Enzyme variation at morphological boundaries in *Maniola* and related genera (Lepidoptera: Nymphalidae: Satyrinae)

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frontispiece 1

Butterflies of the tribe Maniolini - males

(top left to bottom right)

Maniola jurtina - Easdale Island, Argyll, Scotland
M. jurtina (underside) - Easdale Island, Argyll, Scotland
M. chia (paratype) - Kardamila, Chios, Greece
M. megala - Marmaris, Aydin Province, Turkey
M. telmessia - Filerimos, Rhodes, Greece
M. cypricola - Trikoukka, Cyprus
M. nurag - Nuoro, Sardinia
Pyronia tithonus - Le Touquet, Pas de Calais, France
P. bathseba - Calaspara, Alicante, Spain
P. cecilia - Valence, Drome, France
Aphantopus hyperantus - Glen Farg, Perthshire, Scotland
Hyponephele lycaon - La Grave, Hautes Alpes, France
H. maroccana - Oukaimeden, High Atlas, Morocco
H. lupina mauritanica - Azerbaidjan, Iran
H. lupina rhamnusia - Kalavrita, Greece
Cercyonis pegala - Don Valley, Toronto, Ontario

frontispiece 2

Butterflies of the tribe Maniolini - females

(top left to bottom right)

Maniola jurtina - Easdale Island, Argyll, Scotland
M. jurtina (underside) - Easdale Island, Argyll, Scotland
M. chia (paratype) - Nea Moni, Chios, Greece
M. megala - Perge/Aksu, Antalya, Province, Turkey
M. telmessia - Filerimos, Rhodes, Greece
M. cypricola - Platres, Cyprus
M. nurag - Nuoro, Sardinia
Pyronia tithonus - Silecroft, Cumbria, England
P. bathseba - Fountain de Vaucluse, Vaucluse, France
P. cecilia - Gadagne, Vaucluse, France
Aphantopus hyperantus - Kintore, Aberdeenshire, Scotland
Hyponephele lycaon - La Grave, Hautes Alpes, France
H. lupina mauritanica - Platres, Cyprus
H. lupina rhamnusia - Kalavrita, Greece
Cercyonis pegala - Don Valley, Toronto, Ontario

frontispiece 3

Enzyme electrophoretic banding patterns in Maniolini

(top left to bottom right)

Diaphorase (DIA-1, -2): DIA-1 (below) - 1-2 jurtina 100/100, 3 jurtina 100/144, 4 tithonus 141/141, 5 bathseba 141/141, 6 cecilia 141/141, 7 hyperantus 141/141, 8 lycaon 80/100, 9-12 jurtina 100/100: DIA-2 (above) - 1 jurtina 100/113, 2 jurtina 100/100, 3 jurtina 100/100, 4 tithonus 113/113, 5 bathseba 113/113, 6 cecilia 113/113, 7 hyperantus 100/100, 8 lycaon 100/100, 9 jurtina 100/113, 10-12 jurtina 100/100

D(-)3 glycerophosphate dehydrogenase (D(-)3GPDH): 1-2 jurtina 100/100, 3 cypricola 90/93, 4 nurag 93/93, 5 chia 112/112, 6-14 jurtina 100/100

Hexokinase (GK, MK): GK (below) all jurtina - 1-6 100/100, 7 100/109, 8-11 100/100, 12 100/100, 13 100/100, 14-15 100/109: MK (above) - all jurtina - 1-18 100/100

Glutamate oxaloacitate transaminase (GOT-1): all jurtina (1-5 females, 6-23 males) - 1-5 100/100, 6 114/114, 7 100/114, 8-9 114/114, 10 100/100, 11-13 114/114, 14 100/100, 15 100/114, 16 114/114, 17 100/100, 18-20 114/114, 21 100/100, 22 114/114, 23 100/100

Glutamate oxaloacitate transaminase (GOT-1): 1 jurtina 100/100, 2 jurtina 100/114, 3 tithonus 93/93, 4 bathseba 100/100, 5 cecilia 100/100, 6 hyperantus 150/166, 7 lycaon (E) 86/86, 8 lycaon (W) 114/114, 9 lupina rhamnusia 93/93, 10 lupina mauritanica 93/114, 11 maroccana 114/114, 12 pegala 84/84

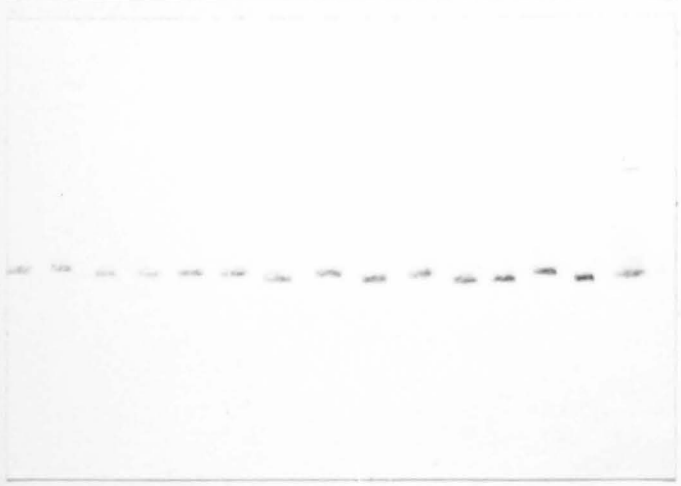
Isocitrate dehydrogenase (IDH-1, -2): IDH-1 (below) all jurtina - 1 44/100, 2-13 100/100: IDH-2 (above) all jurtina - 1-6 100/100, 7 76/100, 8 100/118,9-13 100/100

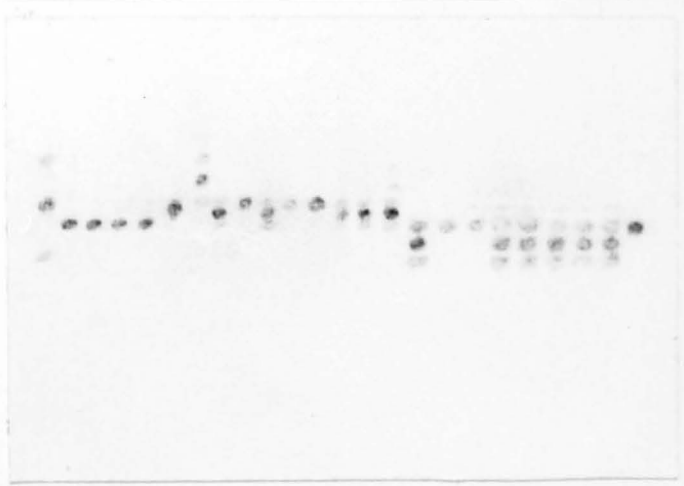
Leucine aminopeptidase (LAP-2): 1 jurtina 100/100, 2-6 jurtina 100/115, 7-8 jurtina 100/100, 9 jurtina 100/115, 10-12 telmessia 90/100, 13-14 telmessia 90/90, 15 telmessia 90/100, 16 telmessia 90/90, 17 telmessia 90/100, 18 telmessia 70/90, 20-23 jurtina 100/100, 24 jurtina 70/115

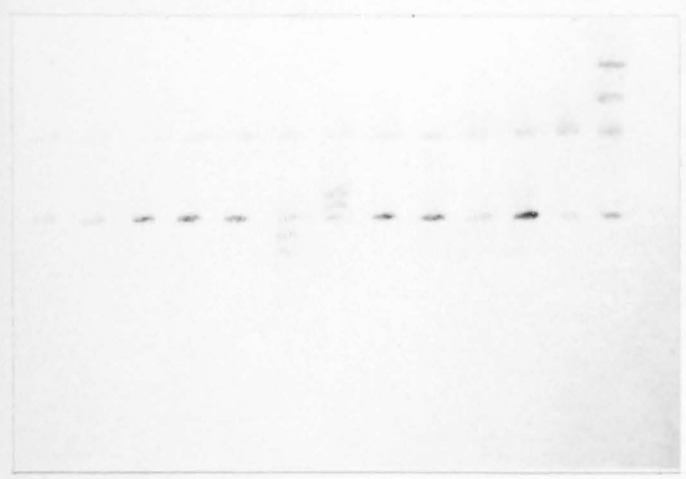
Malic enzyme (ME-1, -2): ME-1 (below) - all jurtina - 1-15 100/100: ME-2 (above) - all jurtina - 1-15 100/100 showing 'mobility flux'

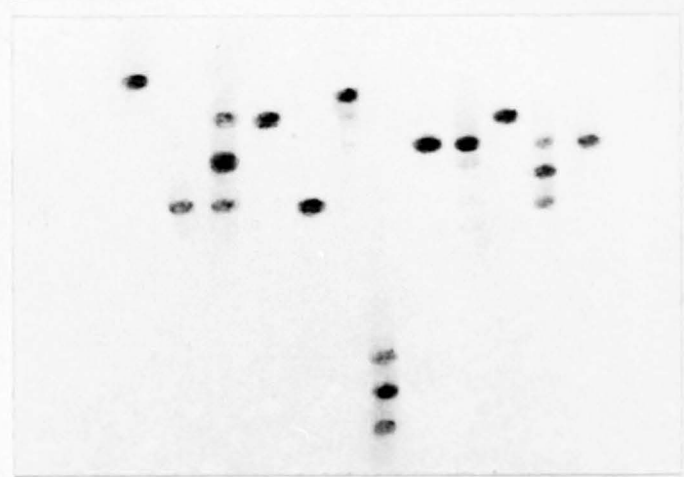
Phosphoglucose isomerase (PGI-2): 1 jurtina 100/100, 2 jurtina 60/100, 3 jurtina 22/100, 4 jurtina 90/119, 5 cecilia 178/178, 6 lupina mauritanica 100/100, 7 lycaon (W) 219/219, 8 lupina rhamnusia 219/219, 9 pegala 240/333

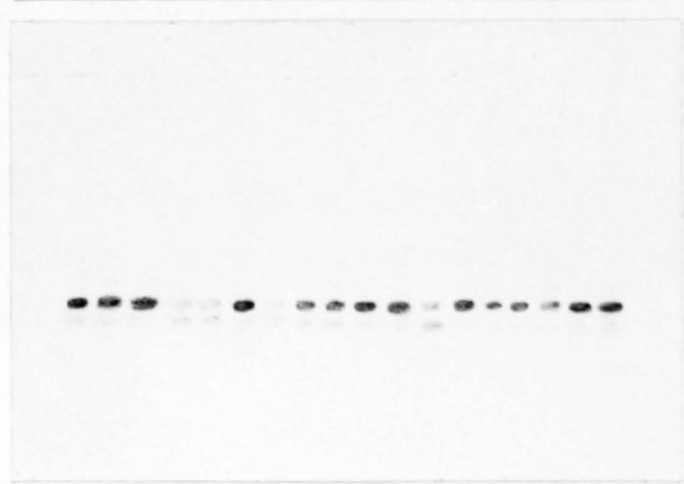
Phosphoglucomutase (PGM): all jurtina - 1 54/100, 2 60/100, 3 78/78, 4 84/100, 5 88/88, 6 96/100, 7 100/100, 8 100/100, 9 100/103, 10 100/113, 11 121/121, 12 121/128, 13 100/100





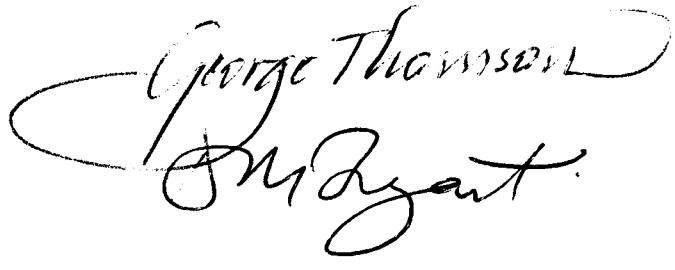






DECLARATION

I declare that this thesis has been composed by myself and that it embodies the results of my own research and its contents have not been included in another thesis. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others and included in the thesis.

Candidate 

Supervisor

CONTENTS

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I acknowledge the generous support of Dr Roger Dennis, especially in developing my still embryonic understanding of some new applications of multivariate statistics and I thank Dr Robert Williams for practical help in running mainframe applications.

Material in the form of live specimens was received from friends and colleagues, too numerous to list, who went to considerable effort on my behalf to send live butterflies for electrophoresis. In particular I should mention Mr Bert Van den Brink, Mr John Coutsis, Dr David Gaskin, Mr Mark Hope, Mr Ian Lorimer, Dr Ayten Kirmiz, Dr Otto Magyar, Dr A. Mohles, Mr Alain Olivier, Squadron Leader Robert Parker, Mr Alex Riemis, Mr Paul Sammut and Dr Roberto Villa.

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I have to thank the Trustees of the Carnegie Trust for the Universities in Scotland for financial help towards collecting trips to Europe and the Greek islands.

Finally, I have to mention my long suffering wife, Elizabeth, who, over a period of six years which included two house moves supported me without reservation.

ABSTRACT

The evolutionary biology of 14 species of Maniolini (Nymphalidae: Satyrinae) was studied.

Electrophoretic analysis of 35 enzyme loci identified a larger number of alleles than and levels of polymorphism similar to those found in other Lepidoptera.

In Maniola jurtina, some populations exhibited a massive heterozygote deficit and sex associated allele frequency differentiation at the GOT-1 locus. Allele frequencies in pre- and post-aestivation jurtina from southern Europe were significantly different. At some loci, significant annual differences in allele frequencies were noted.

A significant correlation between geographic and genetic distance in allele frequencies was observed, but no correlation was detected between heterozygosity and land area in insular populations.

Cluster analysis and nonmetric multidimensional scaling performed on electrophoretic data from populations of Maniola jurtina revealed a dichotomy between 'Eastern' and 'Western' subspecies groups. The analysis of Manioline species fitted existing taxonomies.

Genetic differences between Maniola species were much smaller than those between Pyronia and Hyponephele species.

Ultrastructural studies of the Maniola Jullien organs revealed a species-specific tooth pattern on the inner surfaces. It is suggested that these structures may be sound production mechanisms of great evolutionary significance to the species.

Maniolini ova were studied and it is suggested that their form and chorionic sculpturing owe much to selection induced by oviposition strategy.

Chaetotaxy of first instar larvae was undertaken and morphometric analysis of setal lengths was found to be useful, but not unambiguous.

Multivariate analysis of chaetotaxy data showed a significant correlation with electrophoretic data.

The evolution and zoogeography of Maniola is discussed. It is suggested that disjunction, founder effect, rapid post-glacial colonisation and bottlenecking have played a major roles in effecting rapid speciation. It is further suggested that all Maniola species are very recent, perhaps having evolved within the last 50,000 years, and some species almost certainly have evolved in postglacial times.

Abbreviations

Abbreviations not explained in text

ADP	adenosine-5-diphosphate
ATP	adenosine-5-triphosphate
EDTA	ethylenediaminetetra-acetate
G6PDH	glucose-6-phosphate dehydrogenase
HK	hexokinase
MTT	thiazolyl blue (tetrazolium salt)
NAD (NAD^+)	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADP ($NADP^+$)	nicotinamide adenine dinucleotide phosphate
NP	nucleoside phosphorylase
OTU	operational taxonomic unit
PMS	phenazine methosulphate
tris	tris(hydroxymethyl)aminomethane
XOD	xanthine oxidase

1. INTRODUCTION

1.1 Evolutionary problems

Before this research was undertaken a number of fundamental questions remained unanswered within the Maniolini, a group of butterflies popularly called 'meadow browns'. These were problems of evolutionary, genetic and phylogenetic importance which were not fully understood within this group. It was considered important to determine the broad relationships of species and genera to each other: it was equally important to attempt to identify the evolutionary processes which have led to complex intra- and interspecific variation, especially in the six species of Maniola, which is seen in these butterflies and which offered a great opportunity to investigate these matters.

Maniola jurtina is a species which has attracted the attention of evolutionary biologists (Scali 1971a, 1971b, 1972, Masetti and Scali 1972, 1974, Scali and Masetti 1973, 1975, 1979, Dowdeswell 1981, Brakefield 1979a, b, 1984) and is a species exhibiting outstandingly interesting biological phenomena. It has been the subject of detailed investigations into possible sympatric evolution (Creed, Dowdeswell, Ford and McWhirter 1959) and major studies in ecological genetics (see Dowdeswell 1981).

The species poses a number of problems, the solutions to which might lead in some way towards answering more fundamental questions of phylogenetic relationships and the mechanics of evolution in these butterflies.

The distribution of geographical forms of jurtina is complex (figure 1.1a). Over most of north-eastern, central and south-eastern Europe phenotypic expression in the species is similar, with little variation between populations across vast distances (the subspecies janira L., phormia Fruhstorfer and strandiana Obtraztsov). From central and southern Italy eastwards, jurtina is found in a mosaic of forms, some of which are large (ghilanica Le Cerf, persica Le Cerf, corfiothispulla Graves, and some north-western Turkish races), while others approach the large, brightly coloured phenotypes typical of south-

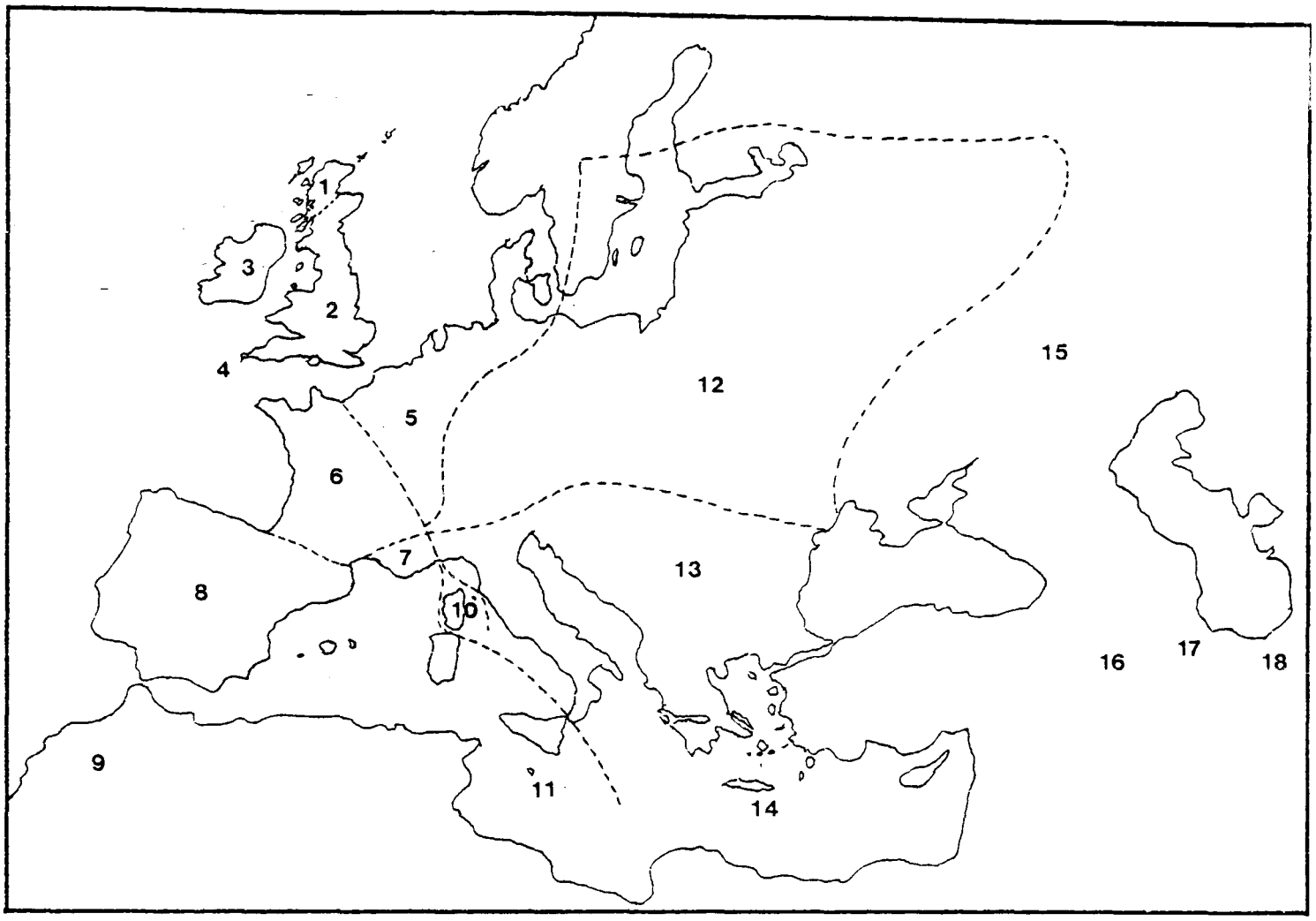


figure 1.1a

Geographic subspecies, races and forms of Maniola jurtina.

1. splendida
2. insularis
3. iernes
4. cassiteridum
5. mirtyllus
6. occidentalis
7. miscens
8. hispulla
9. jurtina
10. emihispulla
11. hyperhispulla
12. janira
13. phormia/praehispulla
14. corfiothispulla
15. strandiana
16. persica
17. ghilanica
18. iranica

western Europe (forms praehispulla Verity, emihispulla Verity, some Greek and Turkish populations). Some of the more northern and western forms (miscens Verity, myrtillus Fourcroy and occidentalis Pionneau) have a superficial resemblance to janira and have been, in the past, considered indistinguishable from it (Graves 1930), although Thomson (1969) has pointed out that there are important differences between these subspecies. In south-western Europe and north Africa, the races miscens Verity, hispulla Hubner, fortunata Alpheraky, hyperhispulla Thomson and, to a lesser extent, cantabrica Agenjo are very large and characterised by extensive fulvous marking in the female. Similar, possibly not homologous, expression is seen in the 'Atlantic' races splendida White, iernes Graves and cassiteridum Graves (Graves 1930).

The occurrence of large, brightly coloured forms throughout southern Europe and Asia Minor has led some Lepidopterists to believe that 'hispulla-form' races belong to a quite distinct species (De Lattin 1950, 1958, Bernardi 1966), but the work of Higgins (1969) and, more decisively, Thomson (1973b, 1976) on genitalia form has shown that the species comprises two distinct eastern and western groups with an intermediate zone between. This variation, found in the male genital armature, was first recognised by Le Cerf (1912, 1913). He examined male genitalia in the far eastern part of the species range (subspecies persica and ghilanica) and found differences between them and what he called jurtina, fortunata and hispulla, although it has since been shown that his 'typical jurtina' were in fact intermediate (Thomson 1973b) and his persica were typical of the eastern form. Muschamp (1915) observed that the genitalia of what he called jurtina and hispulla from southern Europe differed from each other. When De Lattin elevated the hispulla group (from south-west Europe) to species rank, his illustrations of the 'jurtina-type' genitalia, now more correctly called janira (Thomson 1971b), were in fact hispulla-form. Nor did his map (De Lattin 1958) correspond to what is now known of the distribution of the subspecies groups. Details of the supposed distribution of the genitalia types were given by Bernardi (1961, 1966) who confused the issue somewhat, as he clearly did not appreciate fully the individual variability of the structure and, as his findings were based on a few specimens, the value of his work is greatly reduced. Bernardi believed that the range of the 'hispulla-type' extended from the Canary Islands and north Africa, through the

Iberian peninsula to south France and including the Balearic Islands, Corsica, Sardinia, Sicily and Malta. The remaining area of the butterfly's range (and also Corsica) he claimed was populated by the 'species' janira, excepting that part occupied by megala. Higgins (1969) concluded that the range of the 'old hispulla' genitalia form was more westerly than had been presumed, extending from the Canary Islands, through north Africa, Sicily, Corsica, Sardinia, south and west France to Great Britain. The eastern ('jurtina' ie janira) type he reported as being found in Finland, Austria, Italy and eastwards. Between the two regions he found an area in which the two types 'fuse'. Tauber studied the genus in the 'east' (Tauber 1968, 1969, 1970). His work raises some interesting questions, particularly those concerned with the origin of the genus Maniola. Unfortunately, like those of Bernardi, his samples were inadequate.

The results of the work of Thomson (1973b, 1975, 1976) on the genitalia of jurtina are summarised in the map (figure 1.1b). This is compared with that of Bernardi and De lattin. The statistical basis for the map and a discussion on the evolution of the group can be found in Thomson (1973b).

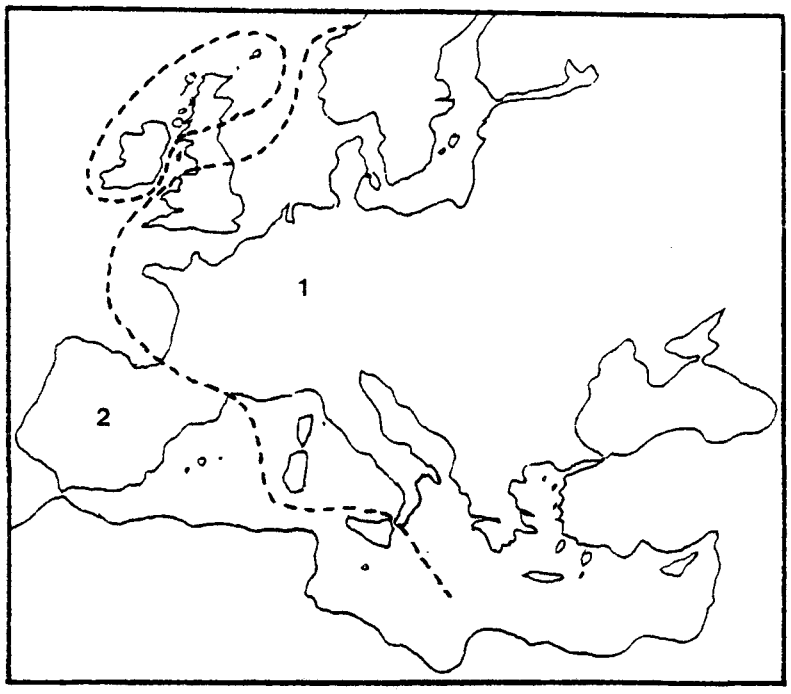
The following problems are raised by these studies.

