direction of higher fitness (Frankham, 1990). If asymmetry was present there are several possible causes (Falconer, 1989, Ch. 12) which include random drift and inbreeding (discussed above). Another possible source of asymmetry is genetic asymmetry due to additive genes not being at the frequency which maximises their contribution to egg length in the base population. These factors can have a gradual accumulative effect on response over successive generations. In contrast, genetic asymmetry of genes with large effects, maternal effects and scalar asymmetry may all lead to asymmetrical responses after just one generation. Such responses were present in this experiment but cannot readily be distinguished from random change. Genes with large effects and scalar asymmetry can be predicted in advance if the offspring - parent regression in the base population is non-linear but this was not determined. The second explanation of bias in the realised heritability is that selection of traits, and especially those with high heritabilities, leads to a rapid reduction in variance in selected lines relative to the base population due to the reduction in the range of phenotypic values (Robertson, 1977) and increase in linkage disequilibrium (Bulmer, 1985). As response is related to the phenotypic variance of the selected

trait X

$$R = ihX\sigma PX \qquad [6.11]$$

where i = selection intensity

σPX = population phenotypic standard deviation of X, this means that the response will decline after the first generation of selection. The decline in response in the second replicate is consistent with this explanation.

The difference in the size of eggs laid late in the oviposition sequence between the final selected generations suggests that genetic and physiological determinants of egg length are the same throughout female lifespan. The greater variation in egg length in the UP line stems from the presence of a few atypically small eggs which may have been laid towards the end of the oviposition period.

6.5.2 Correlated response of fecundity

Using the estimated heritability from the breeding experiment, the predicted correlated response to selection in fecundity was -3.30 eggs per generation (relative to the direction of selection for egg size). The actual correlated response was +3.01 eggs per generation. Thus the genetic correlation between egg length and fecundity was of similar size but opposite sign in the two experiments. Two general reasons may account for the discrepancy between the genetic correlation based on correlated response and that estimated independently from the breeding experiment. Either the difference is an artifact of the low precision and inconsistency generally associated with correlated responses (Falconer, 1989, p. 319) or it is a real effect brought about by genetic changes caused by selection in addition to the expected correlated response.

The heritability estimates from the breeding experiment have large sampling errors and the correlated response may be of similar low precision as is the case in several other studies (Gromko *et al*, 1991). These two sources of variance are combined in the genetic correlation estimate and in this experiment the standard error was larger than the genetic correlation. A second issue which causes separate measures of correlated responses to differ is the general problem of whether a genetic correlation estimate from a selection experiment is relevant to the base population. Changing selection pressures results in the perturbation of equilibrium gene frequencies (Bulmer, 1976; Turelli, 1988) which contribute to the additive genetic variance of traits including the one being selected. The effect on genetic covariances is proportionally greater so that genetic correlations may become unrepresentative of the base population over only a few generations (Bohren *et al*, 1966; Barton & Turelli, 1987). However, in a study of coxal and sternopleural bristle number in *Drosophila melanogaster*, Sheridan & Barker (1974) found little change in the genetic correlation after ten generations of selection but some replicates had significantly changed after 22 generations. In another study on morphological traits in *D. melanogaster*, genetic correlations changed little after 23 generations of selection upwards but changed substantially in the downwards direction of selection (Wilkinson *et al*, 1990). Changes in genetic covariances do therefore not always seem to occur but gene frequencies affecting traits closely associated with fitness may alter at a faster rate.

An important result is that the measured positive correlated response in fecundity is in fact a relative one. In lines for both increased and decreased egg length the absolute fecundity decreased but the reduction was less in the 'increased' line resulting in a relative correlated response. The difference between the selected lines is not quite significant. Three reasons may explain

why there is a reduction in the value of a fitness component, or fitness itself, in both directions of selection. One possibility is that the artificial selection pressures do not have the same effect in the UP & DOWN lines. For example, selection for large egg size may select for increased allocation to each egg and consequently fecundity is reduced as predicted. On the other hand, selection for small egg size may effectively select for small bodied females which have less resources in total and thus lay smaller *and* fewer eggs. Evidence for this explanation amongst the measured correlated responses to selection for egg length is considered in Chapter 7. A second reason for the reduced fecundity in both lines is simply that the small population size normally involved in selection experiments results in inbreeding depression (Bell & Koufopanou, 1986; Falconer, 1989, p.319).. This may have played a part in the response measured but the *a priori* inbreeding estimates indicated that inbreeding would be less than 5% over the duration of the experiment. Thirdly, a multivariate perspective may also account for the decline in fecundity (Pease & Bull, 1988; Charlesworth, 1990). However, if other fitness components have positive genetic correlations with egg size and negative genetic correlations with fecundity they may increase their fitness contribution as fecundity declines. Hence the correlated response of reduced fitness contribution of fecundity may be compensated for in other fitness components and fitness itself may not be altered.

CHAPTER 7

FITNESS CONSEQUENCES OF EGG SIZE

7. Fitness consequences of egg size

7.1 Introduction

The fundamental assumption underlying the predicted trade-off between egg size and fecundity is that large eggs give rise to adults with higher fitness than adults developing from small eggs (Holloway *et al*, 1987). This assumption is also implicit in models of the evolution of offspring size (Smith & Fretwell, 1974) and egg versus clutch size variation (Parker & Begon, 1986; Begon & Parker, 1986). The causal sources of variation in egg size may be environmental and in particular egg size prior to oviposition may be determined by maternal phenotype for body size. Alternatively egg size variation may be due to gene expression. If the trade-off is to have any evolutionary significance then it is not only necessary to show that egg size is correlated with fitness, but to show that when egg size is changed that fitness changes as well. Artificial selection is a way of measuring both the correlation with fitness components, and the effects of manipulation, on egg size in one experiment.