1. Why does jurtina form phenotypically, and possibly biochemically, very similar populations over many hundreds of kilometers, yet, in some parts of its range, there are sudden changes from one subspecies to another. For example, on the basis of genitalia form, jurtina is 'transitional' in Corsica and Elba and 'western' in Sardinia, yet it is 'western' in Capri only a few hundred meters from the Sorrento mainland where jurtina is 'eastern'.

2. What is the nature of the 'eastern' and 'western' subspecies groups - does the jurtina complex comprise one species with two main geographical forms, or are the two geographical forms closely related sibling species? This poses questions of the configuration of the 'transitional zone' between the major groups. Is this zone some sort of area of genetic disturbance, or is it a zone of secondary intergradation between previously allopatric forms?

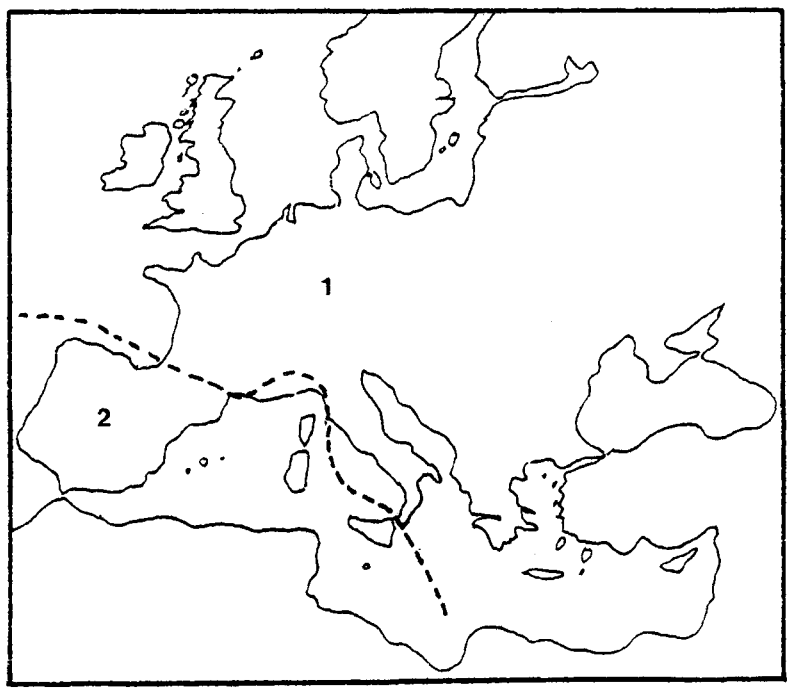
figure 1.1b

The taxonomy of the Maniola jurtina group according to De Lattin (1958), Bernardi (1966) and Thomson (1973).



De Lattin (1958)

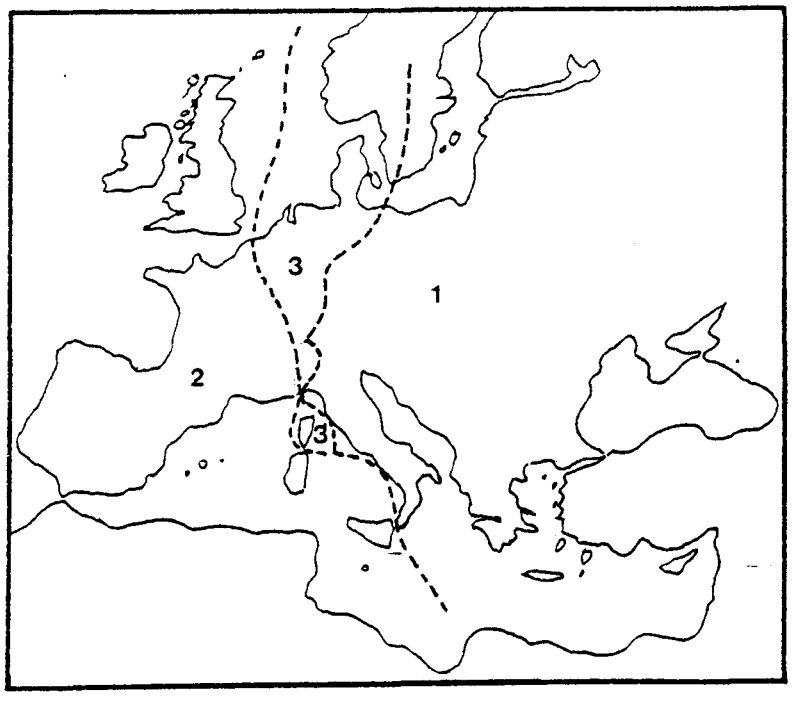
1. Maniola jurtina
2. Maniola hispulla



Bernardi (1966)

1. Maniola janira
2. Maniola hispulla

(sympatric in Corsica)



Thomson (1973)

1. 'Eastern'
2. 'Western'
3. 'Transitional'

(all Maniola jurtina)

3. What are the factors which have led to the four subspecies evolving in the British islands?

From the time of its description in 1852, Maniola nurag was recognised as specifically distinct from jurtina. However, telmessia was associated with jurtina as a subspecies and cypricola was considered to be a form of either telmessia or jurtina. Graves (1928) argued the specificity of cypricola. Maniola telmessia is more usually recognised as an allopatric species (Higgins 1975). Until this research, little was known about the status of Maniola megala and only a few specimens existed in collections. In the course of this study the nature of sympatry and allopatry in Maniola species was clarified to a great extent. M. jurtina and telmessia have been found sympatrically in a very few localities in southern and eastern Turkey (Van den Brink and Van Oorschot 1986), but it has become evident that the ecological requirements of the two species could be different and that this, perhaps more than some other aspect of its biology, is important in maintaining allopatry (Van den Brink pers. comm.). Two males with aberrant genitalia housed in the Instituut voor Taxonomische Zoologie, Zoological Museum, Amsterdam, might represent jurtina/telmessia hybrids. Maniola megala and telmessia are commonly sympatric and appear to fill the same ecological niche. The two species fly together in the same parts of southern and western Turkey (and Lesbos), at the same time, probably utilising the same larval hostplants and aestivating in the same sites. It is indeed possible that the form of the male genitalia in these species represents a case of character displacement. No instance of sympatric jurtina, telmessia and megala is known.

One of the aims of the study was to conduct detailed investigations into interspecific relationships in Maniola, on morphological evidence a group of very closely related species, which includes the widespread jurtina, and five others, some of which are sympatric with jurtina, others island endemics.

As well as considering the relationships of jurtina, telmessia, cypricola, nurag and megala to each other, their relationships with other Manioline species were to be examined.

In the course of these studies, a number of other relevant evolutionary situations were investigated, including the genetics of pre- and post-aestivation populations of jurtina (2.2.2), the effects of altitude (2.3.1), population structure (2.3.2), the genetics of populations on islands (2.4.2), wing marking expression (3.1.1-3), scale morphology (3.1.4), genitalia variation (3.1.5), possible mate location mechanisms (3.1.6), embryonic and pre-imaginal morphology (3.2), the correlation between and relative value of data from biochemical and various types of morphological analysis (4.1), the mechanics of speciation and biogeography in Maniolini (4.2.2).

In Lepidoptera there has not been a study of a major group which has utilised a range of biochemical and morphological techniques. Although evolutionary problems in some major butterfly groups have been tackled, there has been a dichotomy between those based on biochemical genetics (McKechnie et al. 1975, Brussard and Vawter 1975, Vawter and Brussard 1975, Brittnacher et al. 1978, Angevine and Brussard 1979, Geiger 1978, 1980 (1981), 1982, Courtney 1980, 1982, Tebaldi 1982, Geiger and Rezbanyai 1982, Geiger and Scholl 1982a, 1982b, 1984) and morphology (Miller 1968, Eliot 1973, Kitching 1984 and studies based solely on wing markings and/or genitalia, which are too numerous to cite). Fortunately, there is an increasing awareness of the value and importance of approaching evolutionary, and in particular systematic problems from many angles, using a variety of techniques and methods.

Arguments about the relative value of electrophoretic and morphological analyses have verged on the fanatical. More detailed work using these methods has shown that some aspects of morphology, eg genitalia and chaetotaxy (Thomson 1973b, Kitching 1984) appear to be relatively free from environmental influences and therefore reflect the phenotypic and genetic characteristics of an individual, population or species. On the other hand, environmental and physiological changes can result in differential expression of isozymes (Massaro and Booke 1971, Shaklee et al. 1977, Ogihara 1975). It is probably true to say that morphological analysis is more prone to environmental and other external effects than electrophoresis. The effects of temperature and photoperiod on wing pigmentation in some butterflies is well known (see section 3.1.1). Adult behaviour, especially oviposition regimes,

almost certainly affect some aspects of pre-imaginal morphology (see section 3.2.2). However, with appropriate caution, the value of morphological data should not be underestimated. Biochemical analysis utilises proteins which are the primary gene products and have the potential advantage that the genetics of the protein systems is known. It is likely, therefore, that the molecular structure is determined genetically, with relatively little chance of environmental or other influences (Avise 1974, Lewontin 1974, Ayala 1976, Berlocher 1984).

While there are a number of theoretical advantages in the use of electrophoretic data compared with that obtained by conventional (morphological) methods - objectivity and precision, relative constancy with age or sex, deducible genetic information - there are also some disadvantages. Avise (1974) lists these as chance identity of band mobility, difficulties in scoring, non-detected protein differences, non-identified mutational steps and biased sampling of genes.

A broad approach has been taken in this study to the analyses of variation in Maniolini, with more detailed investigations being applied to the very specific evolutionary situations mentioned previously. The utilisation of scale morphology, genitalia morphology, egg morphology and larval chaetotaxy, together with electrophoresis, presented a rare opportunity to resolve evolutionary problems in a complex group of butterflies.

1.2 The study organisms

At the foot of a ladder in the centre of the right hand 'Hell' panel of Hieronymous Bosch's Garden of Earthly Delights painted sometime between 1485 and 1510 a bird-like creature with butterfly wings clings precariously to one of the rungs. The wings are not painted but collaged (possibly unique for that period) and are the real wings from at least one female meadow brown butterfly. Not only is this the earliest illustration of the insect we now know as Maniola jurtina, it can also be considered to be the earliest known 'specimen' of a butterfly in existence. In 1634, Moufet in his Insectorum Theatrum

illustrates jurtina by crude woodcut, although the more accurate original watercolour paintings, from which the woodcuts were made, are housed in the British Library. The translation of Moufet's work appeared as part of Topsell's History of Serpents (1658). The description of jurtina reads

> '...It seems inside and outside all alike; the head and wings look pale; the body is wan, as also the horns; the eyes are flaming red, the shoulders hairy with a pale down.'

Little did these Renaissance men realise that the butterfly and its close relatives would be arguably the most interesting butterfly in evolutionary biology.

Linnaeus (1758) described the male and female jurtina as two distinct species, jurtina and janira.

> "jurtina. 104. P. N. alis subdentatis fuscis:
> primoribus supra litura flava ocello utrinque unico...
>
> ...
>
> Janira. 106. P. N. alis dentatis fuscis: primoribus subtus luteis ocello utrinque unico; posticis subtus punctis tribus..."

The tribe Maniolini Hampson (1918) (Lepidoptera, Satyridae) approximates to the old genus Epinephele Hubner [1819](1816-1826) - type species Papilio janira L. (= Papilio jurtina) designated by Butler (1868). It is a useful, if rather untidy taxonomic group, which has been treated differently by various workers (De Lesse 1952, Miller 1968, Higgins 1975). The majority of the species belong to three generally recognised Palaearctic genera, Maniola Schrank (1801) - type species Maniola lemur (= Papilio jurtina L.), designated by Scudder (1875), Pyronia Hubner [1819](1816-1826) - type species Pyronia tithonus Hubner (= Papilio tithonus L.), designated by Scudder (1875) and Hyponephele Muschamp (1915) - type species Papilio lycaon Rottemburg by monotypy. Two other genera have been treated as members of

the tribe by some workers. Aphantopus Wallengren (1853) - type species Papilio hyperantus (misspelled 'hyperanthus') L. by monotypy was included by Higgins (1975) and the Nearctic Cercyonis Scudder (1875) - type species Papilio alope Fabricius (= Papilio pegala Fabricius) by original designation was included by De Lesse (1950). Aphantopus has been placed within the distantly related tribe Coenonymphini by Miller (1968).

The separation of Epinephele into several genera (De Lesse 1950) was based on the structure of the male and female genital armature. Preliminary electrophoretic analysis showed that both Aphantopus and Cercyonis were well placed in the Maniolini and these genera were, in consequence, included in the study (table 1.2a).

The genus Maniola includes six western Palaearctic species, one widespread species (or species complex), jurtina L. (1758) and five peripherally distributed species, megala Oberthur (1909), chia Thomson (1987), telmessia Zeller (1847), cypricola Graves (1928) and nurag Ghiliani (1852). Pyronia includes four species, the widespread tithonus L. (1771), the south-west European bathseba Fabricius (1793) and cecilia Vallantin (1894), and janiroides Herrich-Schaefer (1852), confined to Africa north of the Atlas Mountains, which was not available for this study. There are over 20 Hyponephele species, mostly from central Asia (Wyatt and Omoto 1966, Sakai 1980). Three western Palaearctic species were included in this research, the southern European lycaon Kuhn (1774) and lupina Costa (1836) and maroccana Blanchier (1908) from Morocco.

Aphantopus hyperantus L. (1758) and the widespread north American Cercyonis pegala Fabricius (1775) represented their genera in this research.

Maniola jurtina is distributed throughout Europe, western North Africa and parts of the Middle East, from the Irish Aran Islands in the west to east of the Caspian Sea in the east, and from central Scandinavia in the north to the Canary Islands in the south. It occurs on virtually all of the islands in the Mediterranean area except the Aegean Dodecanes and some of the Northern Greek Islands where is replaced by telmessia and chia, and Cyprus in which cypricola is the

table 1.2a The study organisms

tribe	genus	species
Maniolini	Maniola	jurtina
		megala
		chia
		telmessia
		cypricola
		nurag
	Pyronia	tithonus
		bathseba
		cecilia
		[janiroides]
	Aphantopus	hyperantus
	Hyponephele	lycaon
		lupina
		maroccana
		[+ about 20 other species]
	Cercyonis	pegala
		[+ about 6 other species]

indigenous endemic Maniola species. M. telmessia largely replaces jurtina in southern and western Turkey, although jurtina still occurs sporadically in that area (Van den Brink and Van Oorschott 1986, Thomson, this study). It occurs in Syria, Lebanon, Jordan, northern Israel, parts of lowland Iran and possibly northern Iraq. Recent discoveries resulting from this research have shown that M. megala is much more widespread than was thought, flying sympatrically with telmessia in southern and western Turkey as well as on the island of Lesbos. The recently described species, M. chia, was discovered on the island of Chios as a result of this research. Maniola cypricola is the only species of the genus in Cyprus. Maniola nurag is restricted to Sardinia, where it flies at moderate altitude with jurtina (Simmonds 1930) (figure 1.2a).

Pyronia tithonus is found from western Europe, including Spain to Asia Minor and the Caucuses. It is widely distributed in western, central and southern Europe including southern Ireland, Britain, Sardinia, Corsica and Elba, but does not occur on the other Mediterranean islands. The ranges of bathseba and cecilia are much more restricted and southern, the former species being found only in north Africa, Spain and south-eastern France, while cecilia can be found in most regions bordering the Mediterranean (Higgins and Riley 1980).

Aphantopus hyperantus is distributed throughout most of the western Palaearctic region except the far south. It does not occur in northern Africa (Higgins and Riley 1980).

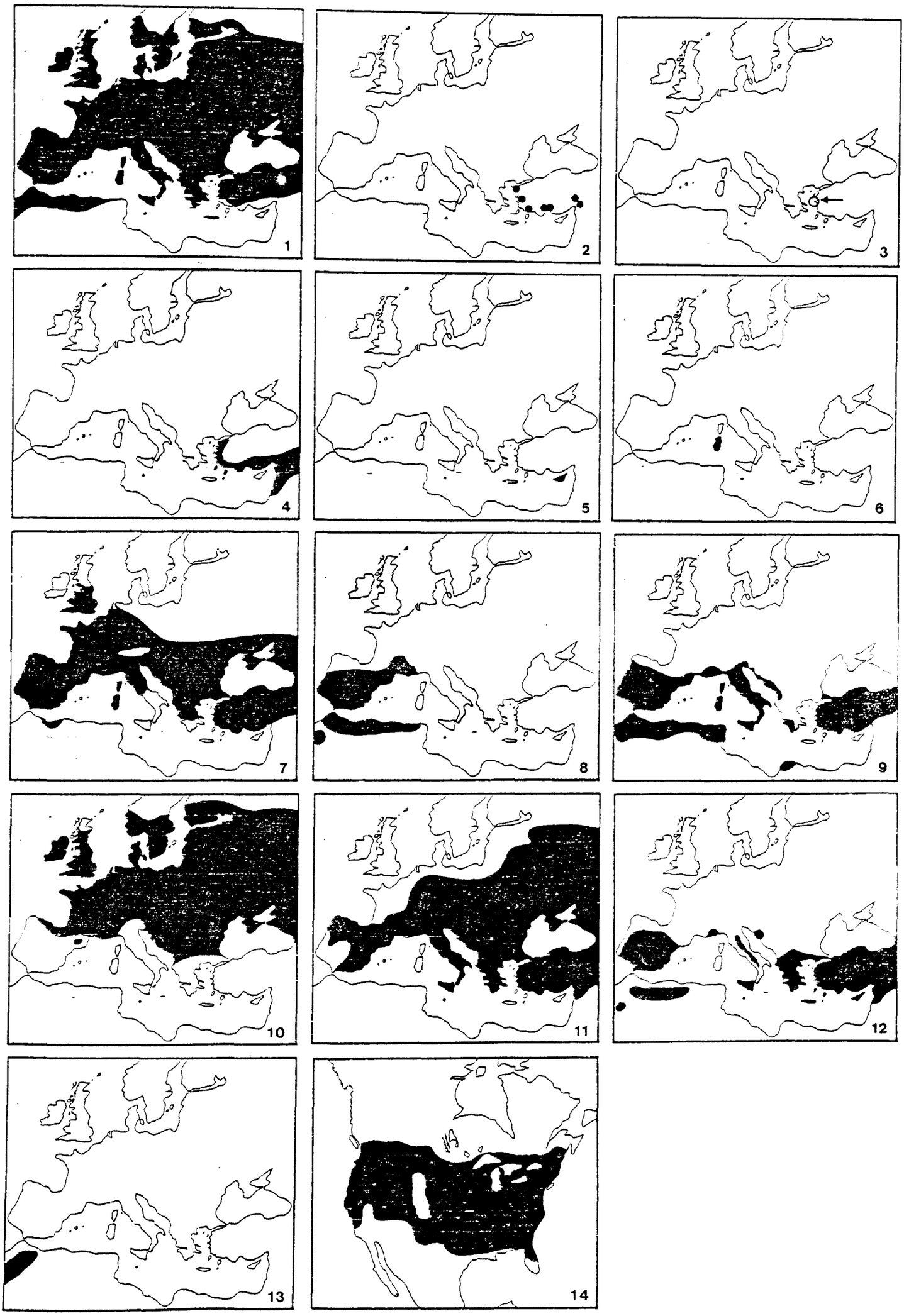
The range of Hyponephele lycaon covers much of Spain, throughout most of southern and eastern Europe and eastwards to Asia. The distribution of the species in Asia is not fully known as many of the forms previously ascribed to lycaon have now been described as distinct species. Similar problems inhibit the determination of the range of lupina, which is found sporadically in most southern parts southern Europe and Asia. The north African maroccana is restricted to the Atlas Mountains of Morocco (Higgins and Riley 1980).

Cercyonis pegala is widespread throughout most of north America except the far south. It does not penetrate beyond the Canadian zone (Emmel 1969).

figure 1.2a

The distribution of the western Palaearctic species of Maniolini.

1. Maniola jurtina
2. Maniola megala
3. Maniola chia
4. Maniola telmessia
5. Maniola cypricola
6. Maniola nurag
7. Pyronia tithonus
8. Pyronia bathseba
9. Pyronia cecilia
10. Aphantopus hyperantus
11. Hyponephele lycaon
12. Hyponephele lupina
13. Hyponephele maroccana
14. Cercyonis pegala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Maniola jurtina and telmessia, Aphantopus hyperantus, Pyronia tithonus, Hyponephele lycaon and lupina have haploid chromosome numbers of n=29 (Lorcovic 1941, Larsen 1975, Higgins 1975), the modal number for Satyridae (Robinson 1971). Pyronia bathseba has a haploid number of n=33 and cecilia n=28. Tauber (1970) speculated that Maniola megala was polyploid because of its size, but, in the light of the present study, this is unlikely. The chromosome numbers of other species have not been determined.

All species fly in a range of grassland biotopes, although some , eg telmessia, megala, Hyponephele, are tolerant and possibly prefer dry, even sunbaked habitats. In the hotter parts of southern Europe and Asia, Maniola utilise scrub, light woodland, caves and rock crevasses as shelter in the hottest parts of the day (Larsen 1974, Parker 1983, Thomson 1985).

Although Maniola nurag in Sardinia inhabits the mountainous areas only, the genus must be considered as a lowland one. M. jurtina's normal altitudinal tolerance is from sea level to 1000m, although in some parts of the southern Alps it can be found at elevations in excess of 1700m (Thomson 1973b). M. telmessia does not appear to occur above 1000m (Thomson, this study) and prefers sites at lower levels. In spite of Tauber's arguments to the contrary (Tauber 1970), megala is not a montane species (Thomson, this study). Similar altitudinal restrictions appear to be the rule in the case of the other Manioline genera, although Pyronia bathseba is found at 1700m in the High Atlas mountains, while lycaon and lupina form colonies at very high altitudes in Turkey (Van Oorschot pers. comm.). Most species of Hyponephele are high altitude forms, occurring at elevations in excess of 4500m in central Asia (Sakai 1980).

The life cycles of all species in this group are not known. All species appear to hibernate as young larvae. The first instar larvae of Hyponephele and Cercyonis do not feed on hatching from the ovum and have their first meal after hibernal diapause: those of Maniola, Pyronia and Aphantopus feed immediately on hatching and hibernate in the early instar stages (Thomson, present study). Cercyonis, and presumably all Hyponephele species, achieve six instars (Emmel 1969). Pyronia and Aphantopus have five (Howarth 1973). The number of larval

instars differ in the genus Maniola. M. jurtina undergoes six instars, but nurag (Simmonds 1930) and telmessia (Van den Brink pers. comm.) have only five.

The life cycle of jurtina is better known than any other species in the group. It is reasonably typical of other species of the genus and has much in common with that of the other genera. Eggs are laid singly on grasses, throughout the summer months. They are more or less fixed to the foodplant or nearby surfaces. The young larvae hatch out and feed slowly through to the third instar (cf. Hyponephele and Cercyonis) and the onset of hibernal diapause. Feeding is diurnal at this stage and changes to nocturnal after hibernation, when the feeding rate increases dramatically. The butterfly emerges after a pupal stage of about 10 to 30 days, depending upon temperature. The larvae suffer heavy losses (more than 90%) from a bacterial pathogen, possibly Pseudonomas fluorescens (McWhirter and Scali 1966, Dowdeswell 1981) and from the parasite Apanteles tetricus (Dowdeswell 1961).

All species of Maniolini are univoltine (Emmel 1969, Thomson 1973a, 1980, Van den Brink and Van Oorschot 1986), in spite of statements to the contrary (Larsen 1975, and many other papers). Maniola fly from April to October, according to geographic location, altitude and weather. Pyronia appear a little later, Hyponephele are more typically July and August butterflies, although in parts of the eastern Mediterranean area they emerge in late May (Thomson 1985).

In the hotter regions of southern Europe and Asia, all Maniola species aestivate (Hemming 1932, Parker 1983, Thomson 1973a) from mid-June to mid-August. Most aestival diapausing individuals are female. Although some males display the same behaviour, most butterflies of that sex die before its onset (Parker 1983, Thomson, this study). It has been shown that, in all jurtina females which have mated before the onset of aestivation, gonadal diapause occurs (Scali 1971b, Masetti and Scali 1972, Scali and Masetti 1979). On the resumption of adult activity, the eggs develop in the females and the ova are laid in late summer and autumn. This diversified and precise regulation of jurtina's reproductive biology has enabled the species to colonise a wide range of habitats and climates (Scali 1971b). The

adaptation to diapause in two development stages of Maniola is rare (Beck 1980) and of great ecological interest (see section 3.1.1).

Geographical variation in wingmarkings and genitalia in jurtina is extensively reviewed by Thomson (1969, 1973b, 1975). A large number of races and subspecies has been described from the external phenotypes and these have been grouped on the basis of genitalia characteristics by Thomson (1973b). Wingmarking variation has resulted in the description of many forms in Pyronia, Hyponephele and Cercyonis (Seitz 1909 - 1932). While similar genitalia variation to that noted in Maniola probably occurs in these genera, it has not received much attention.