In Expt. 3C circumstantial evidence for effects of egg size on fitness components of the progeny that hatched from them were obtained from phenotypic correlations. In the same way that phenotypic correlations cannot reliably recognise trade-offs (section 1.2.1) the use of correlations in this context is unable to distinguish between genetic and maternal effects and hence the information they provide is not conclusive. The correlations in Expt. 3C are based on the variation in egg size occurring in one single population in one environment. Other researchers have correlated phenotypic measures of egg size and offspring fitness traits across ranges of variation expanded in some way. Kaplan (1989) used egg size variation in one population exacerbated by environmental heterogeneity to examine effects of egg size on larval fitness. He

found that large eggs of the frog *Bombina orientalis* result in large larval size during the first few weeks of development which reduces the risk of predation from other tadpoles. Interpopulation and interspecies comparisons have also demonstrated raised values of components of adult fitness arising from larger eggs. These must include some genetic variation but these studies are also unable to distinguish causal genetic and maternal effects. Tessier & Consolatti (1989) studied clones of five *Daphnia* species in which large eggs gave rise to large adults with increased starvation resistance. However, relationships between egg size and fitness using essentially phenotypic analyses are not general (Capinera, 1979). A series of studies of Satyrid butterflies have consistently failed to reveal a fitness consequence of egg size (Wiklund & Persson, 1983; Karlsson & Wiklund, 1984; Wiklund & Karlsson, 1984) although only larval traits were examined and the laboratory environments of these experiments may have excluded the environmental variables that distinguish the role of egg size. In some starfish, egg size was not correlated with energetic content (McEdward & Coulter, 1987) which also illustrates that this trait may not always a good indicator of investment in offspring.

The alternative and altogether preferable method of examining the effect of egg size *per se* is to experimentally manipulate egg size in some way so that egg size variation in a population is randomised with respect to developmental elements of maternal environment. This approach has been effectively used in studies of clutch size in birds as this is a trait that can be easily manipulated before a large proportion of parental investment is made (parental care) by adding or removing eggs from nests (Lessells, 1986; Linden & Moller, 1989). In so doing, clutch size is divorced from components of parental quality. Unfortunately, egg size in *C. maculatus* cannot be manipulated in a similar convenient way as all parental investment in eggs is made before oviposition. Females cannot be made to lay

eggs of different size except perhaps indirectly which would probably affect maternal environment of eggs at the same time.

The selection program (Ch. 6) offered an opportunity to employ the best available analogue of direct manipulation of egg size. Populations that had been artificially selected for increased and decreased egg size can be viewed as representing multiple generation manipulations; the disadvantages of which include all the possible inherent changes in genetic variation and covariation associated with selection which may mean that conclusions about egg size effects are difficult to relate back to the original unmanipulated population (section 6.5). In this chapter, information gathered from the assays of life history traits in the final generations of the divergent selected lines (Ch. 6) are examined to see if the changed egg sizes in these populations are associated with changes in other life history traits (correlated responses to selection). In addition, other parameters of egg size and egg weight were compared between the selected populations to see if they had also responded to selection for egg length.

7.1.1 Aims

1. to asses the intrinsic effects of egg size (length) on subsequent adult life-history traits from correlated responses to selection.
2. to see if large and small eggs are also heavier and lighter respectively after selection.

7.2 Methods

This experiment was conducted by assaying the base and two final selected generations of the selection program in Chapter 6 (lines selected for large and small egg length respectively; Fig. 6.1) These generations were treated as three

populations with different mean egg lengths and are termed the BASE, UP and DOWN populations in this chapter. In each of the populations 28 virgin adult pairs less than 24h old that had been reared in the absence of larval competition were chosen at random and weighed before being mated to non - sib partners. Males were separated after mating had been observed and the females were placed in $20cm^3$ pots with 25 beans. A replacement of five fresh beans were given to each female on the 2nd, 3rd and 4th days after mating. Those beans presented last were left with the females until they died. Fecundity and egg size were recorded and longevity, development time and elytral length were measured in both sexes.

In the UP and DOWN populations in one replicate only, additional measures were taken from eggs to compare fresh and dry weights of eggs with egg length. From each female's egg compliment laid on the first day of oviposition, five eggs less than 2d old were chosen at random for weighing in addition to the eight chosen for size measurements. Eggs were weighed in batches of five as they were too light to weigh individually on the electrobalance. The eggs were then dried to a constant weight and weighed again. Mean fresh and dry egg weights were calculated for comparison with the mean egg lengths measured from different eggs of the same females.

7.3 Results

7.3.1 Correlated changes in adult traits in populations selected for egg length

The three populations were significantly different from each other in egg length (DOWN vs. BASE, $t_{(216)} = -15.1$, $P < 0.001$; BASE vs. UP, $t_{(215)} = -12.25$, $P < 0.001$). Egg breadth was positively correlated with egg length across the

populations ($r = 0.97$, $n = 6$, $P < 0.01$; Fig. 7.1(b)) and although the three populations are significantly different in egg breadth they vary proportionally less than in egg length (length:breadth ratio, DOWN = 1.51, BASE = 1.55, UP = 1.62). The sex of adults developing from eggs was independent of egg length and breadth (Fig. 7.1 (a) & (b)). The longevity of UP population males and females showed no correlated response to selection. Male beetles lived longer than females except in the DOWN population where there was convergence of the sexes due to reduced male longevity (Figure 7.1 (c)). Development rate was significantly reduced after egg length selection in both directions but the reduction was significantly greater in the DOWN population than the UP population ($t_{(215)} = 8.38$, $P < 0.001$). Development rate of males was greater than that of females in the BASE population (Figure 7.1 (d)) but in both selected populations the small sex difference disappeared. Only females in the DOWN population showed a significant difference between populations in emergence weight or elytral length to selection; their emergence weight decreased. Males were significantly lighter at emergence and smaller in terms of elytral length than females in all populations (Figure 7.1 (e) & (f)).

In summary, the three populations manipulated through artificial selection to differ in egg length also differed in egg breadth. Only fecundity (Ch. 6) and development rate differed in the manipulated populations from the unmanipulated population in that both traits were reduced in value in the UP and DOWN populations. In both sexes, the UP and DOWN populations both had lower fecundity and development rate. Males and females differed in longevity, emergence weight and elytral length in all three populations but the populations did not differ in these traits.

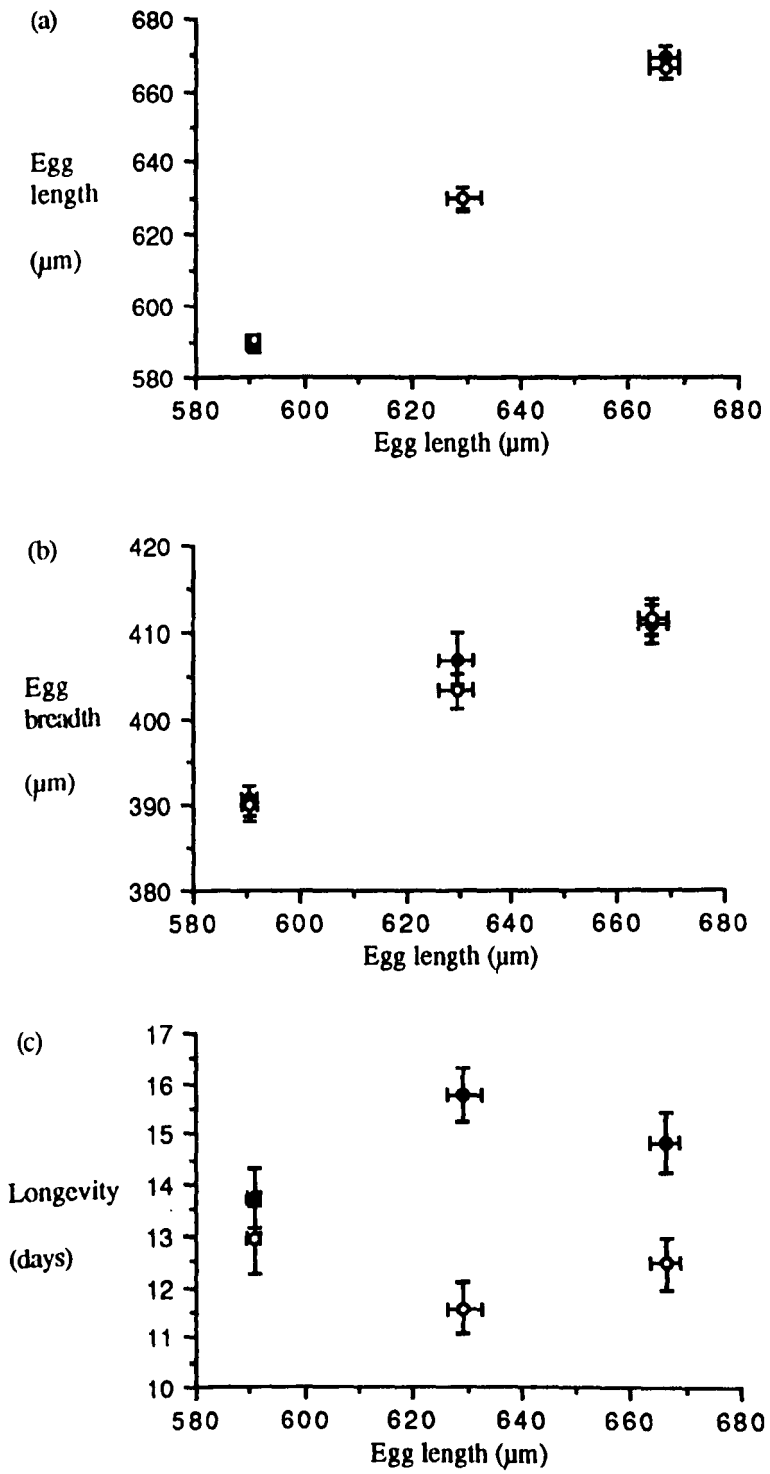


Fig. 7.1 The relationship between life history traits (given on vertical scales) and selected egg length. The three pairs of points are generation means of (from left to right) the final generation selected for short egg length, the base generation, and the final selected for long egg length. Means in each group are given for males (closed circles) and females (open circles) with standard error bars.