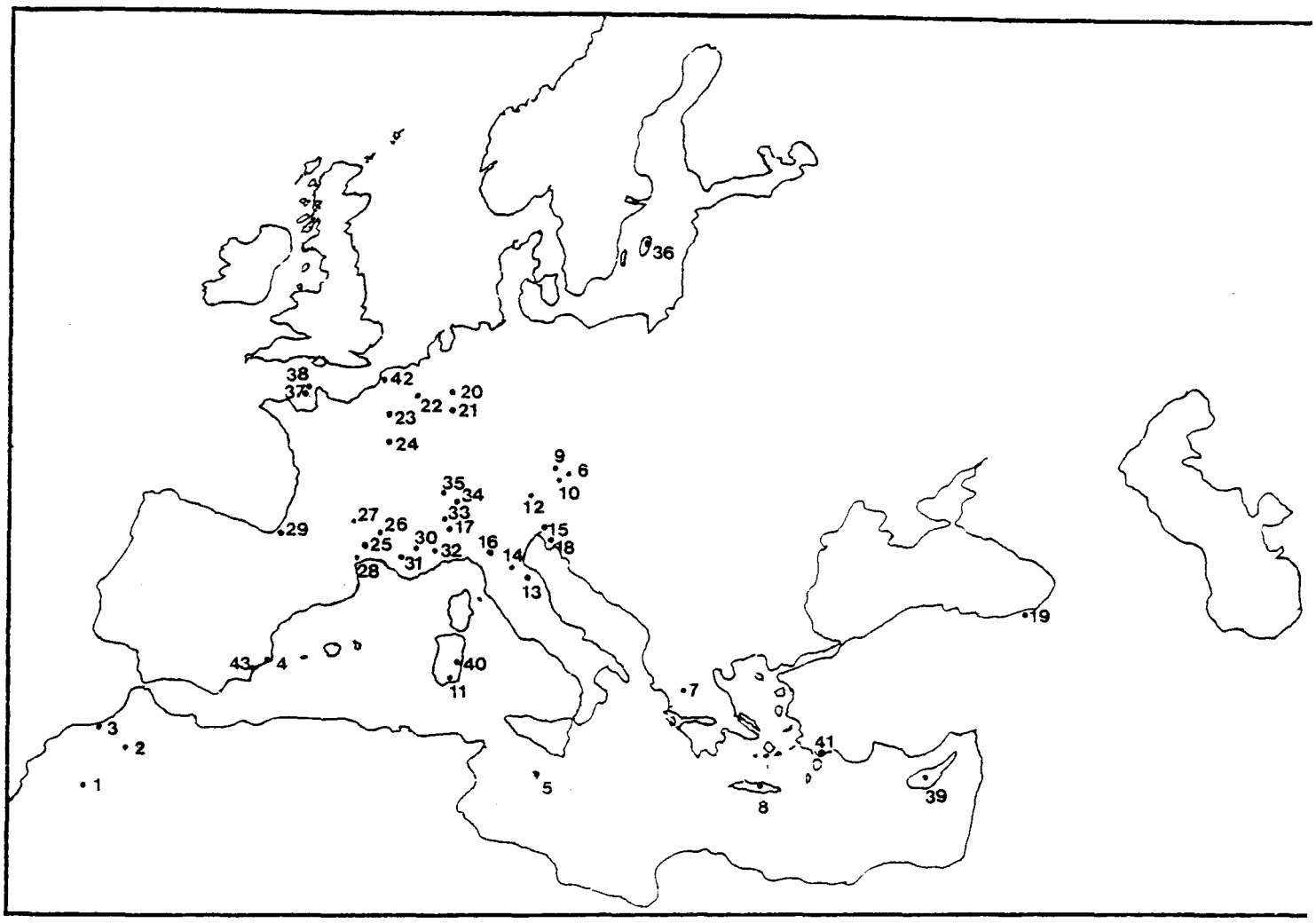


figure 1.3.1a

Sampling sites - Europe, north Africa and western Asia (see section 1.3.1)

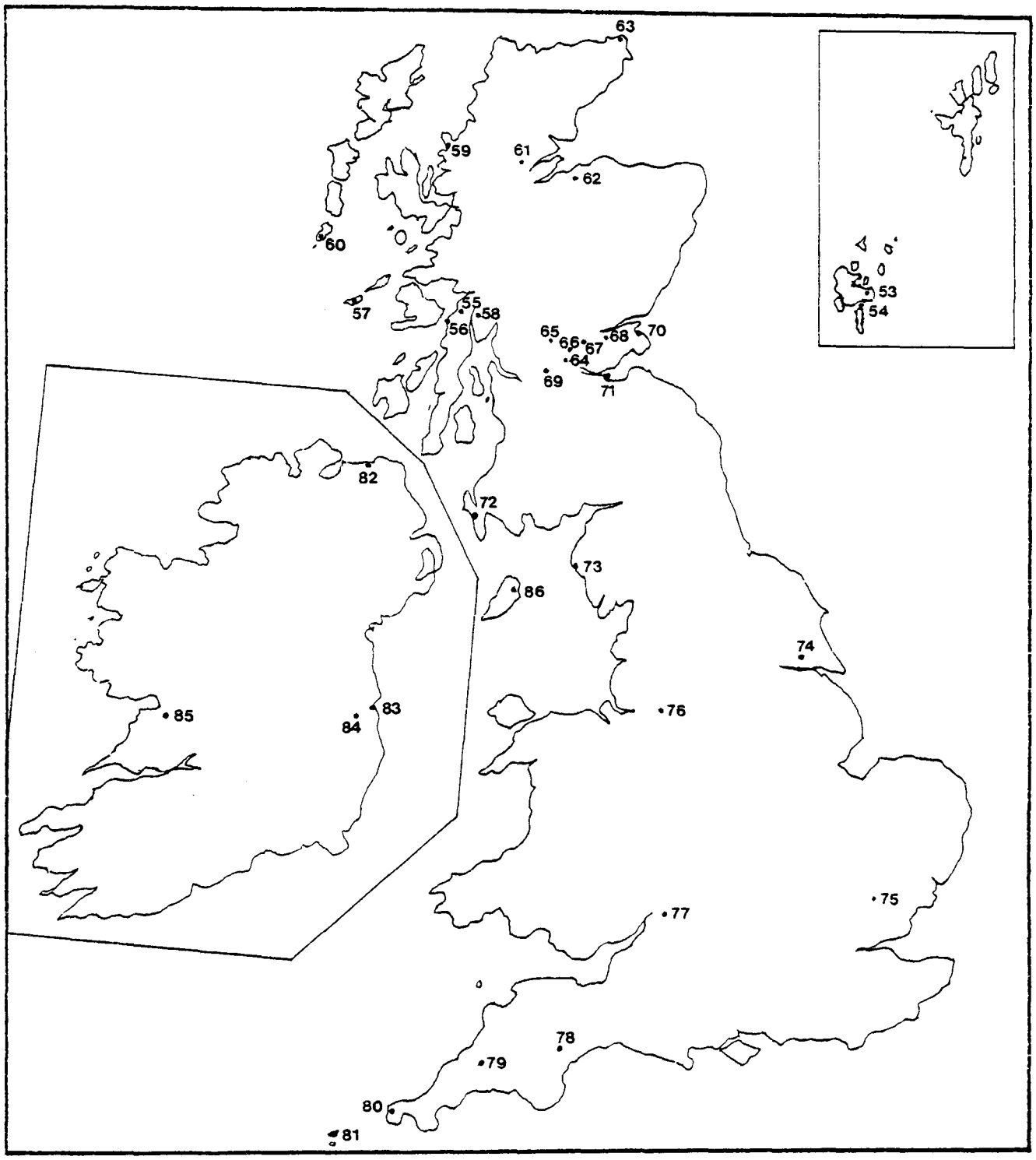
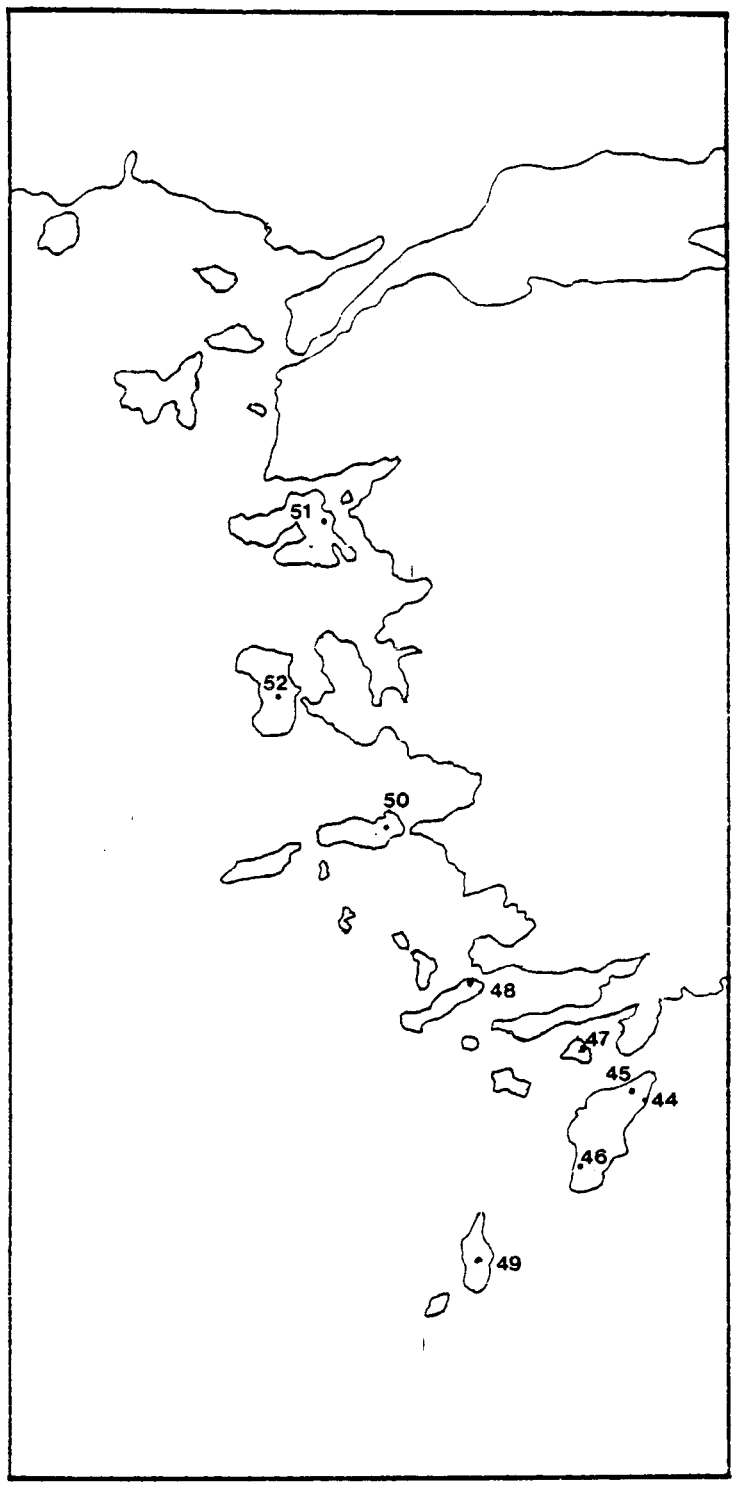


figure 1.3.1b

Sampling sites - Britain and Ireland (see section 1.3.1)



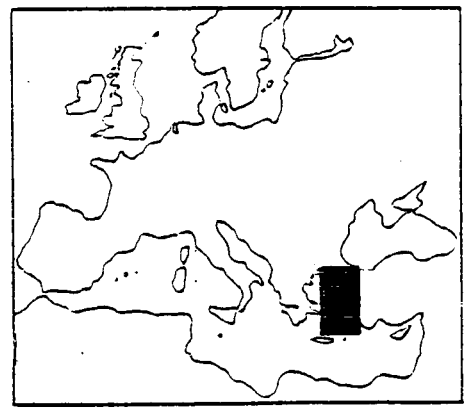


figure 1.3.1c

Sampling sites - western Turkey and the Aegean Islands (see section 1.3.1)

1.3 Materials and methods

1.3.1 Comprehensive list of species and sampling sites utilised.

Site numbers refer to figures 1.3.1a-c. Numbers in parenthesis represent sample sizes. Altitudes given if greater than 100m.

Maniola jurtina

MOROCCO

1 Tizi N'Test Pass, High Atlas, 2100m, 18.8.1984 (5)
1 Oukaimeden, High Atlas, 2450m, 20.8.1984 (23)
2 Azrou, Middle Atlas, 1900m, 21.7.1984 (1)
3 Oulmes, 1000m, 25.5.1985 (15)

SPAIN

4 Denia, Alicante, 20.9.1982 (3)

MALTA

5 Buskett, 19.8. - 4.9.1982 (56)
5 Buskett, 26.6. - 27.6.1984 (48)
5 Rabat, 200m, 2.6. - 3.6.1984 (14)
5 Rabat, 200m, 28.8.1983 (14)

HUNGARY

6 Sopron Umbeg, 300m, 3.6. - 4.6.1982 (41)

GREECE

7 Trikala, Aspropotamus, Pindus Mts., 25.7.1983 (5)
8 Arkadion, N. Rethimnon, Crete, 7.6.1984 (17)
8 Anogia, N. Rethimnon, Crete, 8.6.1984 (16)

AUSTRIA

9 Vienna, 250m, 17.8.1982 (43)
10 Loipersbach, Westumgurm, Burgenland, 400m, 20.6.1984 (12)

ITALY

11 M. Sette Fratelli, Cagliari, 1000m, 10.9.1983 (4)
12 Feldthunes, Brixen, S. Tyrol, 1000m, 31.7.1983 (55)
13 Fiastra, M. Sibillini, Macerata, 700m, 19.8.1984 (18)
13 Lago di Fiastra, Macerata, 800m, 19.6.1983 (26)
14 Marzabotto, M. Sole, Bologne, 500m, 22.6.1983 (15)
15 San Leonardo, Udine, 170m, 12.6.1983 (56)
16 Ponti Lamberti, Parma, 350m, 18.8.1983 (31)
17 Gignod, Valle d'Aosta, 950m, 5.7.1982 (111)
17 Gignod, Valle d'Aosta, 950m, 14.7.1983 (57)
17 Gignod, Valle d'Aosta, 1300m, 5.7.1982 (40)
17 Gignod, Valle d'Aosta, 1350m, 6.7.1982 (41)
17 Gignod, Valle d'Aosta, 1350m, 14.7.1983 (71)
17 Gignod, Valle d'Aosta, 1650m, 6.7.1982 (39)
17 Gignod, Valle d'Aosta, 1650m, 14.7.1983 (41)
18 Gabrovizza, Trieste, 250m, 9.6.1985 (16)

TURKEY

19 Trabzon (various sites), 50m - 500m, 6. - 7.1983 (27)
19 Trabzon (various sites), 50m - 500m, 6. - 7.1984 (30)

WEST GERMANY

20 Mouschau, 24.7.1983 (8)
21 Altenahr, Eifl, 20.7.1983 (21)

BELGIUM

22 Belveaux, 400m, 15.8.1983 (23)

FRANCE

23 Guernanville, Eure, 200m, 26.6. - 3.7.1983 (30)
24 Celon Chateauroux, Eure, 154m, 28.6.1982 (56)
25 Nante, Millau, Eure, 379m, 1.7.1982 (36)
26 Concoules, Lanarce, Ardeche, 1100m, 9 - 10.7.1983 (74)
27 Cahors, Lot, 120m, 30.6.1982 (45)
28 Sorede, Pyrenees Orientales, 25 - 26.8.1983 (13)
29 Sare, Pyrenees Atlantique, 24.6.1983 (32)
30 Digne, Alpes de Hautes Provence, 5.7.1982 (54)
31 Fountain de Vaucluse - north, Vaucluse, 200m, 2.7.1982 (6)
31 Fountain de Vaucluse - north, Vaucluse, 200m, 5 - 7.7.1983 (22)
31 Fountain de Vaucluse - south, Vaucluse, 300m, 7 - 8.7.1983 (37)
32 Vergon, Alpes maritimes, 900m, 2.7.1982 (38)

SWITZERLAND

33 Verbier, Valais, 1200m, 8.7.1982 (25)
33 Verbier, Valais, 1200m, 17.7.1983 (3)
33 Verbier, Valais, 1760m, 17.7.1983 (3)
34 Raron, Valais, 16.7.1983 (11)
35 Bern, 10 - 15.7.1985 (9)
35 Heinisberg, Bern, 16.7.1985 (16)

SWEDEN

36 Paviken, Gottland, 18.7.1983 (8)
36 Stanga, Gottland, 17.7.1983 (8)
36 Masterringe, Gottland, 15.7.1983 (2)
36 Tofta, Gottland, 16.7.1983 (28)
36 Stenhulma, Gottland, 22.7.1983 (25)

SCOTLAND

53 Head of Holland, St Ola, Orkney, 31.7.1983 (10)
53 Head of Holland, St Ola, Orkney, 7.8.1984 (33)
54 Burray Links, Burray, Orkney, 7.8.1984 (8)
55 Glen Lonan, Argyll, 19.7.1982 (5)
55 Benderloch, Argyll, 1.8.1982 (23)

56 Ellanbeich, Seil, Argyll, 19.7.1982 (33)
56 Ellanbeich, Seil, Argyll, 31.7.1982 (14)
56 Easdale Island, Argyll, 19.7.1982 (56)
56 Easdale Island, Argyll, 31.7.1982 (65)
57 Kilkenneth, Tiree, Argyll, 31.7.1984 (65)
57 Kilkenneth, Tiree, Argyll, 2 - 5.8.1983 (51)
58 Cairndow, Argyll, 19.7.1982 (3)
59 North Erradale, Gairloch, Ross and Cromarty, 27.7. - 8.8.1982 (5)
60 Castlebay, Barra, Invernessshire, 25.7.1984 (29)
60 Baghd, Barra, Invernessshire, 24.7.1984 (91)
61 Strathconnon, Invernessshire, 26 - 29.7.1984 (22)
61 Strathconnon, Invernessshire, 8.8.1984 (6)
62 Wester Hardmuir Forest, Morayshire, 29.7.1982 (5)
63 Huna, John O'Groats, Caithness, 5.8.1984 (36)
66 Milour Moor, Dunblane, Perthshire, 18.7.1982 (107)
66 Milour Moor, Dunblane, Perthshire, 4.8.1982 (70)
66 Milour Moor, Dunblane, Perthshire, 7.7.1984 (81)
66 Milour Moor, Dunblane, Perthshire, 11.8.1984 (9)
66 Milour Moor - site 2, Dunblane, Perthshire, 18.7.1982 (95)
66 Milour Moor - site 2, Dunblane, Perthshire, 4.8.1982 (25)
66 Leighills, Dunblane, Perthshire, 17 - 27.7.1982 (69)
66 Leighills, Dunblane, Perthshire,29.6. - 1.7.1984 (9)
66 Tannahill, Dunblane, Perthshire, 1 - 4.7.1984 (62)
65 Torrie Forest, Callander, Perthshire, 20.7.1982 (60)
65 Torrie Forest, Callander, Perthshire, 3.8.1982 (21)
67 Auchterarder, Perthshire, 24.7.1982 (63)
68 Glen Farg, Perthshire, 24.7.1982 (68)
66 Sheriffmuir, Stirling, 200m, 14 - 16.8.1982 (37)
64 Sauchie Crag, Stirling, 3 - 16.8.1982 (11)
69 Kilsyth Hills, Kilsyth, Stirlingshire, 150m, 30.7.1982 (1)
69 Kilsyth Hills, Kilsyth, Stirlingshire, 300m, 30.7.1982 (26)
69 Kilsyth Hills, Kilsyth, Stirlingshire, 300m, 6.7.1984 (32)
69 Kilsyth Hills, Kilsyth, Stirlingshire, 350m, 30.7.1982 (7)
69 Kilsyth Hills, Kilsyth, Stirlingshire, 350m, 6.7.1984 (7)
69 Auchenstarry, Kilsyth, Stirlingshire, 30.7.1982 (24)
70 St Andrews, Fife - site 1, 22.7.1982 (15)
70 St Andrews, Fife - site 2, 22.7.1982 (20)
70 St Andrews, Fife - site 3, 22.7.1982 (38)
71 Cramond Island, Midlothian, 21.7.1984 (101)

72 Portpatrick, Wigtownshire, 19.7.1984 (31)

ENGLAND

73 St Bees, Cumbria, 19.7.1984 (56)
74 South Cave, North Humberside, 5.9.1982 (4)
75 Tiptree, Essex, 21.7. - 2.8.1982 (47)
76 Wilmslow, Cheshire, 21.7.1983 (64)
77 Stroud, Gloucestershire, 2.7.1983 (53)
78 Whiddon Down, Exeter, Devon, 10.7.1984 (54)
79 Ruthvoes, Bodmin, Cornwall, 10.7.1984 (47)
80 Sennen Cove, Land's End, Cornwall, 12.7.1984 (54)
81 Hughtown, St Mary's, Isles of Scilly, 11.7.1984 (56)

IRELAND

82 Portrush, Co. Antrim, 8 - 10.8.1984 (44)
83 Allenwood, Dublin, 15.7.1984 (29)
84 Rosebery, Newbridge, Co. Kildare, 6.7.1984 (41)
85 Kinvara, Co. Clare, 15.8.1984 (47)

CHANNEL ISLANDS

37 Hougue Nicolle, St Sampson, Guernsey, 12.8.1984 (6)
38 Longis Common, Alderney, 29.8.1984 (15)
38 Giffione, Alderney, 29.8.1984 (15)
38 Essex Hill, Alderney, 29.8.1984 (15)
38 Fort Corblets, Alderney, 29.8.1984 (12)

ISLE OF MAN

86 Abbeville 7, 26 - 27.7.1984 (26)
86 Jurba Road, 8.7.1984 (3)
86 Cronk, Ruagh, 6.7.1984 (13)
86 Ramsey, 6.7.1984 (1)
86 Smeale, 6.7.1984 (3)
86 West Kimmeragh, 7.7.1984 (4)
86 North Barrule, 250m 3.8.1984 (19)

Maniola telmessia

GREECE

44 Faliraki, Rhodes. 26.5. - 1.6.1963 (26)
45 Filerimos, Rhodes, 350m, 26 - 28.5.1983 (101)
46 Apolakkia, Rhodes, 27.5.1983 (10)
47 Symi Town, Symi, 150m, 31.5.1983 (55)
48 Kos Town, Kos, 150m, 30.5.1983 (48)
49 Othos, Karpathos, 500m, 12.6.1984 (12)
49 Piles, Karpathos, 300m, 12 - 13.6.1984 (6)
50 Mytillini, Samos, 250m, 24.5.1986 (20)
51 Agiosos, Lesbos, 600m, 5.6.1986 (20)

Maniola cypricola

CYPRUS

39 Episkopi, 17.4.1984 (11)
39 Limassol, 6 - 9.6.1985 (12)
39 Fry Tree Bay, Paralimna, 4.8.1985 (9)
39 Tala, Paphos, 150m, 19.8.1985 (10)
39 Polis, 13.8.1985 (1)

Maniola chia

GREECE

52 Nea Moni Monastery, Chios, 500m, 25.5.1986 (19)

Maniola nurag

ITALY

40 Correboi, Fonnd, Sardinia, 1000m, 9.6.1985 (6)
40 Bronou Spina, M. Gennargentu, 1500m, 6.7.1985 (16)

Maniola megala

TURKEY

41 Marmaris, 8 - 9.6.1986 (16)

Pyronia tithonus

FRANCE

42 Le Touquet, 23.7.1983 (19)

ENGLAND

73 St Bees, Cumbria, 19.7.1984 (14)
80 Sennen Cove, Land's End, Cornwall, 12.7.1984 (7)

CHANNEL ISLANDS

37 Hougue Nicolle, St Sampson, Guernsey, 12.8.1984 (11)

Pyronia bathseba

FRANCE

31 Fountain de Vaucluse, Vaucluse, 200m, 6.7.1983 (19)

SPAIN

43 Vistahermosa, Alicante, 30.6.83 (8)

Pyronia cecilia

SPAIN

43 Vistahermosa, Alicante, 30.6.1983 (4)

Aphantopus hyperantus

ITALY

17 Gignod, Valle d'Aosta - site 3, 1350m, 14.7.1983 (17)

SCOTLAND

66 Doune, Perthshire, 20.6.1983 (2)
65 Drumloist, Callander, Perthshire, 26.7.1983 (3)

Hyponephele lycaon

ITALY

17 Planet, Valle d'Aosta, 1500m, 11 - 17.7.1983 (22)
13 Fiastra, M. Sibillini, 1200m, 19.8.1984 (12)
17 Cogne, Pte de Lavas, Valle d'Aosta, 800m, 18.8.1985 (9)

Hyponephele lupina

GREECE

48 Kos Town, Kos, 150m, 30.5.1983 (4)

CYPRUS

39 Tala, Paphos, 150m, 19.8.1985 (5)

MOROCCO

1 Tizi N'Test Pass, High Atlas, 2100m, 18.8.1984 (1)

Hyponephele maroccana

MOROCCO

1 Tizi N'Test Pass, High Atlas, 2100m, 18.8.1984 (8)
1 Oukaimeden, High Atlas, 2450m, 20.8.1984 (6)
1 Oukaimeden, High Atlas, 2700m, 20.8.1984 (3)

Cercyonis pegala

USA

- Macomb, Illinois - site 1, 23.7.1984 (5)
- Macomb, Illinois - site 2, 28.8.1984 (2)

1.3.2 Electrophoresis

The study of proteins by electrophoresis on supporting gels was pioneered by Smithies (1955,1959) who examined human serum. In the 1950s and 1960s the 'zymogram' technique using substrates specific to certain classes of enzymes was developed thus enabling the protein fractions to be separated qualitatively according to their inherent electric charge and, using more sophisticated techniques, also by their molecular size. However, prior to 1963 most studies described variation in single proteins, but by the mid 1960s electrophoretic techniques were sufficiently refined to permit examination of a large number of different proteins in the same organisms (Hubby and Lewontin 1966, Harris and Hopkinson 1972, Avise 1974). Since then the technique has been directed increasingly towards answering questions of evolutionary biology. The development of insect molecular systematics has been reviewed by Wagner and Selander (1974) and Berlocher (1984).