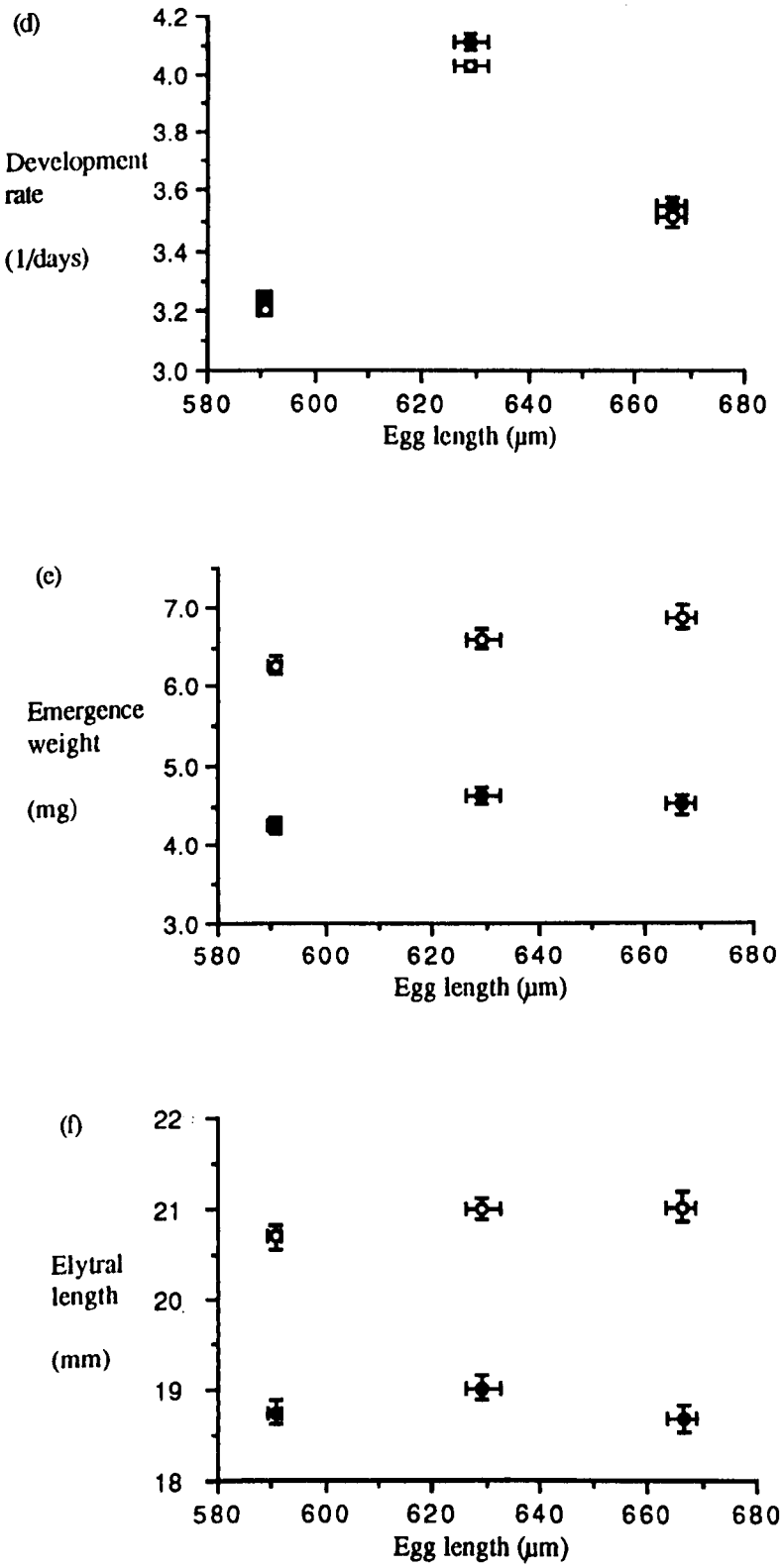

(d)
Development rate
(1/days)
4.2
4.0
3.8
3.6
3.4
3.2
3.0
580
600
620
640
660
680
Egg length (μm)
(e)
Emergence weight
(mg)
7.0
6.0
5.0
4.0
3.0
580
600
620
640
660
680
Egg length (μm)
(f)
Elytral length
(mm)
22
21
20
19
18
580
600
620
640
660
680
Egg length (μm)

Fig. 7.1

7.3.2 Relationship between egg weight and egg length

Direct comparison of egg fresh and dry weights in the UP and DOWN populations did not reveal any difference between them (fresh egg weight, pooled $t_{(27)} = 1.89$, $P = 0.07$; dry egg weight, pooled $t_{(27)} = 1.37$, $P = 0.18$). However, within populations both measures of egg weight are correlated with maternal development time (Table 7.1) which itself differs between the two populations. Females from large eggs complete development in a shorter time than females from small eggs (pooled $t_{(103)} = 8.14$, $P < 0.001$) and this obscured the interpopulation differences in egg weight (Fig. 7.2). After removal of the maternal development time covariation both fresh and dry egg weights were greater in the UP line than the DOWN line (ANCOVA: Table 7.2). Egg length is not correlated with maternal development time although egg size and weight themselves remained highly correlated after selection in both populations (Table 7.3; Figure 7.3). In the UP population only egg length and breadth were not correlated.