The most widely used molecular technique in insect systematics is undoubtedly gel electrophoresis. The proteins used are almost invariably soluble enzymes as the catalytic activity of the enzyme, via enzyme-specific histochemical stains (Hunter and Markert 1957), can be used to mark the position of the enzyme on the gel. The technique has been usefully applied to a number of insect orders including Odonata (Knopf 1977), Orthoptera (Harrison 1979, Sbordoni et al. 1980, Gill 1981), Hemiptera (Guttman et al. 1981, Sluss et al. 1982, Varvio-Aho et al. 1978) Coleoptera (Kuboki 1978, Lokki and Saura 1980), Hymenoptera (Pamilo et al. 1979, Halliday 1981, Ward 1980) and Diptera (Ayala 1975, Eisses et al. 1979, Craddock and Johnson 1979, Johnson et al. 1975, Lakovaara and Keranen 1980, Tabachnick et al. 1979, Miles and Patterson 1979, Steiner et al. 1982, May et al. 1977, Snyder 1981, Ward et al. 1981, Berlocher and Bush 1982, Mcrgante et al. 1980). Gel electrophoresis was used throughout this study.

Adults were collected in the field by net or supplied by colleagues and other contacts. Field collected specimens were transferred to individual small polythene bags which were packed fairly firmly in sealable plastic containers: the tight packing prevented movement of the butterflies while the polythene bags and containers minimised

moisture loss. Specimens were kept as cool as possible (in a refrigerator when possible) until they could be deep frozen or analysed. These procedures extended the life of the butterflies on prolonged collecting trips. Instructions were given to other collectors to follow similar procedures as far as possible. Butterflies of the group under study survived remarkably well under these conditions, up to eight days, which meant that material received by mail was usually in good condition, frequently living. Females retained their enzyme activity better than males. Greatest activity was retained in specimens which were frozen live, or which were electrophoresed fresh. At some loci, enzyme activity was was greatly reduced or absent in specimens which had not been frozen live, especially ALD, D(-)3PGDH, IDH-1, MPI and PGI-1, While activity at some loci appeared to be remarkably long lived. This was the case with AK-1, AK-2, CK, LAP-2 and PGM.

Samples were deep frozen, either at $-70^{o}C$ or $-30^{o}C$ and stored at the higher temperature until utilised. There was no apparent decrease in enzyme activity in specimens frozen at the higher temperature.

Some Maniola (jurtina, telmessia and cypricola) which had been frozen in vivo for 30 months, showed no decrease in enzyme activity. In repeat runs of jurtina samples, neither heat denaturation nor aging induced changes in electrophoretic mobilities at any locus, with the possible exception of ME-2.

Equipment configuration, buffers and staining recipes often must be modified significantly for the satisfactory resolution of apparently homologous loci in different organisms. For example, some of the dehydrogenases are very difficult to resolve in butterflies, yet they are amongst those most commonly located in vertebrates. It was noticeable that activity at some loci in Maniola and Pyronia was far less in Hyponephele and vice versa, even although precisely the same electrophoretic conditions were met. Furthermore, the same methods and formulae which work successfully in one laboratory, do not necessarily work in another. An acceptable explanation for this has not yet been proposed and, in consequence, the resolution and identification of enzyme loci was largely a matter of trial and error.

It has been shown that polyacrylamide can resolve to a much finer degree than starch gel, and thereby reveal 'hidden alleles' at some loci (Coyne 1976). However, starch gel was used in preference to polyacrylamide as it was considered more appropriate for bulk analysis for cost and safety reasons. Horizontal electrophoresis was utilised rather than vertical because of the availability of equipment, but there is no evidence that either configuration produces superior results (Geiger pers. comm.). Certainly electrophoresis is notoriously fickle and it would be difficult to attribute, with any certainty, the benefits or drawbacks of either system without controlled tests.

Electrophoresis, buffers and stains were modified from those of Brewer et al. (1967), Shaw and Prasad (1970), Geiger (1982 and pers. comm.) and McAndrew and Majumdar (1983). The methodology used in this study differed in some important respects from that used by the authors cited.

All equipment was routinely rinsed with distilled water. Contamination, especially of tris-citrate buffer with boric acid, greatly inhibited the activity of many enzymes. On the other hand, the addition of a small amount of boric acid to tris-citrate gel buffer enhanced the activity of GOT-1, ADH-1 and ADH-2. Initially, deionised water was used for electrode, gel and stain buffer solutions, but it was found that distilled water gave much more consistent results. The addition of NAD, NADP and/or NADH to the gel mixture after degassing did not enhance the enzyme activity.

Gels were prepared using 10 to 12.5% starch (Connaught Laboratories), depending upon the product batch. This was mixed with the appropriate buffer. Two gel/electrode buffers were used routinely 1. continuous tris-citrate pH8.0 and 2. TEB pH8.5. However, several enzymes could be resolved more or less satisfactorily on either buffer or other buffer systems, notably Poulik (table 1.3.2a) Constant voltage power supplies were set to 200V by 40mA for tris-citrate and to 250V by 42mA for TEB which gave running times of 3 hours and 3 hour 30 minutes respectively. The buffer pHs, and voltage/mA settings were calibrated so that a full sample could be prepared, run and analysed in a day. After some time it was found that the voltage/mA could be

table 1.3.2a

Gel/electrode buffers: ** - optimal system, used routinely * - other satisfactory systems. CTC - continuous tris-citrate; TEB - tris/EDTA/borate. For enzyme abbreviations see table 1.3.2b.

locus	CTC	TEBpH8.5	Poulik	TEBpH9.1
ADA		**		
ADH		**		
AK	**			
ALD	**			
CK	**		*	*
DIA	*	**		
D(-)3GPDH	*	**		
EST-A	*	**		
EST-C	*	**		
EST-1	*	**	*	*
GK	*	**		
GOT	*	**		
IDH	**			
LAP	**			
MDH		**		*
ME	**			
MPI		**		
MK	*	**		
PGI	**	*		
PGM	**			
PK	**	*		*
SOD	*	**		
αGPDH		**		
6PDGH	**			*

increased to 250V/50mA for tris-citrate and 300mA/54mA which reduced running times by approximately 30%. Electrophoresis was carried out at 2°C.

For routine analysis, only the thorax of butterflies was used. Simultaneous runs using thoraces and heads gave identical results at all loci. More complex patterns were observed from abdomen at several loci. This derived, most probably, from tissue-specific enzymes or loci and there are interesting possibilities for research here. The thoraces were homogenised in a small quantity of distilled water, 50 - 200 µl depending upon the specimen size. The use of gel buffer for this purposed did not enhance activity or resolution. The homogenate was chill centrifuged at 7,000rpm for ten minutes and the supernatant fraction absorbed onto small rectangles of filter paper (Watman Number 1), 3mm X 8mm for routine analysis, allowing 30 specimens per gel, and 8mm X 8mm when gels were prepared for photography. The paper rectangles were applied to the edge of a straight slit cut lengthwise in the gel, 30mm from the end to which the cathode charge was to be applied. The current was transferred from the tanks containing electrode buffer via washed lint wicks overlapping some 10mm on the gel.

For many reactions, composite mixtures were prepared (table 1.3.2b), after standard assays had been established. Stain was invariably applied to the cut surface of the gel. In most instances, the agar overlay method was used. This was not applied to GOT or EST staining, both of which required pre-incubation. The use of agar greatly facilitated the handling of gels and was essential in some reactions where the products at the site of enzyme activity were water soluble. Care was taken to ensure that the agar solution was cool (<40°C) to avoid denaturation of the enzyme. The stain reagents were weighed and mixed only a short time before use, as many of the biochemicals involved were unstable, hygroscopic or both. Special precautions were taken with PGI and D(-)GPDH stain mixtures. Contact with $MgCl_2$ was avoided until the stain buffer was added to the dry products. Enzymes and other reagents in liquid form were added to the mixtures after the other ingredients had been dissolved in the stain buffer. Gels were incubated at 40°C for ten minutes to two hours, until the patterns could be read, and before diffusion confused the banding. The

table 1.3.2b

Enzymes used and Enzyme Commission numbers.

Enzyme	E.C. number
Adenelate kinase (AK)	2.7.4.3
Adenosine deaminase (ADA)	3.5.4.4
Alcohol dehydrogenase (ADH)	1.1.1.1
Aldolase (ALD)	4.1.2.13
Diaphorase (NADH) (DIA)	1.6.2.2
Creatine kinase (CK)	2.7.3.2
D(-)3 glycerophosphate dehydrogenase (D(-)3GPDH)	
Esterase A and C - umbelliferyl acetate (EST)	3.1.1.1
Esterase 1 - α naphthyl acetate (EST)	3.1.1.1
Glucokinase (GK)	2.7.1.1
Glutamate oxaloacetate transaminase* (GOT)	2.6.1.1
Isocitrate dehydrogenase (IDH)	1.1.1.42
Leucine aminopeptidase (LAP)	3.4.11 or 13
Malate dehydrogenase (MDH)	1.1.1.37
Malic enzyme (ME)	1.1.1.40
Mannokinase (MK)	2.7.1.1
Mannose phosphate isomerase (MPI)	5.3.1.8
Phosphoglucomutase (PGM)	2.7.5.1
Phosphoglucose isomerase (PGI)	5.3.1.9
Pyruvate kinase (PK)	2.7.1.40
Superoxide dimutase (SOD)	1.15.1.1
α glycerophosphate dehydrogenase (αGPDH)	1.1.1.8
6 phosphoglucose dehydrogenase (6PGDH)	1.1.1.44

* The name glutamate oxaloacetate transaminase was used instead of the preferred aspartate aminotranferase as it has been adopted exclusively in other butterfly studies.

table 1.3.2c

Isozyme utilised in this study - loci number and subunit structure.

enzyme	loci	nomenclature	structure
ADA	1	ADA	dimeric
ADH	3	ADH-1	dimeric
		ADH-2	
		ADH-3	
AK	2	AK-1	monomeric
		AK-2	
ALD	1	ALD	tetrameric
CK	1	CK	monomeric
DIA	2	DIA-1	monomeric
		DIA-2	
D(-)3GPDH	1	D(-)3GPDH	dimeric
EST	2	EST-A	monomeric
		EST-C	
EST	1	EST-1	dimeric
GOT	1*	GOT-1	dimeric
GK	1	GK	monomeric
IDH	2	IDH-1	dimeric
		IDH-2	
LAP	2	LAP-1	dimeric
		LAP-2	
MDH	1	MDH	tetrameric
ME	2	ME-1	tetrameric
		ME-2	
(MES)	1	(MES)	dimeric
MK	1	MK	monomeric
MPI	1	MPI	monomeric
PGI	2	PGI-1	dimeric
		PGI-2	
PGM	1	PGM	monomeric
PK	1	PK	tetrameric
SOD	3	SOD-1	dimeric
		SOD-2	
αGPDH	1	αGPDH	dimeric
6PGDH	1	6PGDH	dimeric

* A second locus was resolved poorly but not utilised.

patterns were drawn or photographed. All gels were incubated in total darkness except SOD which required post-exposure to light. The frequent incorporation on gels of control homogenate of known electrophoretic mobility minimised the risk of undetected or wrongly identified mobilities. The enzymes used in this work are listed in tables 1.3.2b and c.

Standard conventions were followed in the nomenclature of allozymes. All mobilities were measured in relation to the mobility from the origin of the common allele, 100/100, in Maniola jurtina. For example, the allele 140/140 migrated 40% further than allele 100/100.

The identification of most isozymes was checked by the absence of activity on gels stained without the appropriate substrate. Glucokinase and mannokinase are not usually identified separately in electrophoretic studies. Two hexokinase bands were resolved. MK was identified by replacing glucose with mannose and the addition of PMI and PGI. Fructokinase was not resolved by replacing glucose with fructose and the addition of PGI. Glucokinase was, therefore identified by elimination following the explanation of Jelnes (1971). A low activity locus was resolved by replacing a glycerophosphate with D(-)3 glycerophosphate in an αGPDH staining mixture. This locus had a different mobility from αGPDH and produced two unique loci in Salmo (Stevens pers. comm.) This has been provisionally called D(-)3 glycerophosphate dehydrogenase. An unidentified slow moving dimeric locus was resolved on malic enzyme gels. This has been called (MES) throughout this work.

Frequently in ME-2 and rarely in PGM apparently identical alleles migrated a slightly different distance on repeated runs. Attempts at inducing this 'mobility flux' using different thermal conditions were unsuccessful. Cobbs and Prakash (1977) report similar changes and suggest that these are the result of changes in unit charge induced by a reaction with artifacts in the form of molecules of similar structure.

Formulae and recipes used

(all reagents supplied by Sigma unless otherwise stated)

Electrode/gel buffers

Continuous tris-citrate pH8.0

tris	30.29gms
citric acid (BDH)	11.98gms
H_2O	1.001

(gel buffer - 8% solution of electrode buffer)

TEB pH8.5

tris	60.57gms
EDTA	5.99gms
boric acid (BDH)	15.00gms
H_2O	1.001

(gel buffer - 4.5% solution of electrode buffer)

Discontinuous tris-citrate (Poulik) pH8.7

gel:

tris	9.21gms
citric acid (BDH)	1.05gms
electrode:	
H_2O	1.001

electrode:

boric acid (BDH)	18.55gms
NaOH (BDH)	2.40gms
H_2O	1.001

TEB pH9.1 (Geiger 1982) - 16 hours at 8V/cm

tris	87.00gms
EDTA	1.00gms
boric acid (BDH)	8.70gms
H_2O	1.001

Stain buffers

tris-HCl pH8.0/0.2M

tris	24.22gms
HCl (1N)	95.00ml
H_2O	905.00ml

tris-maleate pH5.2/0.1M

tris	12.10gms
maleic acid	11.60gms
H_2O	1.001

acetate pH5.2/5.2M

sodium acetate	1gm
H_2O	1.001

(pH adjusted with glacial acetic acid)

Agar overlay

1 - 2% agar in H_2O

Reaction mixtures - to make 100ml with the addition of tris-HCl buffer, unless otherwise indicated.

ADA + ADH-1 + SOD-1

sodium arsenate	100mgs
adenosine	30mgs
NAD	40mgs
MTT	5mgs
PMS	5mgs
isopropyl alcohol	5mgs
XOD	100µl
NP	50µl

ADH-2 and ADH-3

sodium arsenate	100mgs
adenosine	40mgs
NAD	40mgs
MTT	5mgs
PMS	5mgs
isopropyl alcohol	200µl
XOD	100µl

AK + CK + PK

phospho(enol)pyruvate	30mgs
glucose	120mgs
ADP	60mgs
KCl	80mgs
$MgSO_4$	80mgs
fructose 1,6 diphosphate	30mgs
NADP	5mgs
MTT	5mgs
PMS	5mgs
$MgCl_2$	20mgs
HK	50µl
G6PDH	50µl

ALD

fructose 1,6 diphosphate	200mgs
sodium arsenate	120mgs
NAD	40mgs
MTT	5mgs
PMS	5mgs
G3PDH	50µl

DIA

2,6 dichlorophenol indophenol	2mgs
H_2O	2ml
NADH	20mgs
MTT	5mgs

The dichlorophenol indophenol was dissolved in H_2O and filtered. The remaining reagents were mixed and the filtered solution added immediately before staining.

D(-)3GPDH

D(-)3 glycerophosphate	100mgs
EDTA	120mgs
NAD	30mgs
MTT	5mgs
PMS	5mgs

EST-A and EST-C

methyl umbelliferyl acetate	4mgs
acetone	1ml
acetate buffer	24ml

Tris HCl buffer was not added. The gels were incubated in acetate buffer for ten minutes at $2^{o}C$. this buffer was drawn off before the

staining mixture was applied. The methyl umbelliferyl acetate was dissolved in acetone before the buffer was added. gels were observed under UV light.

EST-1

α naphthyl acetate	20mgs
acetone	1ml
H_2O	1ml

stain:

fast blue RR	40mgs
H_2O	25ml

75ml tris maleate or tris HCl Buffer and H_2O was added to The α naphthyl acetate, dissolved in acetone. The gel was incubated at 40°C for 15 minutes in the solution before the stain was added.

GK + MK

glucose	200mgs
ATP	80mgs
NADP	10mgs
MTT	5mgs
PMS	5mgs
G6PDH	100µl

GOT

tris	800mgs
L aspartic acid	200mgs
α ketoglutaric acid	60mgs
pyroxidal 5 phosphoric acid	20mgs
PVP	30mgs
H_2O	75ml

stain:

fast blue RR	60mgs
H_2O	25ml

H_2O used in place of buffer. Gel incubated for 5 minutes before stain was added. No activity was induced when fast blue BB was used in place of fast blue RR.

IDH + PGM

Na glucose 1 phosphoric acid	100mgs
Na isocitric acid	100mgs
NADP	5mgs
MTT	5mgs
PMS	5mgs
$MgCl_2$ (BDH)	140mgs
G6PDH	100µl

LAP

L leucylglycine	40mgs
peroxidase	20mgs
L amino acid oxidase	10mgs
O dianisidine	10mgs
$MgCl_2$	10mgs

MDH

DL malic acid	120mgs
NAD	30mgs
MTT	5mgs
PMS	5mgs

ME

L malic acid	300mgs
tris	1200mgs
NADP	5mgs
MTT	5mgs
PMS	5mgs
$MgCl_2$	100mgs

MPI

mannose 6 phosphate	20mgs
NADP	5mgs
MTT	5mgs
PMS	5mgs
G6PDH	100µl
PGI	50µl

SOD-1 was also resolved with this mixture.

PGI

fructose 6 phosphoric acid	40mgs
NADP	5mgs
MTT	5mgs
PMS	5mgs
G6PDH	50µl

α GPDH

α glycerophosphate	400mgs
EDTA	120gms
NAD	30gms
MTT	5gms
PMS	5gms

6PGDH

6 phophogluconate	20mgs
NADP	10mgs
MTT	5mgs
PMS	5mgs
$MgCl_2$	40mgs

This mixture also resolved IDH clearly.

1.3.3 Scanning electron microscopy

All specimens were mounted using double-sided adhesive tape. Conductive lacquer was applied liberally to the mount and tape so that some touched the specimen(s). Scales were first removed onto a glass slide, before being transferred to double-sided tape and the microscope mount. Specimens were given a light coating of gold using an Edwards S150 sputter coater. Experiments with more complicated preparation techniques, including fixation and dehydration, did not improve results. Charging, due to the build up of electrons, was a major problem with some specimens, especially scales which also had a tendency to curl in the vacuum chamber, but generally very good results were obtained from the ova, larval crania, Jullien organs and other structures of the butterflies studied.

SEM work was initially undertaken on a Cambridge Mk1 Stereoscan. Later studies utilised a higher resolution ISA-60A instrument. All images were generated at 2.5 to 4kV.

1.3.4 Larval chaetotaxy

Larvae (fresh or preserved in 70% ethanol) were prepared by removing the head capsule from the body. The tissues were macerated for ten minutes in 10% KOH at 80°C. Bodies were slit laterally (Hinton 1956) to leave the chaetotaxy on one side intact, although the other side was also useful as a check in some instances. The cuticle was lightly stained in mercurichrome, dehydrated in 70 and 100% alcohol rinses and mounted in glycerol or permanently in Euparal (GBI Laboratories). Pressure was applied to the cover slips so that the setae lay flat. Head capsules were prepared in the same way or examined by SEM.

1.3.5 Genitalia preparations

Only male genitalia specifically were examined in this study. Abdomen were broken from dried specimens and macerated in 10% KOH at 80°C for 30 minutes. The structure was cleaned with water and the genitalia carefully removed from the other abdominal segments. Tissues were stained with mercurichrome and dehydrated in 70 and 100% ethanol rinses. Permanent mounts in Euparal were made in all cases. The abdomen were mounted laterally, preserving the Jullien organs in Maniola. The vinculum was cut so that the valves could lie open and the uncus/gnathos laterally (Tauber 1970).

1.3.6 Morphometrics and statistics

Genitalia and larval setae were measured by projection using a monocular microscope with a powerful tungsten halogen light source. Projected images (X50 for genitalia and X500 for setae) were carefully traced and measurements made from the drawings. Care was taken to avoid distortion and stage graticules were used to preset the instrument configuration.

All statistics and cluster analyses, except factor analyses and nonmetric multidimensional scaling, were performed on an Apricot F2 microcomputer. Factor analyses and NMMS were directed through a NUMAC IBM 370 model 168 computer under the control of the Michigan Terminal system at Newcastle University. Factor analyses were extracted via SPSS subroutines (Nie et al. 1975) and nonmetric scaling plots obtained by the MDS(X) program (Coxon 1981).

The following statistical conventions have been adopted throughout this work.

1. Log likelihood χ^2 test (G-test - Sokal 1969, Sokal and Rohlf 1969)

$$\chi^2_G = 2\Sigma obs \log_n(obs/exp)$$

The degrees of freedom are calculated as

$$1/2(n^2 - n)$$

where n is the number of alleles.

2. Standardised variance in allele frequency or inbreeding coefficient (Workman and Niswander 1970, Wright 1978) when samples sizes are equal is calculated as

$$F_{ST} = \delta_p{}^2/p(1 - p)$$

where $\delta_p^2 = \Sigma(p - p_i)^2/k$ and p is the mean allele frequency for k subgroups. This is directly related to the χ^2 contingency statistic by

$$\chi^2 = 2NF_{ST}$$

with k-i degrees of freedom. When samples sizes differ the following formula (Snedecor and Irwin 1933) was used in the computation

$$\chi^2 = [\Sigma(2N_i)p_i^2 - p\Sigma(2N_i)p_i]/p(- p)$$

3. Sanghvi's distance coefficient (Sanghvi 1953, Workman and Niswander 1970) for k alleles at n loci

$$G = \sum_{i=1}^{n} \sum_{j=1}^{k} [(p_{ij} - p'_{ij})^2/(p_{ij} + p'_{ij})]$$

where p_{ij} and p'_{ij} are the frequencies of the jth allele at the ith locus in the two groups. This gives values of 0 when samples are identical. In this work this has been converted to a similarity coefficient

$$g = 1 - G/2$$

4. Nei's genetic identity coefficient I (Nei 1972)

$$I = \Sigma x_i y_i / \sqrt{(\Sigma x_i^2 \Sigma y_i^2)}$$

where x_i and y_i are the frequencies of the ith allele in populations X and Y respectively.

Conversion of polymorphic loci data to overall I values can be performed if large samples have been analysed and there is reasonable certainty of the level of polymorphism detectable by the experimental method. If 95% and 99% levels are accepted, two values can be calcu-

lated, although in practice there will normally be relatively little difference between the figures. Thus

$$I = (S.p) + (1 - p)$$

where S is a similarity coefficient calculated from polymorphic loci and p is the fraction of loci polymorphic across all loci.

I is converted to a distance coefficient thus

$$D = -\log_n I$$

5. Pearson product moment coefficient (Stephenson 1936) between OTUs j and k was computed as

$$r_p = \left[\sum_{i=1}^{n} (X_{ij} - \bar{X}_j)(X_{ij} - \bar{X}_k)\right] / \sqrt{\sum_{i=1}^{n} (X_{ij} - \bar{X}_j)^2 \sum_{i=1}^{n} (X_{ij} - \bar{X}_k)^2}$$

where X_{ij} is the character state value of character i in the OTU j, $\bar{X}_j$ and $\bar{X}_k$ are the means of all state values of OTU_j and OTU_k and n is the number of characters sampled with n-1 degrees of freedom.

6. Euclidean distances were calculated as

$$d_{ij} = \left[\sum_{i=1}^{n} (X_{ij} - X_{ik})^2\right]^{1/2}/n$$

(nomenclature as is r_p).

7. Effective number of alleles was calculated as

$$\frac{1}{\Sigma x_i^2}$$

where x is the frequency of the ith allele. This, unlike actual number of alleles, takes account of the allele frequencies (Ferguson 1980).

2 ELECTROPHORETIC ANALYSIS

2.1 Gene loci and polymorphism

2.1.1 Introduction

The enzymes of a large number of Lepidoptera have been assayed. Jelnes (1974) examined two Thera moth species, Hudson and Lefkovitch (1980) the Amathes c-nigrum complex, Menken (1982) the ermine moth genus Yponomeuta, Pashley (1983) the codling moth Laspyresia pomonella and its relatives and Stock and Castrovilla (1981) Tortricids of the genus Choristoneura. The parthenogenetic primitive moths of the genus Solenobia have been studied electrophoretically by Lokki et al. (1975) and Suomalainen et al. (1981). Electrophoretic work on butterflies was pioneered by Burns and Johnson (1967) working on esterases. They found that populations of the Pierid Colias eurytheme were comprised almost entirely of heterozygotes and suggested that such molecular variation may be invaluable in analysis of 'possible rapid evolution'. Brussard and Vawter (1975) found Euphydryas phaeton to be highly heterozygous. Brittnacher et al. (1978) found that approximately half the loci utilised in his study of Speyeria were monomorphic in most species of the genus. Schrier et al. (1976) found little difference at 11 loci in three samples of Closyne palla. In the Erebia, Geiger and Rezbanyai (1982) assayed some 18 enzymes at 21 loci and found eight systems to be polymorphic. Conversely, they found three loci (AK-1, AK-2 and GAPDH) to be invariant in all taxa examined. AK was found to be polymorphic in Euphydryas editha and E. chalcedonia by McKechnie et al. (1975).

The work of Jelnes (1974, 1975a, b, c) on the sibling Lycaenid species Aricia agestis and A. artaxerxes and that of Angevine and Brussard (1979) on Lethe demonstrated the value of enzyme electrophoresis in assessing the evolutionary relationships between very closely related organisms. It has also been useful in clarifying relationships between closely related butterfly groups in the Pieridae (Geiger 1978, 1980 (1981), 1982, Geiger and Scholl 1982a, b) and in

the identification of previously undetected taxa (Geiger and Rezbanyai 1982, Geiger and Shapiro 1986, Shapiro and Geiger 1986). Utilising seven enzyme loci in an interesting study of the colonising Pieris rapae in north America , Vawter and Brussard (1983) detected little genic differentiation between populations. The recent work by Geiger (1978, 1980, 1982) and Courtney (1980, 1982) on the European Pieridae shows that biochemical analysis agrees well with phylogenies prepared using traditional methods (usually based on morphological characters). Corroborative electrophoretic data confirming existing systematic arrangements are now common in many other groups including birds (Avise et al. 1980, Smith and Zimmerman 1976), deer (Gyllensten et al. 1983) and fish (McAndrew and Majumdar 1984). Ferguson (1980) gives a useful summary.