7.4 Discussion

The absence of any clear correlated responses in longevity, emergence weight and elytral length suggests that they are not genetically correlated with egg length. Development rate decreased in both directions of selection which was not consistent with a correlated response, especially as there was no evidence from the breeding experiment of heritable variation in development rate. The experimental design may have inadvertently permitted direct selection for reduced development rate as a result of the relaxation of the 28 day generation interval enforced in the cultured stocks from which the base generation originated. However, if development rate and egg length are not correlated the

Table 7.1 Pearson correlations between mean egg fresh and dry weights, egg size on first day of oviposition and maternal development time. Correlations were measured in (a) the final generation selected for increased egg length, and (b) the final generation selected for decreased egg length.

	Maternal Development time	Egg fresh weight	Egg dry weight	Egg length
(a) UP line (n = 19)				
Egg fresh weight	0.56**			
Egg dry weight	0.51*	0.60**		
Egg length	0.36+	0.42*	0.62**	
Egg breadth	0.44*	0.63**	0.54**	0.08
(b) DOWN line (n = 14)				
Egg fresh weight	0.64*			
Egg dry weight	0.69**	0.96***		
Egg length	0.16	0.54*	0.54*	
Egg breadth	0.50+	0.88***	0.88***	0.66*

+ $P < 0.1$
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

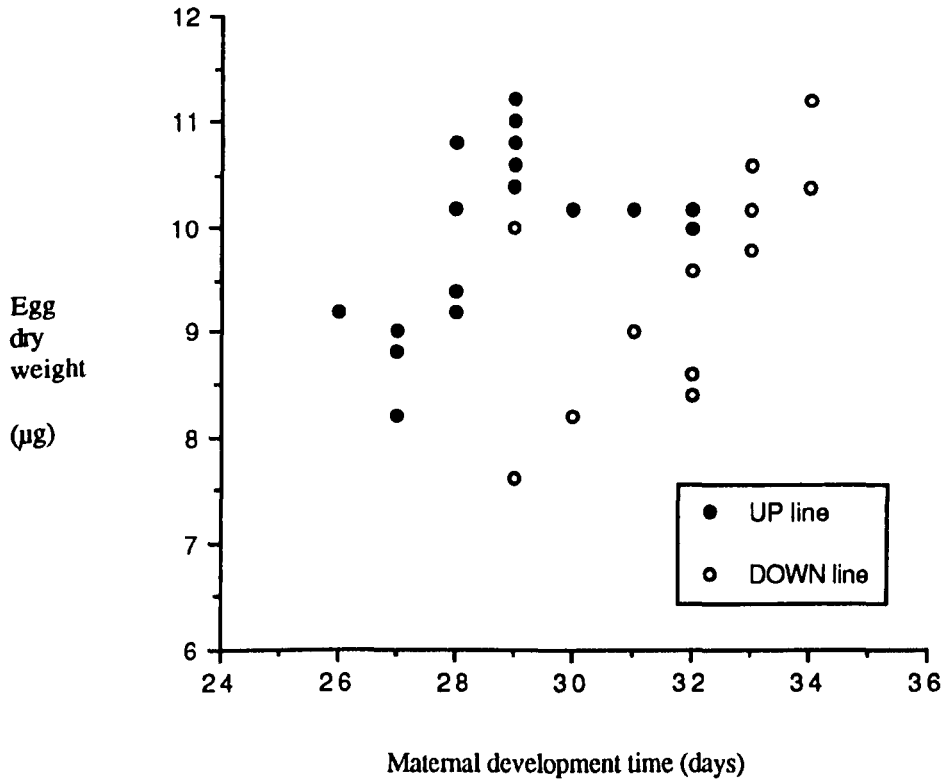


Fig. 7.2 The relationship between dry egg weight and maternal development time in the two final generations selected for increased and reduced egg length.

Table 7.2 Analysis of covariance of egg weight by selection line with maternal development period.

(a) dry egg weight

Test I: are slopes of the two selection lines the same?

Source	SS	df	MS	F
Development period	1.66	1	1.66	2.64
Line	8.71	1	8.71	13.83**
Development period by line	0.63	1	0.63	1.01
Residual	15.74	25	0.63	

Test II: If slopes are the same, are the selection line intercepts the same?

Source	SS	df	MS	F
Development period	8.63	1	8.63	13.70**
Line	8.71	1	8.71	13.83***
Residual	16.38	26	0.63	

(b) fresh egg weight

Test I: are slopes of the two selection lines the same?

Source	SS	df	MS	F
Development period	3.12	1	3.12	1.44
Line	37.88	1	37.88	17.51***
Development period by line	2.55	1	2.55	1.18
Residual	54.07	25	2.16	

Test II: If slopes are the same, are the selection line intercepts the same?

Source	SS	df	MS	F
Development period	29.61	1	29.61	13.60**
Line	37.88	1	37.88	17.39***
Residual	56.63	26	2.18	

** $P < 0.01$ *** $P < 0.001$

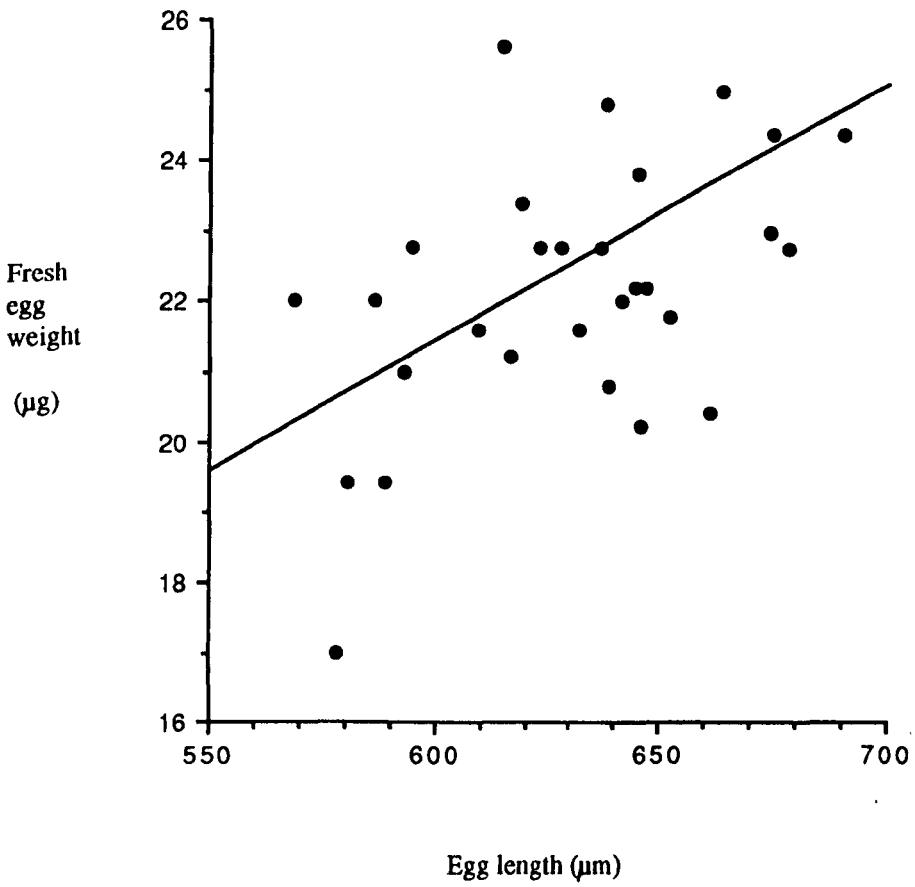


Fig. 7.3 The relationship between fresh egg weight and egg length. Regression: egg weight = 0.11 egg length - 45.58 ($r = 0.53$, $n = 29$, $P < 0.01$).

magnitude of change in both directions of selection would be expected to be the same but the decrease was less in the large egg population suggesting that this change may be partly linked to the change in egg size. Moller *et al* (1990) found additive genetic variation in development rate and evidence for a positive genetic correlation between development rate and both longevity and fecundity in a selection experiment on *C. maculatus*. Their results supported the existence of trade-offs between development rate and longevity and fecundity as a result of reduced emergence weight of individuals with fast development rates. The stock culturing methods used by Moller et al (1990) did not constrain development in the same way as it was in this study and this may account for the differences in genetic variation in development rate.

Fecundity was also reduced in both selected populations relative to the unmanipulated base population (Ch. 6). Thus no fitness consequences consistent with the direct manipulation of egg size were revealed and the phenotypic correlations between egg size traits (Expt. 3C) were not corroborated. In Chapter 6 it was proposed that the decline in fecundity in lines selected for both larger and smaller eggs could be attributable to indirect selection on other life history traits that prevented fecundity being increased in the line selected for smaller eggs. Considering females only (fecundity being a sex-limited trait) a significant difference occurred in emergence weight between the BASE and DOWN populations which could account for the corresponding reduction in fecundity if smaller eggs size also selected for smaller bodied females who had less resource in total and so laid smaller eggs and fewer eggs.

Selection for egg size did result in corresponding changes in egg breadth and egg weight (both fresh and dry). A large genetic correlation between egg length and breadth was also found in the breeding experiment and implied that the two egg size traits have pleiotropic genes in common. These parameters may be

indices of the resources allotted to each egg by the mother but this remains unproven. Egg length and weight do not appear to be entirely equivalent despite their large correlation as the divergence of weight in the two selected lines was not as clear as it was for length. One possibility is that longer eggs are not necessarily greater in volume (and thus possibly weight) and in the selected population with increased egg size the decoupled relationship between egg length and breadth indicates that this may be the case. This notion is also supported by the length:breadth ratios of the populations which showed that long eggs are relatively less broad than short eggs. As a result it is possible that the general absence of correlated responses to selection means that the original support for the assumption that egg length is related to fitness obtained in Chapter 3 is not in fact true.

Ideally, the fitness value of egg size might be tested in future experiments through direct manipulations if suitable techniques can be developed. Recently, a range of developmental methods have been devised and employed in several taxa. These provide perhaps the most convincing evidence for the intrinsic properties of egg size. Sinervo & McEdward (1988) experimentally manipulated egg size in the sea urchin *Strongylocetrotus droebachiensis* by partitioning blastomeres in the early embryo to produce small, mature eggs. These developed into smaller than normal larvae which developed more slowly, although the effects were not carried over into adult traits. More recently, experimental reduction of yolk content of shelled eggs has been achieved. Yolk reduction in the iguanid lizard *Sceloporus occidentalis* resulted in smaller hatchlings which, as juveniles had relatively slow sprint speeds which reduced their survivorship (Sinervo, 1990). Experimental manipulation of egg size in invertebrates remains unattempted but it may be feasible in *Callosobruchus* using egg constriction techniques described by van der Meer (1979).