Handford (1972, 1973a, 1973b) studied esterases in populations of Maniola jurtina in south-west England and the Isles of Scilly, reporting a null allele at the Est-A locus. Interestingly, Burns and Johnson (1967) claimed to have located an esterase null allele in the Pierid butterfly Colias eurytheme. Bullini et al. (1975) examined the enzyme PGM in a number of moths as well as Maniola jurtina. Masetti and Scali (1975a, b, 1976, 1977, 1978, Scali and Masetti 1979) also used PGM in a study of Maniola jurtina in central Italy and the Tuscan Archipelago, with special reference to larva/adult selection. They demonstrated that larval allele frequencies differed from that of the adult (Scali and Masetti 1979). Courtney (1980) traced what he termed 'juvenile enzyme' at the PGM locus, which differed from that found in the adult. Other aspects of Masetti and Scali's work on the PGM locus in jurtina will be discussed in the appropriate sections.

Other electrophoretic studies on butterflies include those of Johnson (1971, 1976), Watt (1977, 1983a, b), Eanes (1978), Tebaldi (1982), Turner et al. (1979), Cullenward et al. (1979), Ehrlich and White (1980), Hughes and Zalucki (1984) and Watt et al. (1983).

Although the Lepidoptera can be considered to be a comparatively well worked group electrophoretically, there have been only a few studies of genetic variation in butterflies in both depth and breadth. Only two electrophoretic investigations of this nature have been undertaken, both on the Pieridae (Geiger 1978, 1980, 1982, Courtney 1980).

Geiger used 20-22 loci from 23 species forming nine genera, while Courtney used 22 loci from 19 species in six genera. The value of Courtney's work, vis a vis inter-species comparisons, is somewhat lessened by the fact that, by using mainly laboratory bred material from only a few females (Courtney 1982), his data approximate to very small samples, significantly affected by distortion in allele frequencies due to simple Mendelian segregation in the F_1 generation. Geiger used wild-caught samples. Nevertheless, there is strong concordance in their results.

Estimates based on electrophoretic data are undoubtedly gross approximations, considering the small fraction of the genome sampled and the fact that not all loci or alleles are detected. However, the work of Handford, Masetti and Scali, Courtney and Geiger suggests that enzyme electrophoresis can provide a relatively independent and objective basis for assessing the evolution of closely related butterfly groups.

2.1.2 Isozymes and allozyme variants in Maniolini

Over a period of five years 13 species (plus one new species described as a result of this research) from four Palaearctic genera and one Nearctic genus were studied electrophoretically (section 1.3.2). Major subspecies or geographic forms were examined, in some instances, where divergence was suspected. Special attention has been given to the species in the genus Maniola, especially jurtina and telmessia. Eight populations of telmessia and 67 populations of jurtina were sampled. Several samples from the same sites were collected at different stages of the flight period and a number of populations were sampled in successive years (section 1.3.1). Of the 35 isozyme loci identified, representing 24 enzymes, four were not used routinely (ADH-1, ADH -2, ADH -3 and PGI-1) as they could not be reliably and consistently resolved and scored. The enzymes ADA, ADH, CK and DIA have not been noted in butterflies before. Additionally, two new isozymes, provisionally named D(-)3GPDH and (MES), (section 1.3.2) were discovered and used routinely.

table 2.1.2a

Comparison of the number of alleles found at presumed homologous enzyme loci in some butterfly studies. Doubtful homologies are marked with an asterisk.

	Maniolini	Pieridae[1]	Euphydryas[2]	Speyeria[3]
species	13	19	2	10
enzymes	22	17	8	10
loci	31	22	8	11
AK-1	1	4	6*	-
ALD	2	6	-	-
EST-1	3	5*	-	-
GK	10	7*	3*	-
GOT-1	13	8	5	6*
IDH-1	10	-	-	3*
IDH-2	10	1	-	-
LAP-1	7	4	-	-
LAP-2	15	5	-	-
MDH	13*	1	-	6*
ME-1	3	3*	-	4*
ME-2	4	1*	-	-
MK	7	1*	-	-
PGI-2	13	4	9	8*
PGM	14	-	6	7
SOD-1	4	-	5*	-
α GPDH	4	4	4	5
6PGDH	1	7	-	-

[1]Courtney (1980) [2]McKechnie et al. (1975) [3]Brittnacher et al. (1978)

At loci which have been used by other workers, a far greater number of alleles were resolved in the Maniolini (table 2.1.2a), far more than by Courtney or Geiger in the Pieridae, in spite of the fact that more Pierid species were examined by these researchers, and the total maximum genetic distance between the most distantly related species in both groups appears to be similar. This could be explained by improved technique or the fact that the Maniolini are, indeed, more polymorphic and heterozygous.

What is believed to be a null allele was located in one population of jurtina, IDH-2_{80} in the Tannahill population. This manifests itself as a missing band in the homozygote and as a two-banded heterozygote. The dimeric locus IDH normally produces a three-banded heterozygote pattern. It occurs in the population at a frequency of 0.039 (±0.019).

At the PGM locus six allozymes were found in jurtina at low frequencies, four with mean frequencies across all populations of less than 0.001, one with a frequency of 0.001 and one with a frequency of 0.003. These occur at much higher levels in some populations (maximum frequencies - PGM_{60} 0.031, PGM_{84} 0.043, PGM_{96} 0.009, PGM_{103} 0.024, PGM_{128} 0.069). Six other more common allozymes were found which were, presumably, homologous with those identified by Bullini et al. (1975), Masetti and Scali (Masetti and Scali 1975a, b, 1976, 1977, 1978, Scali and Masetti 1979), who are the only others to have undertaken an electrophoretic study of PGM in a butterfly belonging to this group.

All populations of jurtina and telmessia were tested for Hardy-Weinberg equilibrium. Less common alleles were pooled to give expected values greater than five. Although there were some non-significant deviations at the PGM and GK loci, most populations fulfilled Hardy-Weinberg expectations. However, there were exceptions (table 2.1.2b). At the MPI locus one population of jurtina and a population of telmessia showed highly significant deviations, while one population of telmessia deviated significantly at the LAP-2 locus. One locus, GOT-1, was notable both in deviating greatly from expected values and showing a marked association between allele frequencies and sex in some populations of jurtina, telmessia, cypricola and nurag. The

table 2.1.2b

Maniola jurtina and M. telmessia - significant levels of deviation from Hardy-Weinberg expectations.

Maniola jurtina	n	χ^2_G	df
GOT-1			
Cahors, Lot	57	24.54	3 ***
Fountain de Vaucluse (north)	27	14.72	3 **
Gignod, Valle D'Aosta site 1	168	98.52	1 ***
Gignod, Valle D'Aosta site 3	163	138.87	3 ***
Gignod, Valle D'Aosta site 4	80	41.74	3 ***
Vienna	39	32.33	3 ***
Buskett, Malta	92	79.07	3 ***
Allenwood, Dublin	20	18.18	2 ***
Kinvara, Co. Clare	57	50.66	3 ***
MPI			
Sopron Umbeg, Hungary	41	21.15	3 ***
Cramond Island	101	18.58	3 ***
Maniola telmessia			
GOT-1			
Filerimos, Rhodes	98	101.42	1 ***
Kos	46	9.03	1 **
LAP-2			
Kos	46	5.06	1 *
MPI			
Simi	65	8.93	1 **

*** $p<.001$
** $p<.01$
* $p<.05$

table 2.1.2c

Maniola jurtina (Cramond Island and Sopron Umbeg) and M. telmessia (Simi and Kos) - observed and expected genotypes in populations which deviate from Hardy-Weinberg expectations.

MPI	Cramond (jurtina)		Sopron Umbeg (jurtina)		Simi (telmessia)	
genotype	obs	expected	obs	expected	obs	expected
76/76	-	-	0	0.02	-	-
76/96	-	-	1	0.34	-	-
76/100	-	-	1	1.54	-	-
76/105	-	-	0	0.07	-	-
84/84	1	0.02	-	-	-	-
84/96	0	0.88	-	-	-	-
84/100	1	1.92	-	-	-	-
84/105	0	0.16	-	-	-	-
96/96	13	8.62	2	1.20	-	-
96/100	31	37.68	9	10.76	-	-
96/105	2	3.21	0	0.51	-	-
100/100	47	41.19	25	24.20	12	6.91
100/105	3	7.02	3	2.30	15	23.05
100/109	-	-	-	-	0	2.13
105/105	3	0.30	0	0.05	23	19.21
105/109	-	-	-	-	4	3.55
109/109	-	-	-	-	1	0.16
	n=101		n=41		n=55	

LAP-2	Kos (telmessia)	
genotype	obs	expected
74/74	0	0.01
74/90	1	2.08
74/95	0	0.08
74/100	0	0.32
74/107	0	0.02
90/90	11	15.19
90/95	8	4.50
90/100	23	17.44
90/107	0	1.13
95/95	0	0.33
95/100	0	2.58
95/107	0	0.17
100/100	3	5.01
100/107	2	0.65
107/107	0	0.02
	n=51	

characteristics of this locus are so unusual that it will be discussed separately in section 2.1.3. One of the reasons which have been suggested for such deviations from expectations is that the sample or samples may have been collected from a population which includes more than one subpopulation, each having different allele frequencies (Cavali-Sforza and Bodmer 1971). In the case of MPI at Cramond the highly significant deviation from expected values (χ^2_G=18.58 df3 p<.001) arises primarily from an excess of homozygotes 96/96 and 100/100 (table 2.1.2c). Cramond Island, which hosts a massive population of jurtina, possibly often exceeding 20,000 individuals (Brakefield pers. comm.), comprised of a number of fairly compact subpopulations (demes). The Cramond sample was collected from various parts of the island. If the allele frequencies in these subpopulations differed from one deme to the other, it could result in a heterozygote deficit in the sample (Wahlund effect). The same explanation can possibly be made for a heterozygote deficit in the Sopron Umbeg sample (χ^2_G=20.95 df5 p<.001). As will be seen, differential allele frequencies in closely adjacent or subpopulations of jurtina are not uncommon. The Simi sample shows a deficit of heterozygote 100/105 and an excess of a homozygote 105/105. The telmessia sample was also collected in various parts of the island, which probably accounts for the Hardy-Weinberg deviations (χ^2_G=8.93 df1 p<.01). The same reasons cannot be forwarded for the significant heterozygote excess at the LAP-2 locus in the Kos sample (χ^2_G=5.06 df1 p<,05), which might be due to selection in favour of the 90/100 heterozygote, but more evidence for this is required.

Of the 31 loci studied extensively, 24 (77%) were polymorphic in one or more species at the 95% level and 26 (nearly 84%) were polymorphic at the 99% level. It is possible that most, if not all loci will, ultimately, be shown to be polymorphic. The mean percentage of loci polymorphic across all Manioline genera was 33.64 at the 95% level and 43.06% at the 99% level, but there was a wide range of polymorphism values among the genera from 22.22 : 37.04% in Aphantopus to 44.44 : 48.15% in Cercyonis. Polymorphism in species ranges from 22.22 : 25.93% in Maniola nurag to 51.85 : 55.56% in Hyponephele lycaon. Mean heterozygosity across all species was 0.115 with that for species ranging from 0.062 in Aphantopus hyperantus to 0.174 in Hyponephele lycaon. The effective number of alleles ranged

table 2.1.2d

Percentage polymorphism, mean heterozygosity and mean effective number of alleles in species and genera of Maniolini - standard errors in parenthesis.

Genus or species	polymorphic 95%	99%	heterozygosity (mean)	effective number of alleles (mean)
Maniola	33.64	43.06	0.100 (0.032)	1.183 (0.099)
jurtina	40.74	51.85	0.090 (0.026)	1.182 (0.059)
telmessia	29.63	51.85	0.085 (0.028)	1.188 (0.072)
cypricola	37.04	44.44	0.109 (0.027)	1.180 (0.248)
nurag	22.22	25.93	0.093 (0.038)	1.207 (0.087)
chia	37.04	40.74	0.118 (0.034)	1.191 (0.065)
megala	33.33	33.33	0.102 (0.037)	1.149 (0.060)
Pyronia	38.27	45.68	0.122 (0.036)	1.212 (0.067)
tithonus	29.63	44.44	0.099 (0.029)	1.186 (0.064)
bathseba	48.15	55.55	0.129 (0.035)	1.268 (0.082)
cecilia	37.04	37.04	0.139 (0.043)	1.183 (0.056)
Aphantopus	22.22	37.04	0.062 (0.023)	1.141 (0.076)
hyperantus	22.22	37.04	0.062 (0.023)	1.141 (0.076)
Hyponephele	40.74	43.52	0.128 (0.046)	1.267 (0.084)
lycaon	51.85	55.56	0.174 (0.037)	1.415 (0.106)
lupina	25.93	25.93	0.102 (0.065)	1.158 (0.065)
maroccana	33.33	37.04	0.109 (0.035)	1.228 (0.082)
Cercyonis	44.44	48.15	0.151 (0.039)	1.458 (0.146)
pegala	44.44	48.15	0.151 (0.039)	1.458 (0.146)

from 1.141 in H. lupina to 1.415 in the congeneric lycaon, with a mean for the Maniolini of 1.260. (table 2.1.2d). This level of polymorphism and heterozygosity corresponds with the averages reported in butterflies (eg Geiger and Scholl 1984), although, as with some other butterfly groups, the Pieridae for example (Courtney 1980, Geiger and Scholl 1984), levels of heterozygosity were a little lower than in other insects (Wagner and Selander 1974) and most invertebrates (Hamrick 1979).

It is important to note that it is still not possible to be certain of isozyme homologies and to compare directly certain aspects of the results of different electrophoretic studies. This is particular so with the esterases. Neither of the two dimeric esterases (Est-A and Est-B) found by Handford (1972, 1973a, 1973b) in jurtina is likely to be homologous with the EST-1, EST-A or EST-C used here, as in general configuration, eg very different allele frequencies and the presence of a common null allele in Handford's esterases, they have little in common. It is often impossible to tell which, if any, of the loci in a multi-locus enzyme corresponds with a single locus system identified by another worker. In the present situation it is wise to view with some caution suggested homologies of most of the enzyme loci used in different electrophoretic studies and to consider results independently.

2.1.3 The GOT-1 locus

The enzyme glutamate-oxaloacetate transaminase, which catalises the reaction a ketoglutarate to l-glutamate and l-aspartate to oxaloacetate, produced electrophoretic data which were extremely interesting and, at the same time, unexpected and anomalous. The resolution with this locus was consistently clear and unambiguous. Denaturation and other forms of organic deterioration (eg resulting from material not being frozen until several days after death), which can lead to misinterpretation of homozygotes as heterozygotes, produced a 'false heterozygote' pattern on the gels. These were easily identified from true heterozygotes by the presence of the middle band (homodimer), which was invariably close to the slower migrating band, not centrally placed as in a normal dimeric pattern and by the much darker

staining of one of the bands which indicates the homozygote mobility. Geiger located two loci, one migrating anodally which he called GOT-1. The second, GOT-2, migrated cathodally and is believed to be mitochondrial (Geiger pers. comm.). GOT-1 has proved to be a particularly useful enzyme in butterfly studies, being diagnostic for a number of closely related species in the Pieridae (Geiger, 1978, 1980 (1981), Geiger and Scholl 1984). Two loci were resolved in Maniolini, although GOT-2 was unreliable and not utilised. These were presumed to be homologous with the GOT loci used by Geiger.

In most Maniolini, GOT-1 was highly polymorphic. Some 13 alleles were identified in the group, of which nine were found in Maniola. However, only five alleles occurred at a frequency greater than 0.1 in any Maniola species and only two at a frequency of more than 0.5, $GOT\text{-}1_{100}$ and $GOT\text{-}1_{114}$ (table 2.1.3b).

In the genera Pyronia, Aphantopus, Hyponephele and Cercyonis, all populations examined were in Hardy-Weinberg equilibrium, as were populations of Maniola megala and chia, although one sample only from these species was available. A complete breakdown of expectations occurred in all populations of cypricola and nurag, and in a significant minority of jurtina and telmessia. Moreover, in most of these populations there was highly significant male / female heterogeneity. In the majority of telmessia populations, the frequency of the common allele, $GOT\text{-}1_{100}$, was about 0.85 - 0.90 in both males and females. In jurtina populations the frequencies were similar, although there was a greater range of values (about 0.80 - 0.95). This was, to some extent, associated with geographical variation. The alleles $GOT\text{-}1_{67}$, $GOT\text{-}1_{84}$ and $GOT\text{-}1_{114}$ in these populations appeared as the next most frequent. In 20 of the 68 jurtina populations, two of the eight telmessia populations and all of the cypricola and nurag populations there were obvious deviations from equilibrium values, highly significant in the case of jurtina (χ^2_G=1218.44 df1 $p<.001$), telmessia (χ^2_G=228.57 df1 $p<.001$) and nurag (χ^2_G=7.638 df1 $p<.001$) and significant in cypricola (χ^2_G=4.230 df1 $p<.05$). The deviations accrued through a deficit of the 100/114 heterozygote in males and, to some extent, in the female. Simultaneously with this in the deviant populations, there was a marked sex-associated allele frequency differential. The characteristics of these populations can be summarised.

table 2.1.3a

Observed and expected genotypes at the GOT-1 locus in two populations of Maniola jurtina at Gignod, Italy.

Gignod site 1

allele	males			females		
	obs	exp	%	obs	exp	%
100/100	4	0.14	3.57	12	6.00	50.00
100/114	0	7.71	0.00	0	12.00	0.00
114/114	108	104.14	96.43	12	6.00	50.00
	n=112			n=24		

Gignod site 2+3

allele	males			females		
	obs	exp	%	obs	exp	%
84/100	1	0.27	0.87			
84/107	0	0.02	0.00			
84/114	0	0.71	0.00			
100/100	24	8.09	20.87	40	32.65	81.63
100/107	0	1.06	0.00			
100/114	12	42.23	10.44	0	14.69	0.00
100/121	0	0.27	0.00			
107/107	1	0.03	0.87			
107/114	2	2.83	1.74			
107/121	0	0.02	0.00			
114/114	74	57.76	64.35	9	1.65	18.37
114/121	1	0.71	0.87			
	n=115			n=49		

1. The two most frequent alleles are almost always GOT-1_{100} and GOT-1_{114}.

2. There is a great deal of variation in the frequencies of these alleles. In males, the most common situation is when GOT-1_{114} has a frequency of between 0.6 and 0.8, although GOT-1_{100} is sometimes the more frequent with a frequency of about 0.6. In some populations the frequency of these two alleles is approximately equal.

In females, GOT-1_{100} is usually by far the most frequent, usually greater than 0.9, but in some populations (telmessia in Karpathos, jurtina in the Atlas Mountains) the situation is reversed and GOT-1_{114} has an equally high frequency, ie 0.9 or higher (table 2.1.3b).

3. Always in males and usually in females, there is a deficit of the 100/114 heterozygote, in some populations reaching an exceptional level (table 2.1.3a).

There appears to be no distributional pattern in the occurrence of sex-associated heterozygote deficit populations (figure 2.1.3a). This is particularly apparent in areas where geographically close populations express their allozyme configuration differentially. For example, the Faliraki and Filerimos populations of telmessia in Rhodes are separated by a maximum distance of 20km: one population is normal and the other deviant. In Ireland two jurtina iernes populations, located only 10km apart (Rosebery and Allenwood), were similarly divergent at this locus. Clearly the phenomenon is largely independent of broad environmental gradients. The allelic configuration at the locus must be in some way determined by influences within the populations themselves. However, it would seem that a population must carry the potential for these traits. Significantly, although more populations of jurtina were sampled in Britain than in any other single area of equivalent size, no population was deviant on the British mainland, Isle of Man or Isles of Scilly. This would suggest that the genetic arrangement required, whatever it may be, was not inherited by the founders of these British populations. Much less likely, the British populations could have lost this characteristic. It is probable, of course, that the group of populations which have been referred to as 'deviant' is

table 2.1.3b

Frequencies of the alleles GOT-1_{100} and GOT-1_{114} in sex-associated and heterozygote deficient populations of Maniola jurtina and M. telmessia.

	population	allele	males	females
telmessia	Filerimos	100	0.296 (0.037)	0.861 (0.058)
		114	0.697 (0.037)	0.056 (0.038)
	Karpathos	100	0.312 (0.082)	0
		114	0.688 (0.082)	1.000
cypricola	Cyprus	100	0.917 (0.046)	0.804 (0.022)
		114	0.083 (0.046)	0.174 (0.056)
nurag	Sardinia	100	0.381 (0.075)	1.000
		114	0.597 (0.076)	0
jurtina	Atlas Mts.	100	0	0.043 (0.030)
		114	1.000	0.957 (0.250)
	Malta	100	0.563 (0.051)	0.792 (0.033)
		114	0.417 (0.050)	0.156 (0.029)
	Theyner Hohe	100	0.227 (0.089)	0.768 (0.056)
		114	0.636 (0.103)	0.107 (0.041)
	Fiastra	100	0.579 (0.080)	1.000
		114	0.395 (0.079)	0
	Trabzon	100	0.490 (0.049)	-
		114	0.490 (0.049)	-
	Crete	100	0.471 (0.086)	0.833 (0.108)
		114	0.529 (0.086)	0.167 (0.108)
	Belveaux	100	0.200 (0.126)	0.974 (0.026)
		114	0.800 (0.126)	0
	Celon	100	0.636 (0.130)	0.900 (0.055)
		114	0.364 (0.130)	0
	Nant	100	0.305 (0.051)	0.636 (0.103)
		114	0.659 (0.052)	0.227 (0.089)
	Cahors	100	0.547 (0.054)	0.893 (0.058)
		114	0.360 (0.052)	0
	Sorede	100	0.667 (0.111)	0.250 (0.153)
		114	0.333 (0.111)	0.750 (0.153)
	Digne	100	0.102 (0.031)	0.750 (0.150)
		114	0.857 (0.035)	0.250 (0.150)
	Fountain de Vaucl.(N)	100	0.361 (0.080)	0.944 (0.054)
		114	0.674 (0.389)	0.063 (0.054)
	Fountain de Vaucl.(S)	100	0.283 (0.066)	0.813 (0.069)
		114	0.674 (0.069)	0.063 (0.043)
	Vergon	100	0.109 (0.039)	0.900 (0.095)
		114	0.891 (0.039)	0
	Verbier	100	0.414 (0.065)	0.500 (0.250)
		114	0.569 (0.065)	0.500 (0.250)
	Gignod	100	0.155 (0.015)	0.701 (0.096)
		114	0.832 (0.016)	0.299 (0.036)
	Allenwood	100	0.409 (0.105)	0.972 (0.027)
		114	0.545 (0.106)	0
	Kinvara	100	0.714 (0.049)	0.900 (0.055)
		114	0.214 (0.045)	0

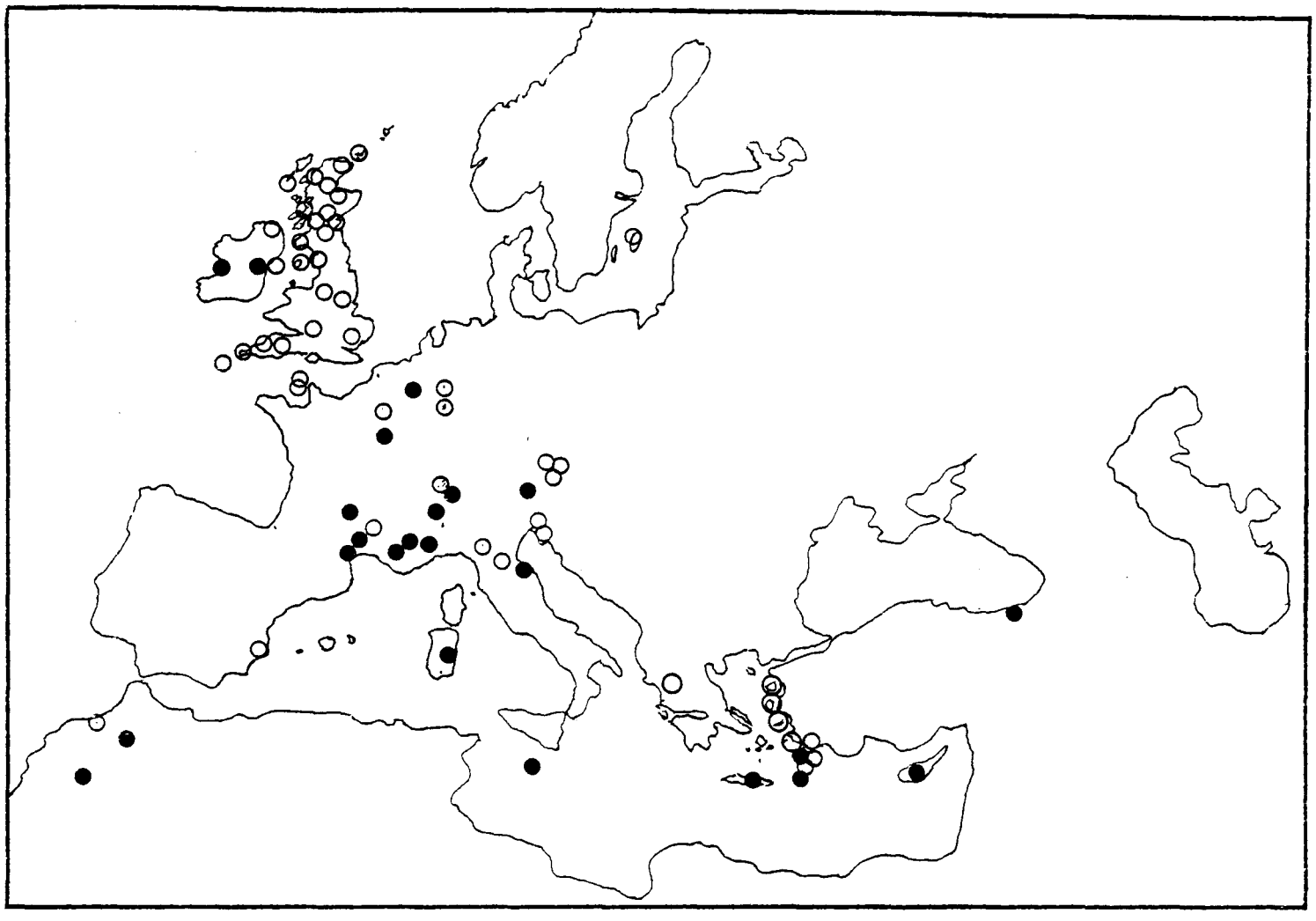


figure 2.1.3a

The GOT-1 locus - distribution of 'normal' and sex-associated heterozygote deficient (SAHD) populations in Maniola jurtina, megala, chia, telmessia, cypricola and nurag.