CHAPTER 8

GENERAL DISCUSSION

8. General Discussion

Life history theory is built on the assumption that trade-offs occur between at least some life history traits. Adult female *C. maculatus* have a fixed amount of resources to allocate between their offspring, because all resources are obtained as larvae, and this creates a situation where a trade-off between egg size and fecundity is expected to exist. The principal aim of this study was to investigate what information could be obtained about the trade-off between egg size and fecundity by means of phenotypic and quantitative genetic experiments. The quantitative genetic methods are utilised as an empirical tool to examine the trade-off in the absence of any alternative experimental method. Direct manipulation of egg size was not possible, so instead the prediction that trade-offs should give rise to negative genetic correlations between traits was utilised by attempting to quantitatively measure such a correlation as evidence for the existence of the trade-off. The basic premise underlying the proposed trade-off between egg size and number is that variation in both traits has consequences for fitness. The fitness value of fecundity has been demonstrated previously in *C. maculatus*. For example, females that lay the most eggs produce the most adult progeny even when larval competition is high (Bellows, 1982a). The relationship between egg size and fitness is less clear and so the second aim of this study was to search for evidence of the intrinsic consequences of egg size on the fitness of progeny that hatched from those eggs.

8.1.1 Interpretation of the results

A trade-off between egg size and fecundity in *C. maculatus* may indeed be real but the results from the breeding and selection experiments were not conclusive, but neither were they incompatible with the trade-off. Both egg size and fecundity contained significant amounts of additive genetic variation. A negative

genetic correlation, consistent with the existence of the trade-off, was determined in the breeding experiment. Conversely, the selection experiment revealed a positive genetic correlation between the traits in direct contradiction to the former estimate. However, the selection experiment estimate of the genetic correlation, which is based on the correlated response in fecundity to selection for egg size, was relative and absolute values of fecundity declined in response to selection for both large and small eggs. This actual response to selection is perhaps more relevant than the genetic correlation itself in detecting the trade-off mechanism, because it directly reveals what happens when egg size is changed (Stearns, 1989). Selection for increased egg size resulted in a response of decreased fecundity. This was consistent with the trade-off. Selection for reduced egg size also led to a reduction in fecundity but this may not be related to any trade-off response and may be caused by another indirect response to the change in egg size. Egg size was positively phenotypically correlated with body weight and development rate of the offspring that hatch from them and both these traits also declined when egg size was reduced by selection. This provides two possible explanations for the unexpected decrease in fecundity. Firstly, small eggs give rise to small adults which may have less resources in total to allocate to progeny. These small females may thus lay smaller eggs *and* fewer eggs. Secondly, small eggs develop slowly, and fecundity has a positive phenotypic correlation with development rate whilst longevity has a negative phenotypic correlation with development rate. In other words, females with a long development appear tend to live longer as adults but lay fewer eggs than females with a short development time.

The final generations of the lines selected for increased and reduced egg size differed from each other and from the unselected base generation in mean egg size. These three groups were expected to provide three points lying on the

trade-off curve between egg size and fecundity. However, the corresponding changes in body size and development rate that occurred in the line selected for reduced egg size may mean that the coordinate representing egg size and fecundity in this group lies below the trade-off curve. However, the other two points from the unselected and upwards selected groups may lie on the trade-off curve and their positions are consistent with the expected pattern (i.e. a negative slope).

The main problem with interpreting the genetic correlations themselves was their poor statistical precision. Both the breeding and selection experiment estimates of the genetic correlation were not significant in a statistical sense due to their large standard errors. This underlines the need to measure large numbers of individuals to determine quantitative genetic statistics precisely. However, the use of half-sib families in the breeding experiment meant that the genetic correlation estimate should be reliably accurate despite the low precision because it avoided the incorporation of possible sources of bias, namely maternal and non-additive genetic effects.

Although the results can be interpreted as being consistent with the existence of a trade-off between egg size and fecundity it is worth considering the reasons why this trade-off may in fact be absent or positive.

Firstly, egg length on the first day of oviposition may not have been an appropriate measure of egg size and may not have any close relationship to the fitness of progeny. Egg length may have been inappropriate because it may not have been related in any way to the egg resources which were the currency of the trade-off. The initial phenotypic correlations between egg length and breadth with egg weight were positive, but in the group selected for increased egg size in the selection experiment egg length and breadth were independent. Whether

egg weight was a good measure of egg resources was not determined. Also, egg length on the third day of oviposition was phenotypically correlated with more life history traits than egg length on the first day of oviposition, possibly as a result of increased variation in the latter measure due to contributions to the eggs from male ejaculate. However only egg length on the first day of oviposition clearly contained significant heritable variation necessary for measuring genetic correlations, so it was chosen as a general measure for this reason. Egg length did appear to be related to some components of progeny life history although these were by no means heritable effects. Phenotypic correlations showed that large eggs developed fastest and gave rise to large females which themselves laid large eggs. Longevity and fecundity in offspring were not correlated with egg size. However, the selected groups with different mean egg sizes did not reveal any differences in longevity, body size or weight.