○ 'normal' populations ● SAHD populations

descended from an ancestral Maniola posessing these characteristics, otherwise they would not occur in several species in the genus. Parallel evolution of such a remarkable feature is unlikely in such a geographically widespread and ecologically diverse group.

Sex linkage at electrophoretically detected loci in butterflies is rare and has not been described at a GOT locus. A heterozygosity deficit appears to be less common than heterozygote excess (Ferguson 1980). Cavali-Sforza and Bodmer (1971) suggest six possible explanations for a deficit in heterozygotes in a population

1. heterogeneity of the population which may consist of a number of independent subpopulations

2. inbreeding

3. selection against heterozygotes

4. presence of a 'silent' (null) allele that masks heterozygotes making them indistinguishable from any of the types of homozygotes

5. errors in the classification of phenotypes

6. positive assortitive mating.

Watt et al. (1983b) report differential mating success of a PGI allozyme genotype in Colias. This too, in appropriate circumstances, could lead to a heterozygote deficit. The fact that both this deficit and sex-associated allozyme frequencies occur simultaneously makes it unlikely that any of the above could account for the situation in Maniola. It will be demonstrated that jurtina (and probably other Maniola species) forms discrete subpopulations. But it will also be shown that an effective level of gene flow between these subpopulations must be usual. Furthermore, contiguous populations not separated by obvious barriers have little tendency to differentiate genetically (section 2.3.2). Some populations may be subject to large scale immigration, but this cannot explain the sex-associated allozyme frequencies. A sibling species coexisting with jurtina, having

very different allele frequencies, could result in a heterozygote deficit, but prerequisites of this would be 1. the species must have different allele frequencies at GOT-1_{100} and GOT-1_{114} and 2. they must have different emergence times so that, by chance, mainly males of one species and females of the other were taken. Samples in a number of populations were taken at different times: this would effectively eliminate the possibility of this sort of 'sampling error'. The chances of at least four sibling species evolving in association with such closely related species as jurtina, telmessia, cypricola and nurag must be remote in the extreme. Inbreeding is unlikely to be a factor as most jurtina populations are very large (eg Gignod).

Selection against the heterozygote 100/114 would assume that the sex-association of allele frequencies was independent of it or acting very differently in either sex. It would also leave the question of the apparent random distribution of deviant populations and assume that only certain populations have the potential for the selection mechanism and sex-association of frequencies.

There is no evidence for the presence of a null allele. More than 4000 specimens were studied electrophoretically and neither unstained bands, identifying null allele homozygotes, nor two banded heterozygotes, identifying normally dimeric three-banded heterozygotes, appeared in any sample. GOT zymograms were easily read and the amount of error due to misclassification of genotypes must be negligible. Positive assortitive mating and differential mating success would explain the deficit of heterozygotes, but not the sex-associated allele frequencies. The possibility of an inherited chromosomal aberration must not be overlooked. While it is not difficult to make simple preparations from butterflies for chromosome counts, karyotyping and more detailed information of chromosome morphology has so far been elusive (Lorkovic 1941, Emmel 1968, Larsen 1975). Chromosomes of butterflies almost invariably appear as amorphous blobs with only size differences and little shape being apparent. Until cytological techniques with Lepidoptera have improved, the study of Maniola chromosomes will be delayed and any implications in the present context must be speculative.

2.2 Intra-population allozyme variation

The usually highly polymorphic loci CK, GK, GOT-1, IDH-1, IDH-2, LAP-2, MPI, PGI-2, PGM and PK were used in the analysis of intra-population variation. Wright's F statistics (section 1.3.6) were used as a measure of the variance in allele frequency within populations. It should be noted that a standardised variance or inbreeding coefficient, F_{ST}, of 0.050 or greater for among subdivision variance suggests significant genic differentiation (Wright 1978).

2.2.1 Sex differences

In all species of the genus Maniola and at all genetic loci, with the notable exception of the GOT-1 populations which exhibit an association of allele frequencies with sex, only a single population at one locus revealed a significant level of male/female heterogeneity, the GK locus in the Baghd (Barra) sample (χ^2=6.41 df1 p<.05). However, this could be due to sampling error (n=39) and little importance can be attributed to it (table 2.2.1a).

2.2.2 Changes during flight period

Two aspects of temporal allozyme variation during the flight period of jurtina were investigated. Three non-diapausing populations of jurtina (Milour Moor, Torrie Forest and the Easdale/Ellanbeich complex) were sampled in early July and early August, representing the early and late part of the brood. In some British populations, jurtina displays a bimodal emergence (Brakefield 1979a, b), sometimes to a remarkable degree (Thomson 1971a). In the southern part of the species range, possibly in association with the Mediterranean climatic sub-zone, all Maniola species diapause during the hot summer period, usually from about mid-June until early August. The importance of aestivation in jurtina and its biological function in relation to reproduction has been investigated in depth by Scali and Masetti (Scali 1971b, Scali and Masetti 1975, 1979, Masetti and Scali 1972, 1976). They also investigated PGM allozyme variation (Scali and Masetti 1979, Masetti and Scali 1975a, b, 1976, 1977, 1979) in the

table 2.2.1a

Maniola jurtina and M. telmessia - significant levels of male / female heterogeneity.

	n	χ^2	df
Maniola jurtina			
GK			
Baghd, Barra	39	6.41	1*
GOT-1			
jurtina: sex-linked populations+	338	133.31	1***
Buskett, Malta	48	5.76	1*
Maniola telmessia			
GOT-1			
Filerimos, Rhodes	98	26.98	2***

*** p<.001 + all other sex-linked populations show
** p<.01 significant levels of heterogeneity
* p<.05

butterfly in central Italy and suggested that changes in allele frequencies at this locus before and after imaginal diapause indicated selection in favour of the 'C/D' heterozygotic genotype. Many years earlier Dobzhansky and Ayala (1973) reported seasonal variation in allele frequencies in PGM-1 and ME-2 in Drosophila pseudoobscura and D. persimilis. Berger (1971) found an increased frequency of an allele at the α GPDH locus from June to September.

The Torrie Forest samples taken in 1982 showed little difference between the early and late material at the loci examined (χ^2=4.625 df9 p>.80), although the standardised variance for genic differentiation at PGM (F_{ST}=0.050) was a little high. The Milour Moor population was sampled in both 1982 and 1984 and the sub-population (site 2) in 1982 only. Significant levels of differentiation between early and late samples were noted in the main population in 1982 at the GOT-1 locus (χ^2=4.334 df1 p<.05) and in the subpopulation at the LAP-2 locus (χ^2=4.015 df1 p<.05). However, over all loci the early/late figure was very similar (χ^2=5.702 df10 p>.80). High levels of genic differentiation were observed at the PGM locus in the main population in 1984 (F_{ST}=0.155) and in site 2 at GK (F_{ST}=0.054) and IDH-1 (F_{ST}=0.069). Early and late jurtina on the island of Easdale were significantly different at the MPI locus (χ^2=4.079 df1 p<.05) and were genetically diversant at GOT-1 (F_{ST}=0.136), but on the nearby mainland, the early/late samples from Ellanbeich were relatively homogeneous (χ^2=7.068 df10 p>.70, although the standardised variance was rather high at the GOT-1 locus (table 2.2.2a)

Pre- and post-aestivation jurtina hyperhispulla from Malta gave significant levels of differentiation at GOT-1, LAP-2 and PGI-2 and highly significant levels at the PK locus, although the standardised variance was significant at PK only (table 2.2.2b). This was a consequence of a large increase in the heterozygotes 58/100 and 100/139, with corresponding changes in allele frequencies and heterozygosity (table 2.2.2c).

It can be concluded, therefore, that there is little evidence for significant differentiation between early and late jurtina in wild populations where diapause is absent. The differences seen in the

table 2.2.2a

Allozyme differentiation in early/late samples from British populations of Maniola jurtina.

	Milour Moor (2 sites) 1982+1984			Torrie Forest		
locus	F_{ST}	χ^2	df	F_{ST}	χ^2	df
CK	0.002	0.373	1	0.000	-	-
GK	0.001	0.293	1	0.022	0.940	1
GOT-1	0.008	1.698	1	0.000	0.017	1
IDH-1	0.012	2.409	1	0.000	0.003	1
IDH-2	0.000	0.075	1	0.000	0.004	1
LAP-2	0.000	0.004	1	0.017	0.703	1
MPI	0.000	0.028	1	0.004	0.163	1
PGI-2	0.001	0.305	1	0.005	0.212	1
PGM	0.001	0.273	1	0.050	2.110	1
PK	0.001	0.249	1	0.000	0.003	1
	mean	total		mean	total	
	0.003	5.707	10	0.012	4.625	9

	Easdale			Ellanbeich		
locus	F_{ST}	χ^2	df	F_{ST}	χ^2	df
CK	0.000	-	-	0.011	0.295	1
GK	0.000	0.038	1	0.026	0.716	1
GOT-1	0.002	0.275	1	0.136	3.812	1
IDH-1	0.006	0.665	1	0.005	0.151	1
IDH-2	0.015	1.654	1	0.028	0.779	1
LAP-2	0.001	0.105	1	0.004	0.104	1
MPI	0.036	4.079*	1	0.000	0.000	1
PGI-2	0.021	2.358	1	0.000	0.000	1
PGM	0.004	0.474	1	0.018	0.495	1
PK	0.007	0.788	1	0.026	0.716	1
	mean	total		mean	total	
	0.012	11.688	9	0.025	7.068	10

* $p<.05$

table 2.2.2b

Allozyme differentiation in pre- and post-aestivation samples from Maltese populations of Maniola jurtina hyperhispulla.

locus	F_{ST}	χ^2	df
CK	0.000	0.001	1
GK	0.001	0.169	1
GOT-1	0.045	6.266*	1
IDH-1	0.006	0.772	1
IDH-2	0.000	0.010	1
LAP-2	0.035	4.885*	1
MPI	0.000	0.042	1
PGI-2	0.040	5.666*	1
PGM	0.002	0.219	1
PK	0.085	11.854***	1
	mean	total	
	0.021	29.885***	10

*** p<.001
* p<.05

---

pre- and post-aestivation samples from Malta add weight to the proposition made by Scali and Masetti that seasonal adaptation, manifested in selection in favour of heterozygotes, is a feature of the species in southern Europe, although there was little change in pre/post aestivation heterozygozity at GOT-1. Watt (1977) found that Colias adults, early in the flight period, showed genotype frequencies in Hardy-Weinberg equilibrium, but heterozygote excess developed in the insects with age. He suggests that simple directional selection and large scale population mixing are unlikely to be the cause of this, although he admits that several other selection modes remain possible. As the late Milour Moor and Easdale samples inevitably included a higher proportion of 'older' individuals compared with the early material and no significant difference was noted between them, it seems unlikely that heterozygote excess is age dependent, although it must be emphasised that female longevity is far greater in diapausing than in non-diapausing jurtina.

table 2.2.2c

Allele frequencies (standard errors in parenthesis) at the loci GOT-1, LAP-2, PGI-2 and PK in pre- and post-aestivation sample from Maltese populations of Maniola jurtina hyperhispulla.

locus	allele	pre-aestivation	post-aestivation
GOT-1	84	-	0.049 (0.028)
	93	0.028 (0.013)	-
	100	0.690 (0.038)	0.863 (0.043)
	107	0.017 (0.009)	-
	114	0.265 (0.037)	0.088 (0.037)
heterozygosity		0.180	0.076
LAP-2	90	0.031 (0.018)	0.033 (0.016)
	95	-	0.016 (0.011)
	100	0.969 (0.018)	0.861 (0.031)
	115	-	0.074 (0.024)
	130	-	0.016 (0.011)
heterozygosity		0.063	0.246
PGI-2	22	-	0.074 (0.024)
	40	0.019 (0.018)	-
	60	0.019 (0.018)	-
	100	0.833 (0.051)	0.680 (0.042)
	119	-	0.016 (0.011)
	165	0.130 (0.046)	0.197 (0.036)
	219	-	0.033 (0.016)
heterozygosity		0.333	0.459
PK	58	0.007 (0.007)	0.091 (0.022)
	100	0.993 (0.007)	0.811 (0.024)
	139	-	0.028 (0.013)
heterozygosity		0.013	0.193

2.2.3 Annual fluctuations

Samples from five jurtina populations were taken over more than a single season. Three populations at Gignod in the Italian Alps were sampled in 1982 and again in 1983, the Milour Moor population in 1982 and 1984, and the Kilsyth Hills population in the same years. Significant and highly significant differences were found between 1982 and 1983 in all of the Gignod populations, reaching highly significant levels at GOT-1 and IDH-2 in site 1, GK in site 2+3 and MPI in site 4, and a significant level at LAP-2 at site 2+3. The overall difference between the years was highly significant in sites 1 and 4 and significant at site 2+3 (table 2.2.3a). However, pooling data from all Gignod sites shows a reduced level of significance (χ^2=18.813 df10 $p<.05$). This could suggest that jurtina in sites 1 to 4 form one very large and widespread panmictic population. This hypothesis was tested in 1983 when 500 jurtina (250 males and 250 females) from site 2+3 were marked and released on the same day using the marker pen technique of Brakefield (1979a, b). Two days later, 300 jurtina (sexes not noted) were collected in site 1 and 200 in site 4. Not one marked individual was taken. It had been expected that habitat disturbance would have forced the butterflies to move in the direction of the other sites: the meadows in which jurtina flew were cut the day after the butterflies were marked. Although sites 2+3 and site 4 are separated by a maximum of 200m, this is consistent with the view that there is very little movement between the populations at Gignod and that the species is relatively sedentary (Brakefield 1979a, b, Dowdeswell 1981).

The Milour Moor population produced highly significant differences between 1982 and 1984 at GK and PK, and a significant level of difference at LAP-2. The mean difference was highly significant. The Kilsyth Hills population did not show the same degree of differentiation, LAP-2 being the only locus which produced any difference between the two years (table 2.2.3b).

This would suggest that there are marked changes in allozyme expression from year to year in jurtina. Mean heterozygosity in Milour Moor populations decreased between 1982 and 1984 and in the Kilsyth Hills populations over the same period. In the latter, there was also a

table 2.2.3a

Allozyme differentiation in Gignod samples Maniola jurtina collected in 1982 and 1983.

	Gignod site 1			Gignod site 2+3		
locus	F_{ST}	χ^2	df	F_{ST}	χ^2	df
CK	0.001	0.080	1	0.000	-	1
GK	0.000	0.011	1	0.049	7.004**	1
GOT-1	0.317	36.182***	1	0.002	0.322	1
IDH-1	0.014	1.591	1	0.000	-	-
IDH-2	0.068	7.709**	1	0.008	1.071	1
LAP-2	0.000	0.055	1	0.028	3.971*	1
MPI	0.024	2.706	1	0.000	0.035	1
PGI-2	0.001	0.082	1	0.016	2.206	1
PGM	0.000	0.016	1	0.008	1.126	1
PK	0.007	0.836	1	0.012	1.650	1
	mean	total		mean	total	
	0.043	49.268***	10	0.017	19.466*	8

	Gignod site 4			Gignod all sites		
locus	F_{ST}	χ^2	df	F_{ST}	χ^2	df
CK	0.000	-	-	0.002	0.603	1
GK	0.001	0.003	1	0.021	7.512**	1
GOT-1	0.000	0.013	1	0.013	4.472*	1
IDH-1	0.012	0.925	1	0.000	0.049	1
IDH-2	0.000	-	-	0.002	0.595	1
LAP-2	0.009	0.695	1	0.004	1.305	1
MPI	0.265	20.682***	1	0.001	0.288	1
PGI-2	0.024	1.854	1	0.006	2.061	1
PGM	0.000	0.001	1	0.001	0.337	1
PK	0.001	0.078	1	0.005	1.591	1
	mean	total		mean	total	
	0.040	25.137**	8	0.005	18.813*	10

*** p<.001
** p<.01
* p<.05

table 2.2.3b

Allozyme differentiation in British samples Maniola jurtina collected in 1982 and 1984.

	Milour Moor			Kilsyth Hills		
locus	F_{ST}	χ^2	df	F_{ST}	χ^2	df
CK	0.000	-	-	0.001	0.075	1
GK	0.049	8.840**	1	0.003	0.195	1
GOT-1	0.007	1.340	1	0.027	1.753	1
IDH-1	0.000	-	-	0.001	0.053	1
IDH-2	0.000	0.075	1	0.014	0.869	1
LAP-2	0.035	6.267	1	0.073	4.650*	1
MPI	0.001	0.211	1	0.001	0.033	1
PGI-2	0.000	0.019	1	0.017	1.100	1
PGM	0.000	0.000	1	0.003	0.194	1
PK	0.049	8.770**	1	0.029	1.857	1
	mean	total		mean	total	
	0.018	25.857**	8	0.017	10.778	10

*** p<.001
** p<.01
* p<.05

table 2.2.3c

Mean heterozygosity and effective number of alleles in populations of Maniola jurtina showing year to year differentiation (standard errors in parenthesis), based on 10 loci - CK, GK, GOT-1, IDH-1, IDH-2, LAP-2, MPI, PGI-2, PGM and PK.

Population and year		mean heterozygosity	effective number number of alleles
Milour Moor	1982	0.215 (0.054)	1.301 (0.134)
Milour Moor	1984	0.160 (0.058)	1.301 (0.134)
Kilsyth Hills	1982	0.190 (0.052)	1.327 (0.118)
Kilsyth Hills	1984	0.168 (0.052)	1.168 (0.052)
Gignod 1	1982	0.217 (0.077)	1.497 (0.196)
Gignod 2	1983	0.232 (0.085)	1.542 (0.204)
Gignod 2+3	1982	0.177 (0.062)	1.400 (0.141)
Gignod 2+3	1983	0.217 (0.076)	1.503 (0.173)
Gignod 4	1982	0.241 (0.089)	1.536 (0.232)
Gignod 4	1983	0.199 (0.078)	1.434 (0.173)

drop in the effective number of alleles. However, at Gignod, while mean heterozygosity decreased at site 4 between 1982 and 1983, it increased in sites 1 and 2+3 over the two years. The effective number of alleles in all Gignod populations remained similar (table 2.2.3c).

While sampling error could have contributed to these differences in the Kilsyth Hills data (1982 n=34, 1984 n=32), it is unlikely in the case of Milour Moor (1982 n=177, 1984 n=90) or Gignod (1982 n=256, 1983 n=175).

Highly significant fluctuations in genotype frequencies were reported by Cullenward et al. (1979) in a massive population of Euphydryas anicia at the PGI, PGM, BDH and MDH loci. Jelnes (1974) reported year to year variation in the moth Thera variata, suggesting selection against a PGM heterozygous phenotype. Geiger (pers. comm.) found only one population of Pieris napi in which allele frequencies changed significantly from year to year. This he believed was due to severe bottlenecking (Maruyama and Fuerst 1984). The Milour Moor and Kilsyth populations could have suffered significant reductions in numbers between 1982 and 1984, thus reducing genic diversity. Another explanation could be that differential environmental pressures in the two years have affected the allozyme configuration. However, the difference in the direction of heterozygosity expression between the two years and among the Gignod samples is consistent with genetic drift being the reason for annual fluctuations in genic makeup in these, and possibly also the Milour Moor populations.

2.3 Inter-population allozyme variation

2.3.1 Altitude

It is very difficult to isolate the effects of altitude on allozyme frequencies from the factors which contribute to the overall the genic differentiation which can be detected between geographically close populations (see section 2.3.2). McKechnie et al. (1975), working on Euphydryas editha and E. chalcedonia, noted what he believed was a strong correlation between allozyme frequencies and some environmental variables, including altitude. Between the Auchenstarry and Kilsyth Hills sites (the Kilsyth Hills population is the highest known colony of jurtina in Britain) there is an altitudinal rise of some 300m (50m to 350m above sea level). These two populations are highly homogeneous (χ^2=3.669 df9 p>.90). The three sites at Gignod cover the altitudinal range of 950 to 1650m (site 1 is at 950m, site 2+3 at 1300m and site 4 at 1650m). Highly significant differences were found between some of the sites at four loci, GK, GOT-1, LAP-2 and MPI. However, when the samples for both years are pooled, only GK (χ^2=7.233 df1 p<.01) and GOT-1 (χ^2=23.647 df1 p<.001) at sites 1 and 2+3 differed, although over all loci these two sites differed to a highly significant level (χ^2=37.038 df9 p<.001 - table 2.3.1a).

Allele frequencies did not reveal any trends which could be related unambiguously to increasing altitude (figure 2.3.1a). In both years at Gignod, the frequency of the common allele at the MPI locus (MPI_{100}) increased and that of the common allele at PK (PK_{100}) decreased from site 1 to site 4, but, as the other loci did not display similar variation trends over the two years, there is no evidence which can demonstrate a link between allozyme variation and altitude in this butterfly.

It is by no means a simple matter to demonstrate a relationship between gene frequency differentiation and geographical/topographical factors such as altitude as they are themselves, directly or indirectly, associated with other environmental variables including temperature, humidity and photoperiodism. Evidence for temperature associated variation is accumulating, although it has still to be demon-

table 2.3.1a

Allozyme differentiation between Gignod samples Maniola jurtina collected at various altitudes - 1982 and 1983 samples pooled.

	Gignod sites 1 and 2+3			Gignod sites 1 and 4		
locus	F_{ST}	χ^2	df	F_{ST}	χ^2	df
CK	0.000	-	-	0.005	0.774	1
GK	0.024	7.233**	1	0.001	0.237	1
GOT-1	0.078	23.647***	1	0.018	2.841	1
IDH-1	0.003	1.017	1	0.004	0.689	1
IDH-2	0.005	1.479	1	0.003	0.415	1
LAP-2	0.002	0.589	1	0.002	0.243	1
MPI	0.006	1.733	1	0.006	1.032	1
PGI-2	0.003	1.062	1	0.003	0.528	1
PGM	0.000	0.003	1	0.000	0.006	1
PK	0.001	0.275	1	0.005	0.528	1
	mean	total		mean	total	
	0.014	37.038***	9	0.005	7.293	10

	Gignod sites 2+3 and 4		
locus	F_{ST}	χ^2	df
CK	0.005	0.801	1
GK	0.014	2.306	1
GOT-1	0.023	3.602	1
IDH-1	0.015	2.454	1
IDH-2	0.009	1.489	1
LAP-2	0.000	0.004	1
MPI	0.000	0.004	1
PGI-2	0.000	-	-
PGM	0.000	0.013	1
PK	0.001	0.110	1
	mean	total	
	0.007	10.781	9

*** $p<.001$
** $p<.01$

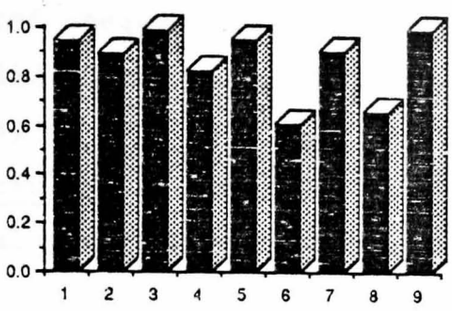
figure 2.3.1a

Frequencies of the 100/100 alleles in Maniola jurtina in 1982, 1983 and 1984 at high altitude sites - see text for explanation. Frequencies at Milour Moor given for comparison.

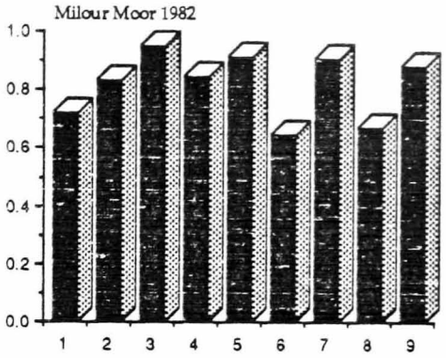
Loci utilised (bars)

1. GK
2. GOT-1
3. IDH-1
4. IDH-2
5. LAP-2
6. MPI
7. PGI-2
8. PGM
9. PK

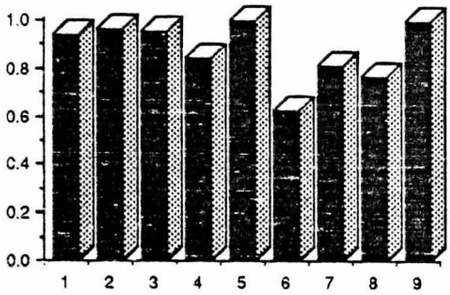
Milour Moor 1984



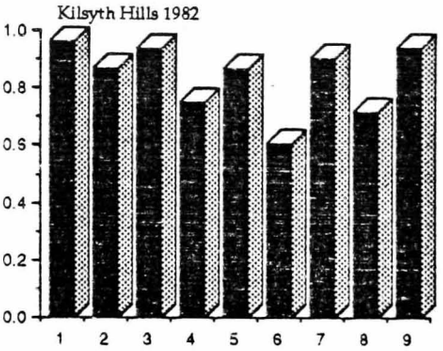
Milour Moor 1982



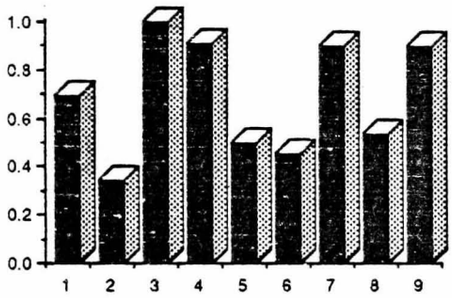
Kilsyth Hills 1984



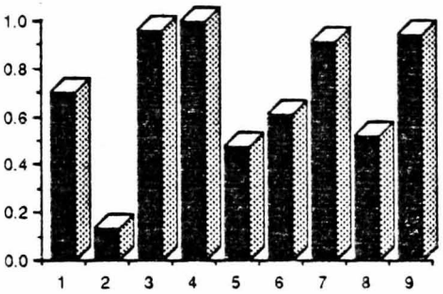
Kilsyth Hills 1982



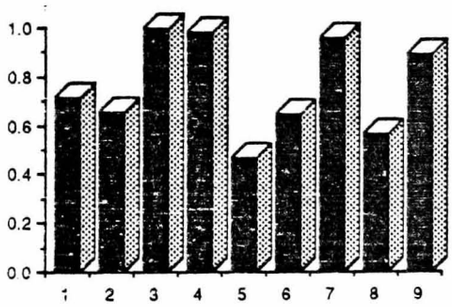
Gignod site 1 1983



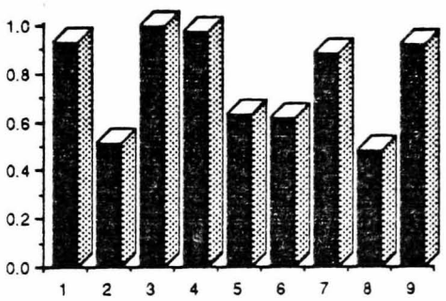
Gignod site 1 1982



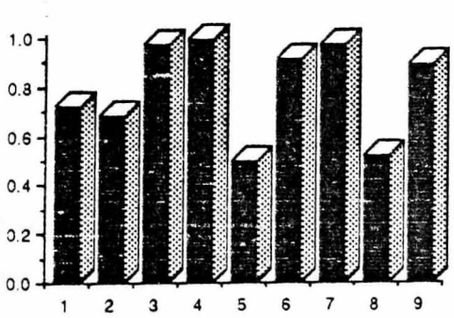
Gignod sites 2+3 1983



Gignod sites 2+3 1982



Gignod site 4 1983



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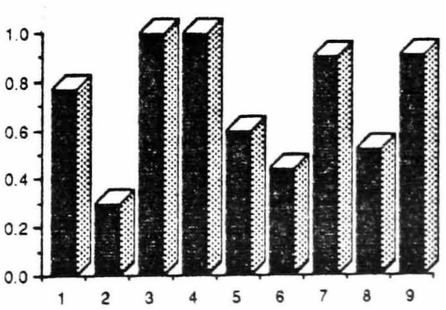


strated unequivocally that allelic frequencies or allozyme expression in wild populations and temperature are directly correlated. Johnson (1974) in his study of two sympatric Menidia species, berylina and peninsulae, claims that their similar response to the same environmental gradient was strong evidence for the effect of a thermal related variable on allelic variation, although he did emphasise the importance of present day and historical restrictions to gene flow and its effect on population divergence.

2.3.2 Quasi-contiguous populations

The dynamics of Maniola jurtina populations have been well studied (Brakefield 1977, 1979, 1979a, b, Dowdeswell 1981, Pollard 1981a, b). Most field observers have noted periodic changes in dispersal behavior in the butterfly (Thomson 1980). Ford and his colleagues, working on the species on the small island of Tean in the Isles of Scilly, noted that, for the duration of their research, not a single female crossed a short stretch of unsuitable land (bracken) between the two colonies (Dowdeswell 1981). Clearly, dispersal behaviour is modified from time to time by influences which have not so far been identified.

Populations of jurtina are frequently complex in structure. They may be isolated, single colonies as is the rule in the northern part of its range. More commonly, the butterfly forms 'multiple' populations, often separated by very narrow stretches of territory which are, apparently, 'hostile' to the insect. In these situations the population (or subpopulation) sizes can be approximately equal, or comprise a large main or 'parent' colony associated with a much smaller 'satellite' population. These population complexes are here termed 'quasi-contiguous'. The structure and relationships between such population groups is of great interest in terms of population dynamics and the evolution of infra-specific divergence.

Five population complexes of this kind were studied (figure 1.3.1b).

1. Milour Moor / Milour Moor site 2 - The population size of the main colony at Milour Moor is very large, probably greater than 25,000

individuals (Brakefield 1979a). The area comprises about 4ha of mixed herb meadow, rush bog and rough grazing land, while site 2, extending to about 0.5ha, is rush bog exclusively. The two areas are separated by bracken and, partially, by woodland across a stretch of about 100m. The area has remained unchanged for many years.

2. Leighills / Tannahill - The Leighills site comprises a complex of rough grazing and railway embankment, rich in herbs. The Tannahill site is 200m distant and was reclaimed building land in 1982-3. The first jurtina were seen there in 1984, although it is likely that a few 'founders' colonised the area in the previous year. The sample was taken in 1984: jurtina did not occur in the adjacent field prior to 1983.

3. Easdale / Ellanbeich - Easdale, a small island rich in highly calcareous soils, is well known for its slate quarries and supports a sizable population of jurtina. It is separated from the large island of Seil (Ellanbeich) by about 250m of relatively calm water. The Ellanbeich population is also large and is restricted to a raised beach area of mainly acid rush bog.

4. St Andrews sites 1, 2 and 3 - The three adjacent St Andrews sites lie partly on a disused railway line and partly on a golf course. However, jurtina appears to be confined to discrete subpopulations across the area.

5. Fountain de Vaucluse north and south - One population is restricted to the northern slopes of the Sorgue valley and the other to the southern side. Both populations fly in olive groves and appear to be confined to the tree-shaded areas.

These sites were selected to create the opportunity to examine the degree of genetic divergence, if any, which has taken place between populations which are contiguous, adjacent, separated by a barrier of 'hostile' territory or very recently colonised.

The comparison between the two Milour Moor populations was inconclusive, not reaching significance levels (χ^2=10.781 df9 p<.30) across

table 2.3.2a

Heterozygosity and effective number of alleles in the Leighills and Tannahill populations, with frequencies of alleles found uniquely in the Tannahill populations (standard errors in parenthesis).

	heterozygosity	effective number of alleles
Leighills	0.196 (0.048)	1.334 (0.115)
Tannahill	0.158 (0.051)	1.293 (0.124)

Alleles unique to Tannahill population with frequencies

$IDH\text{-}2_{80}$	0.039 (0.019)	$LAP\text{-}2_{95}$	0.048 (0.019)	MPI_{84}	0.008 (0.008)
$PGI\text{-}2_{22}$	0.040 (0.017)	$PGI\text{-}2_{165}$	0.008 (0.008)	$PGI\text{-}2_{219}$	0.008 (0.008)
PGM_{103}	0.024 (0.014)				

---

all loci, being extremely homogeneous (F_{ST}=0.006). Only the locus GK differed significantly (χ^2=5.70 df1 p<.02). The difference between the Easdale and Ellanbeich populations proved to be less (χ^2=9.224 df10 p<.70, F_{ST}=0.010). Once again only a single locus, IDH-2, produced a significant level of differentiation (χ^2=4.843 df1 p<.05). A comparison of the Leighills and Tannahill populations was similarly inconclusive (χ^2=12.446 df10 p<.50, F_{ST}=0.010), although two loci differed significantly, PGI-2 (χ^2=4.020 df1 p<.05) and PK (χ^2=5.045 df1 p<.05). Mean heterozygozity was a little lower in the Tannahill population compared with what was thought to have been the 'parent' population at Leighills. A correspondingly lower level of effective number of alleles was also noted. However, the actual number of alleles was greater in the Tannahill population: seven alleles located in the Tannahill population, some at relatively high frequencies for uncommon alleles, which were not noted in the Leighills samples (table 2.3.2a). This would suggest that the 'new' population was colonised from several directions rather than one as was suspected.

table 2.3.2b

Allozyme frequencies at the EST-1 locus at Fountain de Vaucluse - standard errors in parenthesis.

allele	Fountain north	Fountain south
100	0.778 (0.098)	0.063 (0.098)
142	0.222 (0.061)	0.938 (0.061)

The difference between the north and south Fountain de Vaucluse populations corresponded with that found in the British samples (χ^2=9.225 df9 p<.50, F_{ST}=0.018). It is interesting to note, however, that the allele frequencies at the EST-1 locus in Pyronia bathseba differed markedly between the northern and southern populations at Fountain (table 2.3.2b).

The St Andrews populations were highly homogeneous - sites 1/2 (χ^2=2.832 df8 p>.90, F_{ST}=0.012), sites 1/3 (χ^2=5.917 df9 p>.70, F_{ST}=0.022) and sites 2/3 (χ^2=5.280 df9 p>.80, F_{ST}=0.015). These sites correspond with those utilised by Brakefield (1979a, b) in which he claimed to have detected a possible 'boundary region' based on spotting frequencies, similar to the well known south-west England phenomenon. Clearly, there is no evidence here of genic differentiation across this transect.

Hughes and Zalucki (1984) found much higher levels of genic differentiation in subpopulations of non-migratory Danaus plexippus in Australia (F_{ST}=0.032), although unfortunately they do not state the actual geographic distances between the subpopulations. They suggest that this is due to some mechanism other than low gene flow, possibly selection by the butterflies of particular 'patch' (microhabitat) types. However, Vawter and Brussard (1983) found little difference in the degree of genetic differentiation between geographically close and distant populations of north American Pieris rapae, although there was a significant difference (p>.5) at the MDH locus in one instance. Ehrlich and White (1980), working on Colorado Euphydryas but-

terfly populations which they believed had been separated for at least 7,000 generations, suggested that the high levels of cohesion between the populations was due to similar selection pressures or neutral genetic inertia, and not the unifying effect of gene flow.

From the present study, it is clear that there is neither significant divergence nor a high degree of homogeneity in genetic identity between populations which appear to be self-contained units and which are separated by relatively weak isolating barriers. The difference between these quasi-contiguous populations is small, too small to suggest that they have been effectively isolated for any length of time. Yet there is a degree of genic divergence. Where barriers do not exist (St Andrews), even when the butterflies appear to flying in subunits of the main population, gene flow does not seem to be restricted significantly.

It is suggested that such quasi-contiguous populations experience a low level of gene flow from nearby (adjacent) populations. Late in the flight period in 1984, many females were seen flying round the outer margin of the bracken in the direction of site 2 where they had not been observed in previous years. Such periodic dispersal would maintain a level of coherence between these population complexes. Much of the time this would be enough to maintain a close identity between them, although there must be relatively little movement between jurtina populations. It has already been mentioned that, within large populations, the species forms clearly defined sub-units. Geiger and Scholl, working on the alpine Colias phicomone in a population covering an area 2.5km by 600m and an altitudinal range of 1300 - 1850m, marked and released 378 butterflies. No less than 80% of the marked philodice were taken less than 50m from the previous point of capture. Endler (1977) puts it concisely suggesting that very few organisms live in continuously distributed populations. He points out that they are often clumped in sub areas of favourable microclimate and microhabitat, separated by subareas of low population density with even more localised breeding areas.

However, it is reasonable to suppose that there are degrees to which this is the case. Nearly 20 years agao, Ehrlich and Raven (1969) presented a powerful argument against the traditional view that gene

flow was the primary factor in holding together biological units, in species in particular (Mayr 1963). They suggested that gene flow in nature is much more restricted than commonly thought, and, even when it does occur, genic novelties may not be permitted to enter the gene pool because of selection. They also argued that selection is by far the most important factor in maintaining similarities and differences between populations, although they do admit that, in some species, gene flow will be found to be an important factor in keeping populations of the same species relatively undifferentiated. What is important in jurtina is that it would not require a great increase in the effectiveness of isolating mechanisms, whatever their nature, to permit significant genic divergence to take place. For example, the difference between jurtina populations in Orkney and their nearest neighbouring populations at Huna, John O'Groats is highly significant over all loci (χ^2=27.511 df9 $p<.01$, F_{ST}=0.042), although the founder principle might be effective here (section 2.4.2).

2.4 Geographical aspects of genetic variation

2.4.1 Geographical distance and genetic distance

If genic differentiation increases in relation to the degree of isolation, barriers to gene flow, however caused, and increased time from last contact between populations can lead to increased genetic divergence. Both the molecular clock theory and the whole concept of phylogenetic independence largely depend on this hypothesis. Before biochemical analytical techniques were developed, the concept of a correlation between genic divergence and the potential for allopatric speciation embodied the now obvious fact that geographical and other physical barriers, including distance, could and do result in population differentiation through selection and, perhaps, other evolutionary means. Few studies have been directed to the question of correlation between geographical and genetic distance. Electrophoretic techniques have been used to investigate more intimate problems of intra-population genetics, evolutionary mechanisms (the neutrality/selection argument, heterozygosity etc.) and to evolutionary relationships between species groups, although the problems are in some ways inseparable. Both Johnson (1974) and McKechnie et al. (1975) recognised the difficulties of interpreting geographic / genetic variation and associated an environmental factor (temperature) with allozyme variation. Populations along a latitude or longitude gradient are inevitably prone to the effects of climatic fluctuations.

The possible correlation of geographical distance with genetic distance in Maniolini, calculated from electrophoretic data, was examined in two ways

1. an analysis of the overall correlation, ie each population compared with its genetic and geographical distance from every other population in both jurtina and telmessia, and

2. an analysis of three transects of jurtina populations a) Alicante, Spain to Belveaux, Belgium (eight populations), b) Atlas Mountains to Belveaux (nine populations) and c) Stroud to Huna (11 populations),

taking the geographical and genetic distances from one extremity of the transect to each population in the transect. These were selected because they represented relatively straight line transects, while a and b included the possible dispersal and genetic isolating barriers of the Pyrenees and Atlas Mountains.

Genetic distances were calculated as the complement of the similarity coefficient g (1-g). Geographical distances were measured as straight line vectors from one population to the other and, in the case of transects, from the 'source' population to other populations. Regression analysis was performed for each data set and the results are summarised in figures 2.4.1a - g.

The overall correlation between geographical and genetic distance in Maniola telmessia samples (figure 2.4.1a) was significant ($r=0.295$ $df54$ $p<.05$). When the distinct Karpathos population was excluded from the data it was slightly more so ($r=0.512$ $df20$ $p<.02$). It is remarkable that the geographical and genetic distances from such diverse islands which, possibly, were colonised from different parts of mainland Turkey (section 4.2) should show such a high correlation. Unfortunately, data were not available for western Turkish telmessia: the relationship between the data from these islands and the adjacent mainland populations would have been, perhaps, more instructive. In the British populations of Maniola jurtina (figure 2.4.1b), there was a significant correlation between genetic distance and geographic distance ($r=0.220$ $df495$ $p<.001$)[1]. A similarly significant correlation was evident in continental European populations ($r=0.236$ $df594$ $p<.001$)(figure 2.4.1c)[1]. Subjecting the genetic distances in both data sets to logarithmic transformation ($\log_{10}$) induced no improvement in the European correlation figure, but a slight improvement in that for Britain ($r=0.284$)and in the telmessia data ($r=0.370$).

The south to north transect in Britain (figure 2.4.1d) exhibited a high correlation between geographic and genetic distances ($r=0.861$ $df10$ $p<.001$), but that from Spain to Belveaux (figure 2.4.1e) did not ($r=0.389$ $df6$). Nor did the geographic and genetic distances between the Atlas Mountains population and Belveaux (figure 2.4.1f) which, conversely, gave a negative correlation coefficient ($r=-0.289$ $df7$). Past events and the evolutionary history of the species, including

[1] Although significant, an r value of 0.20-0.25 represents only 4-6% of the variance accounted for in the linear trend.

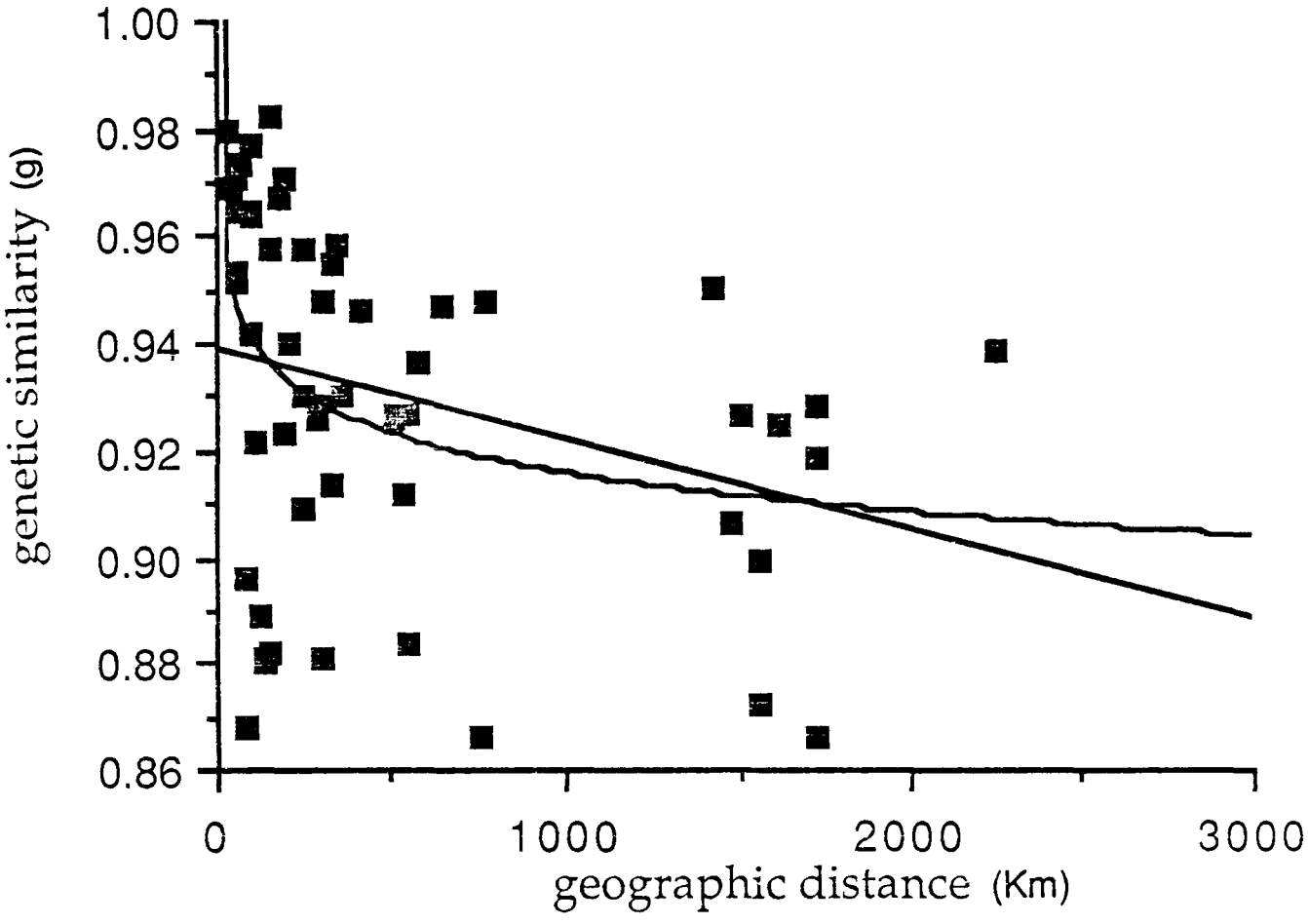


figure 2.4.1a

The relationship between geographic distance and genetic similarity in Maniola telmessia populations. The fitted regression lines are $y=0.939 - 1.666 \times 10^{-5}x$ and $y=0.992x^{-0.012}$.

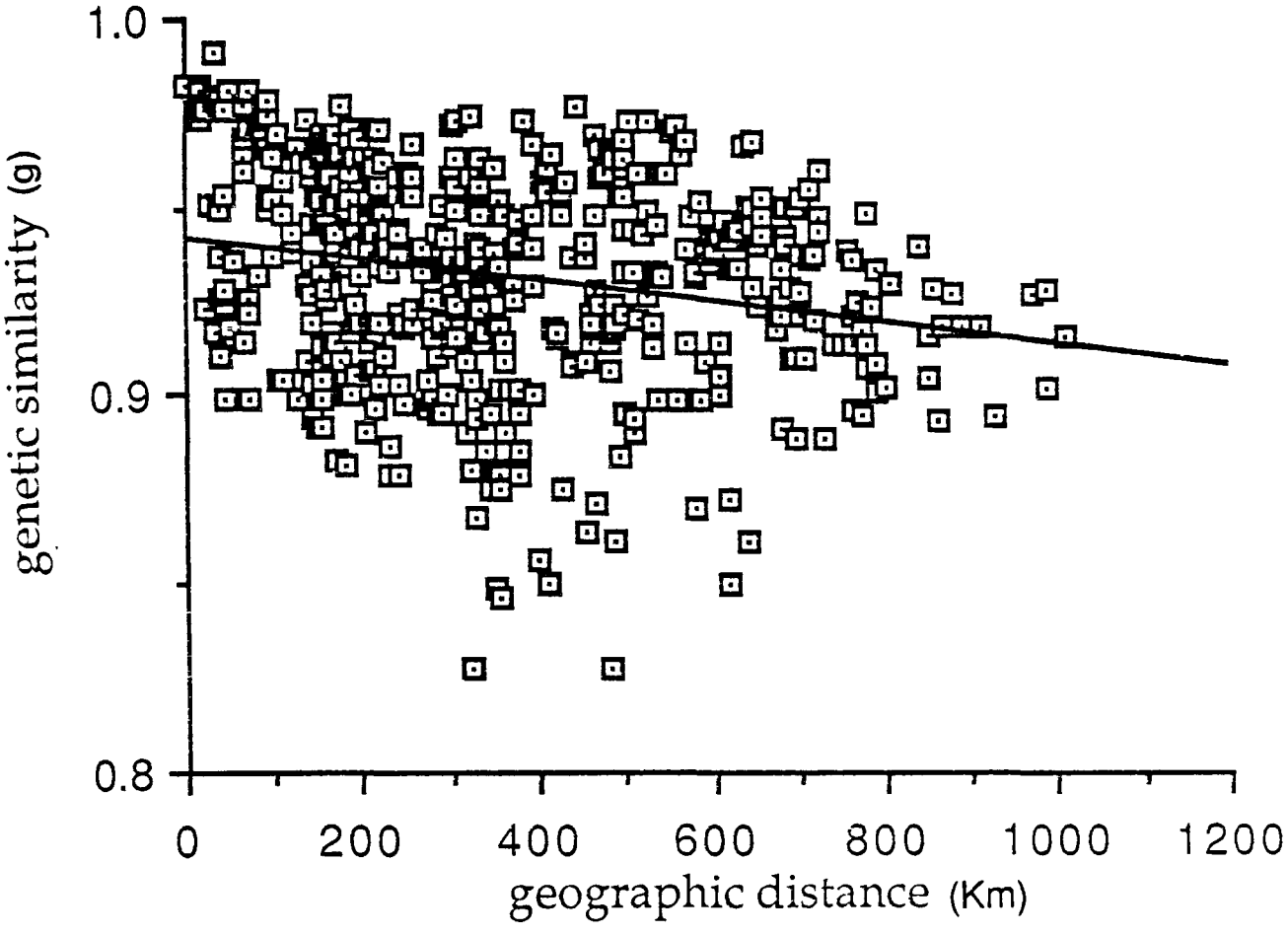


figure 2.4.1b

The relationship between geographic distance and genetic similarity in Maniola jurtina populations in Britain. The fitted regression line is $y=0.942 - 2.702 \times 10^{-5}x$.