A second reason why a trade-off between egg size and fecundity might not be expected is that the traditional optimisation approach of considering two traits at a time may be too narrow a perspective. Trade-offs (and genetic correlations) may exist between egg size or fecundity and other life history traits. Thus the trade-off of interest here may form part of a matrix of trade-off relationships and genetic constraints and some of these may have net neutral or positive values (Pease & Bull, 1988). The positive genetic correlation between longevity and fecundity found in this study and Moller et al (1989) is an example of positive covariation occurring between two traits that optimality models predict to show a trade-off (Bell & Koufopanou, 1986). Even if a trade-offs does exist between a pairs of life history traits, it is possible that mutations in allocation genes which cause those traits to be positively correlated may obscure the underlying negative genetic correlation (Charlesworth, 1990; Houle, 1991). This emphasises the

limited value of studies of the genetic variance-covariance matrix alone (Maynard Smith, 1989; Parker & Maynard Smith, 1990).

8.1.2 Comparison of breeding and selection methodologies

The breeding experiment had several practical advantages over the selection experiment for determining the genetic correlation. The experiment was of shorter duration, being completed in two generations and as a result it was easier to ensure relative constancy of environmental conditions throughout the experiment (Reznick, 1985; Stearns, 1989). Additional information on non-additive genetic and environmental sources of variation was obtained from the breeding design with little extra effort to estimate dominance, epistasis, environmental and phenotypic correlations. The paternal half-sib design used in this study also has the merit of being free from sources of common environmental variance including maternal effects which are often a major source of bias in estimating genetic correlations. However, the large number of individuals measured was insufficient to obtain precise estimates. Sample sizes of one to two thousand may be required to obtain a clear measure of the genetic correlation, which would clearly be impossible to achieve in many species. In contrast, selection experiments need to be of least three of four generations duration. The selection program in this study did not provide much additional quantitative genetic information beyond the response and correlated response themselves. A major problem with selection experiments is that even responses over a small number of generations may quickly become unrepresentative of the base generation due to changes in gene frequency during the course of the experiment. Even more significantly, selection may result in changes in several traits making it difficult to attribute any one trait response to the selected trait itself. One of the main advantages of the selection experiment was that sample

size required was smaller than that for the breeding experiments allowing data to be gathered more easily for a given level of precision in the results. This means that this approach may be applicable to a wider range of species. This is enhanced by the fact that only a sample of all selected individuals have to be measured allowing scope to maintain populations at size that preclude serious inbreeding effects (Hill, 1971). Although selection experiments may generate less quantitative genetic statistics than breeding experiments, they furnish a wealth of additional information of a phenotypic nature that is perhaps of greater use if the question being asked is whether or not a trade-off is operating between a trait pair. The effective manipulation of egg size in this study yielded some of the most interesting results. In summary, breeding experiments can provide a greater sum of information on trait variation relevant to interpretation of genetic correlation estimates which are useful to genetic studies of life history evolution. However, selection experiments can be argued to provide more information relevant to phenotypic questions about the mechanics of existing trade-offs. This is because they provide not only a measure of the genetic correlation but also facilitate direct observation of the effects of changes in one trait in response to changes brought about in another.

8.1.3 Measurement of trade-offs in the future

The employment of breeding and selection experiments in this study has been based on long established analytical techniques described by Becker (1984) and Falconer (1989) that were developed originally for use in selective breeding. Recent developments in statistics and computing have opened up more powerful analytical methods that are only now being adopted by quantitative geneticists, and have yet to be fully utilised by life history pheneticists. The main problem with the methods used here is the large standard errors of genetic variances.

Non-parametric resampling techniques such as the Jacknife (Arvensen & Schmitz, 1970; Miller, 1974) and Bootstrap (Efron, 1982) offer alternative ways of obtaining more precise estimates. Furthermore, use of restricted maximum likelihood estimation (REML) (Patterson & Thompson, 1971; Henderson, 1988) can replace least squares analysis altogether and generally offers a more flexible analysis of genetic estimates (Shaw, 1987). However these methods offer only improved precision and robustness in estimates of genetic correlations which may be of great value to evolutionary geneticists, but for pheneticists interested in trade-off curves knowledge of the genetic correlation is only a means to an end.

Quantitative genetic methods were employed in this study because egg size could not be directly manipulated. Perhaps more significant than the improvements in analytical techniques for future research is the recent advances in developmental and physiological manipulative methods (Bernardo, 1991; Parker & Maynard Smith, 1990) discussed in Chapter 7. These may ultimately be applicable to a wide range of species including *C. maculatus*. Direct manipulation remains the most promising method for testing optimisation models and investigating trade-offs (Charnov, 1989). Ultimately, responses to experimental manipulations may throw more light on long term evolutionary processes than quantitative genetics as results from the latter are always restricted by their high specificity (in terms of population gene pool and environment (Stearns, 1989)) and can only have short term interpretive and predictive value. The scope for future empirical studies of trade-offs remains a largely unexplored landscape.

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Appendix A

Operational definitions of measured traits and limits of lowest precision.

Trait	Definition	Precision
Egg length	diameter along longest axis	± 12μm
Fecundity	total no. of eggs laid by a female during her lifetime when provided with an excess of host beans (> 25)	± 1egg
Development rate	1/development period development period is the time elapsed from oviposition to emergence of the adult from the host bean	± 48h
Longevity	adult lifespan from emergence until death	± 48h
Residual dry body weight	dried weight of carcass after natural death	± 0.26mg

Appendix B

Schematic plan of the paternal half sib design used to estimate heritabilities and genetic correlations of six life-history traits. The 30 sires were mated in succession to 3 females each. The life-history traits were recorded from 8 female progeny from each of these matings. A single sire family is shown.