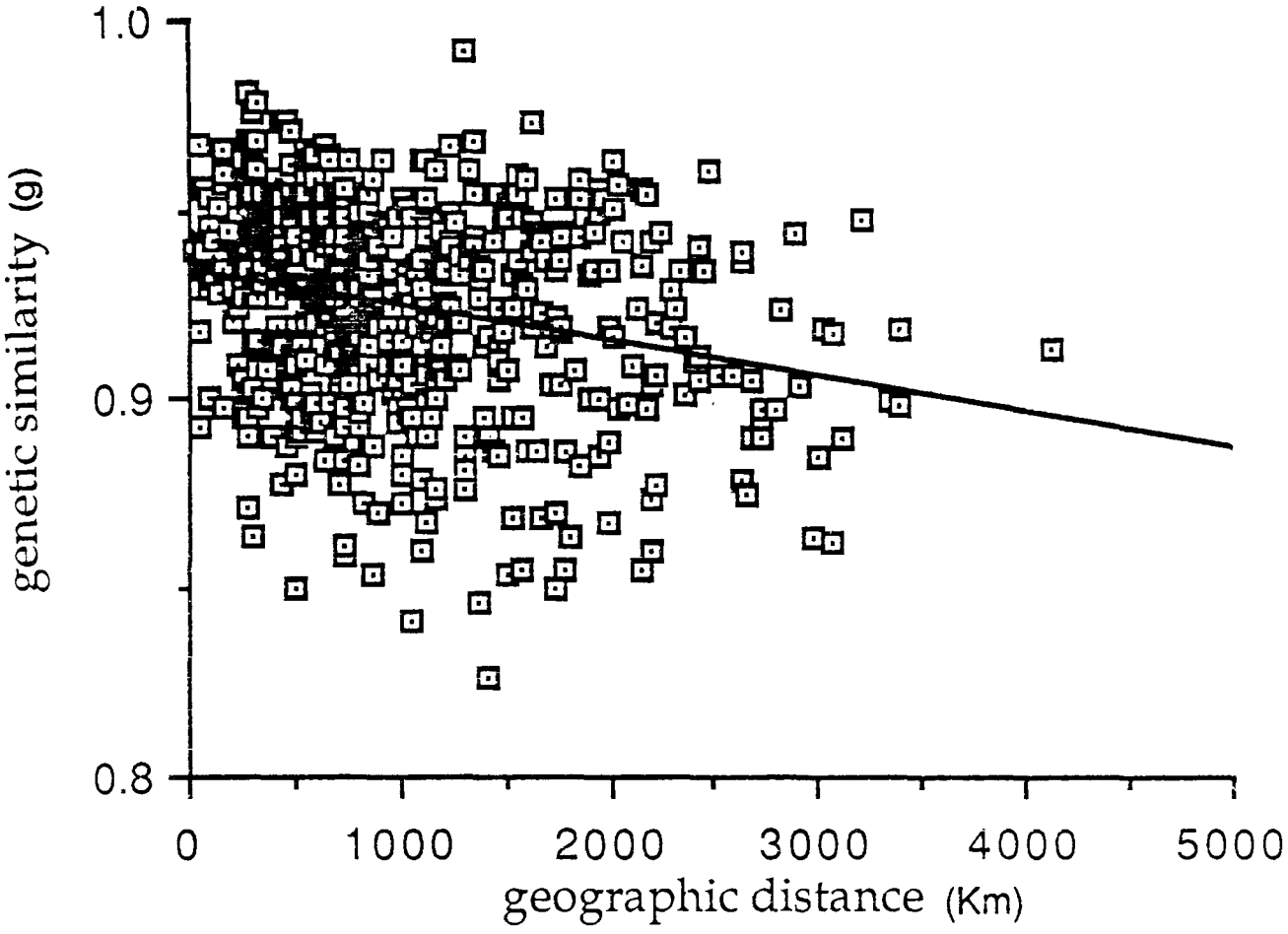


figure 2.4.1c

The relationship between geographic distance and genetic similarity in Maniola jurtina populations in Europe. The fitted regression line is $y=0.934 - 9.140 \times 10^{-6}x$.

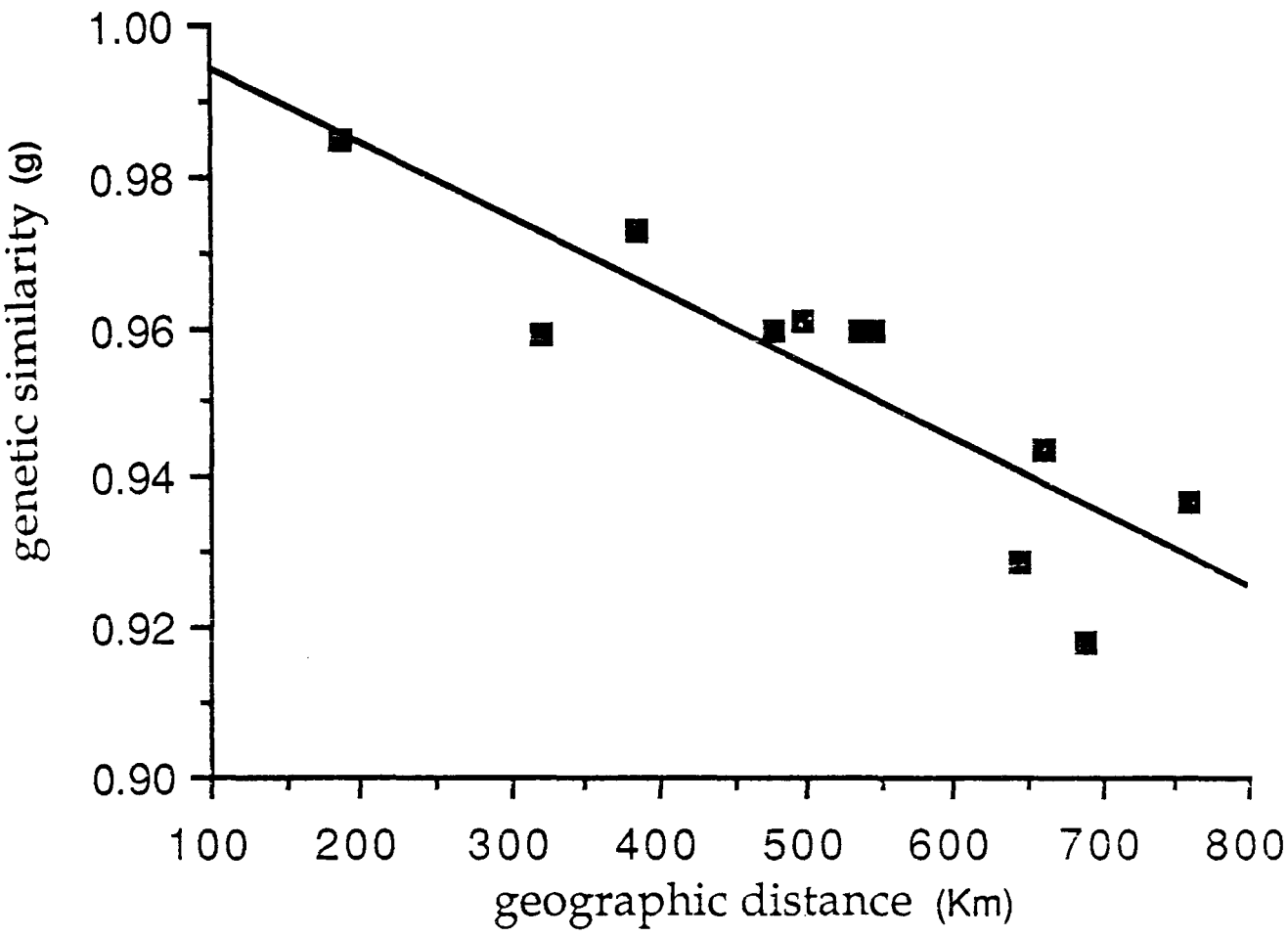
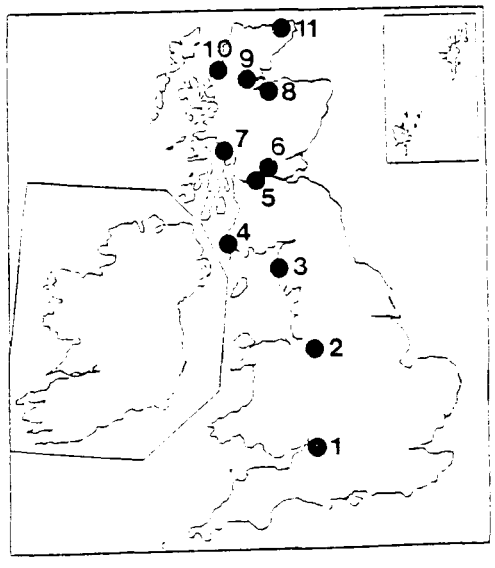


figure 2.4.1d

The relationship between geographic distance and genetic similarity in British populations of Maniola jurtina across a transect from Stroud to Huna. The fitted regression line is $y=1.004 - 9.831 \times 10^{-5}x$.

Transect populations

1 Stroud
2 Wilmslow
3 St Bees
4 Portpatrick
5 Kilsyth
6 Dunblane
7 Oban + Benderloch
8 Wester Hardmuir Forest
9 Strathconon
10 North Erradale
11 Huna



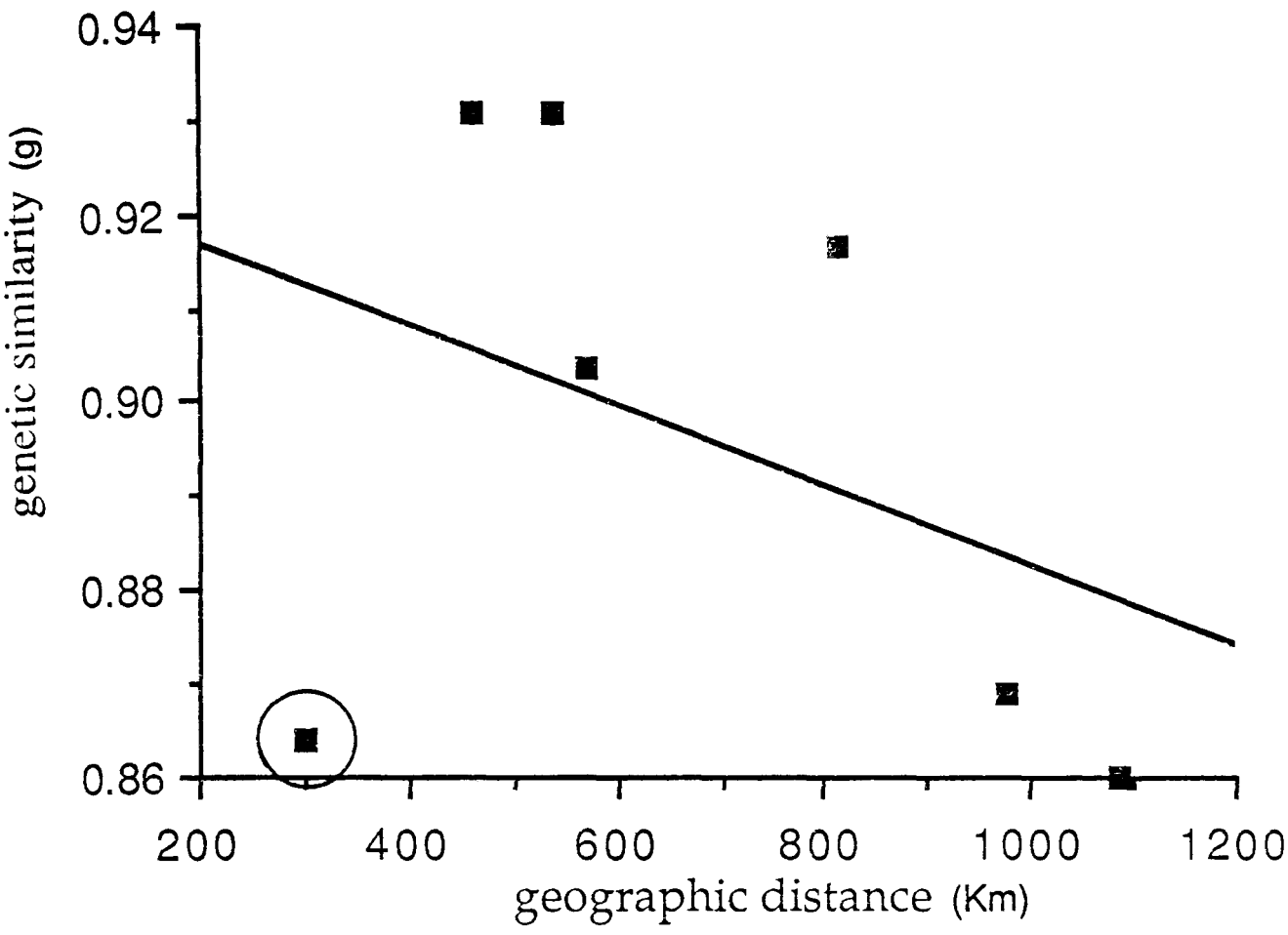


figure 2.4.1e

The relationship between geographic distance and genetic similarity in European populations of Maniola jurtina across a transect from Denia, Alicante to Belveaux. For transect populations see figure 2.4.1f. The isolated point represents the Alicante population. The fitted regression line is $y=0.926 - 4.290 \times 10^{-5}x$.

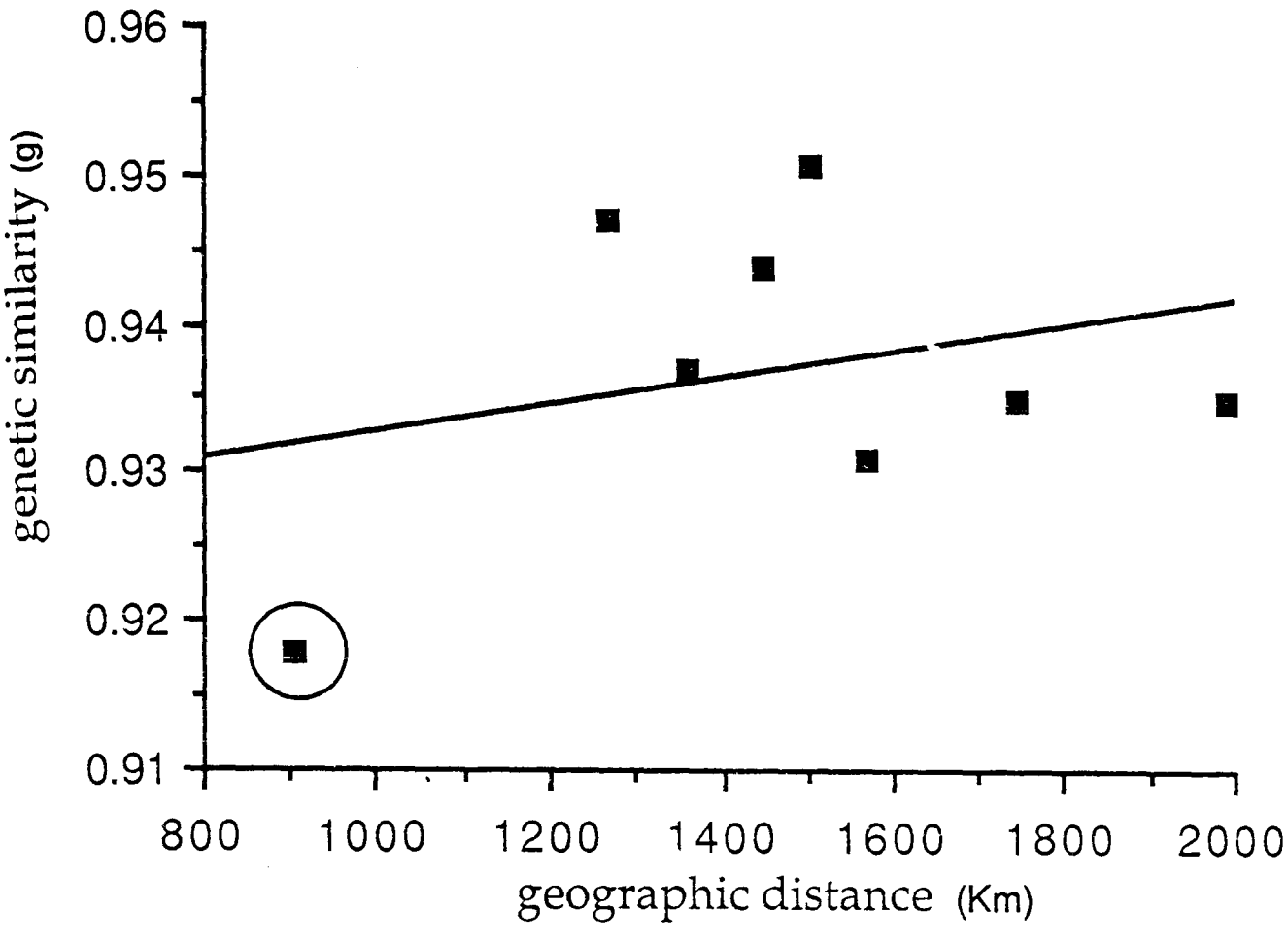
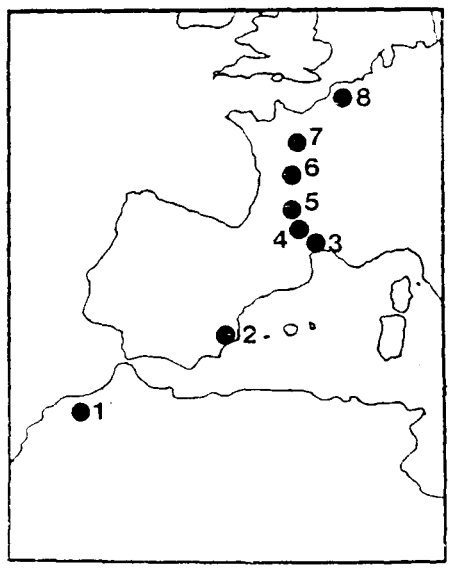


figure 2.4.1f

The relationship between geographic distance and genetic similarity in north African and European populations of Maniola jurtina across a transect from the Atlas Mountains to Belveaux. The isolated point represents the Atlas Mountains population. The fitted regression line is $y=0.924 + 9.270 \times 10^{-6}x$

Transect populations

1 Atlas Mountains
2 Denia, Alicante
3 Sorede
4 Nante
5 Cahors
6 Celon
7 Guernanville
8 Belveaux



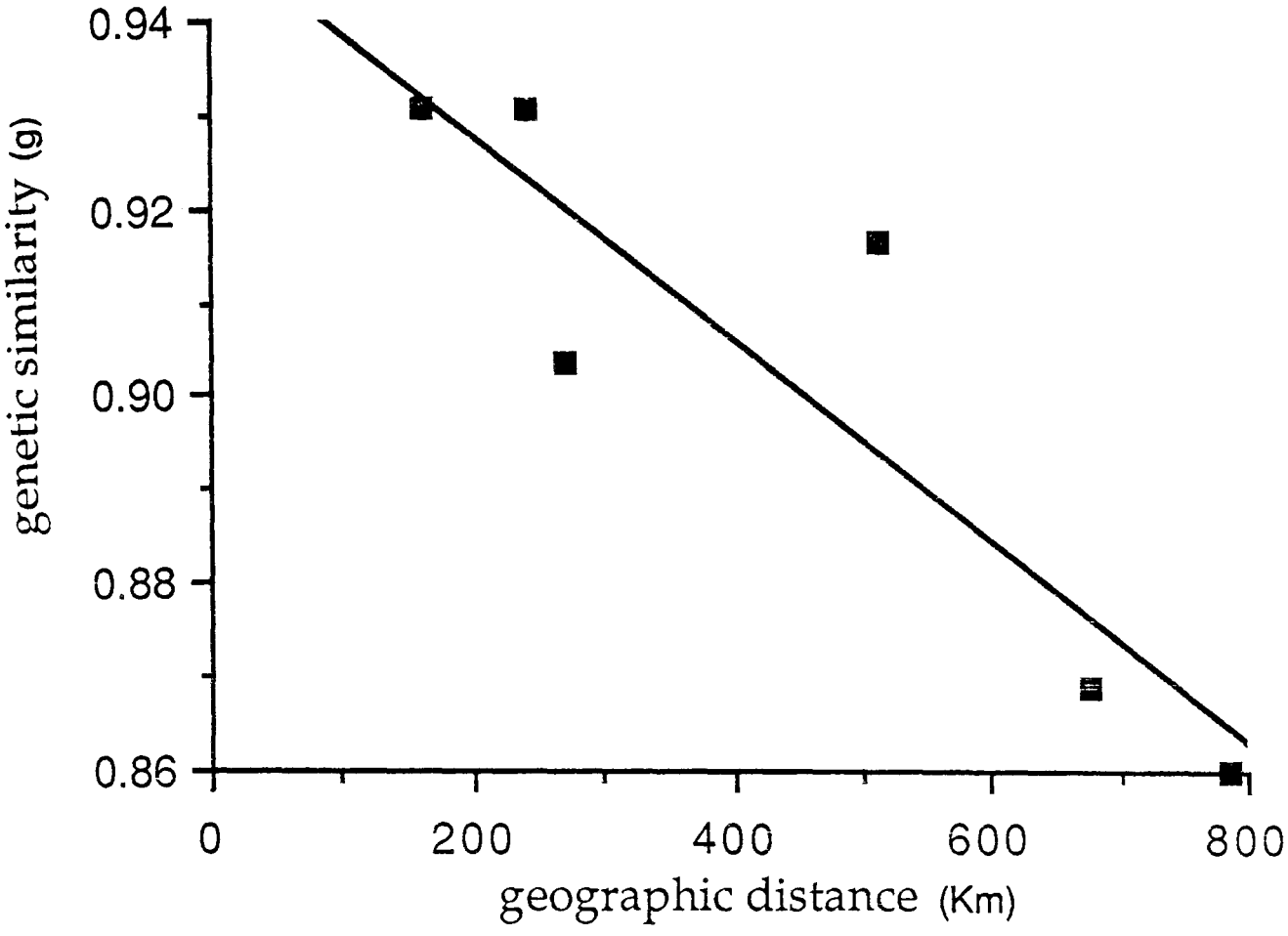


figure 2.4.1g

The relationship between geographic distance and genetic similarity in European populations of Maniola jurtina across a transect from Sorede to Belveaux. For transect populations see figure 2.4.1f. The fitted regression line is $y=0.950 - 1.086 \times 10^{-4}x$.

the location of the glacial relict populations from which Europe was colonised in post-glacial times, must have a bearing on these data. These implications will be discussed in section 4.2.

The corresponding data from the British transect would appear to indicate that geographical distance, from a 'terminal' population in a transect which lies from south to north Britain, makes an extremely important contribution to the differentiation of populations of the species. In Europe, the situation is more complicated. The effective difference between the British and European transects is that, in the former, there are no major physical barriers to dispersal, whereas the Spain to Belveaux transect encompasses the Pyrenees which now and, probably more importantly, during immediate post-glacial times, must have formed and still form major barriers to gene flow. Taking a transect through populations north of the Pyrenees only (ie omitting Alicante from the data set)(figure 2.4.1g) converted the correlation figure to a highly significant value ($r=0.899$ $df5$ $p<.001$). Thus, geographical factors, other than distance, are and have been important in the genetic differentiation processes between Iberian and north African populations and those north of the Pyrenean chain.

2.4.2 Genetic variation on islands

The classical theory of island biogeography (see Carlquist 1965 for a synthesis), the theory of 'dynamic equilibrium' between immigration and extinction in island faunas (MacArthur and Wilson 1963, 1967) and related theories, for example, the importance of 'catching angle' (Lindroth 1960), notwithstanding more recent modifications of the hypothesis, hinge more or less on a correlation between faunal diversity and island size, first suggested by Munroe (1948). Hockin (1980) claims to have found that 75% of butterfly faunal diversity can be accounted for by area alone and, if minimum distance is included, this figure rises to 93%. These conclusions are rather remarkable, as they are based on that author's knowledge of species number in the Mediterranean islands: it is not generally known that this number is a gross underestimate of the actual number, especially on some of the smaller islands (Olivier, pers. comm.). Reed (1982) made a similar massive underestimate of butterfly species numbers in his analysis of

faunal diversity on islands in Britain (Thomson 1980). Udvardy (1969) states

> '... The correlation between size and faunal richness becomes a statistically measurable phenomenon only if the statistical sample is large enough and if it follows those regressional equations botanists, zoologists and biostatisticians have established regarding both mainland and island biota.'

To what extent faunal depauperation is related to reduced variability within a species is not clear. If it does, it is then also dependent upon the variables involved in the island biogeography hypothesis vis a vis species diversity, together with immeasurable others which are associated with the ecological genetics of the species. Gorman et al. (1975), in a study of insular Adriatic lizards of the genus Lacerta, found that the amounts of genetic variation were

1. greater in mainland populations than in putatively relict island populations and
2. [greater] on large islands than on small islands.

However, Gill (1976), working on Californian Channel Island populations of deer mice (Peromyscus maniculatus), found that an island habitat does not necessarily lead to low levels of heterozygosity.

The hypothesis that variability in jurtina and telmessia would be greater on larger than on smaller islands was tested by comparing heterozygosity and effective number of alleles in these species with island area. Maniola jurtina was collected on ten British islands which ranged from the tiny Easdale Island (area 0.20 km^2) to the large Isle of Man with an area of 572 km^2). M. telmessia was sampled on the eastern Aegean islands ranging from Simi (90 km^2) to Rhodes (1404 km^2).

Regression analysis performed on the British island populations revealed conclusively that there was no correlation between island area

table 2.4.2a

Heterozygosity and effective number of alleles (standard error in parenthesis) in insular populations of Maniola jurtina and M. telmessia, based on 10 loci - CK, GK, GOT-1, IDH-1, IDH-2, LAP-2, MPI, PGI-2, PGM and PK.

Maniola jurtina

island	area km^2 (approx)	heterozygosity	effective number of alleles
Mainld. Orkney	490*	0.166 (0.059)	1.448 (0.107)
Seil	115	0.247 (0.072)	1.661 (0.162)
Easdale	0.2	0.209 (0.068)	1.582 (0.132)
Isle of Man	572*	0.200 (0.054)	1.571 (0.126)
St Marys	20	0.010 (0.046)	1.346 (0.084)
Cramond	0.3	0.199 (0.076)	1.603 (0.164)
Tiree	77*	0.281 (0.076)	1.804 (0.227)
Barra	90*	0.263 (0.073)	1.878 (0.347)
Alderney	72*	0.221 (0.072)	1.604 (0.160)
Guernsey	63*	0.244 (0.079)	1.660 (0.173)
jurtina overall		0.228 (0.076)	1.627 (0.163)

Maniola telmessia

island	area km^2 (approx)	heterozygosity	effective number of alleles
Lesbos	1600	0.200 (0.068)	1.603 (0.156)
Samos	650	0.176 (0.066)	1.558 (0.139)
Kos	1100	0.221 (0.082)	1.697 (0.227)
Simi	90	0.166 (0.062)	1.492 (0.132)
Rhodes	1404**	0.168 (0.073)	1.528 (0.148)
Karpathos	288**	0.262 (0.125)	1.682 (0.196)
telmessia overall		0.199 (0.079)	1.594 (0.167)

* data from Reed (1982)
** data from Hockin (1980)

note

The data configuration illustrated in these regression plots, with the large islands creating outlying points, suggests that these analyses are invalid. Further analysis, excluding the two large area values, gives $r=0.601$ df7 $p<0.1$ for area/heterozygozity and $r=0.589$ df7 $p<0.1$ for area/effective number of alleles, both just reaching significance levels. This is barely sufficient to suggest a relationships between island area and either heterozygozity or effective number of alleles.

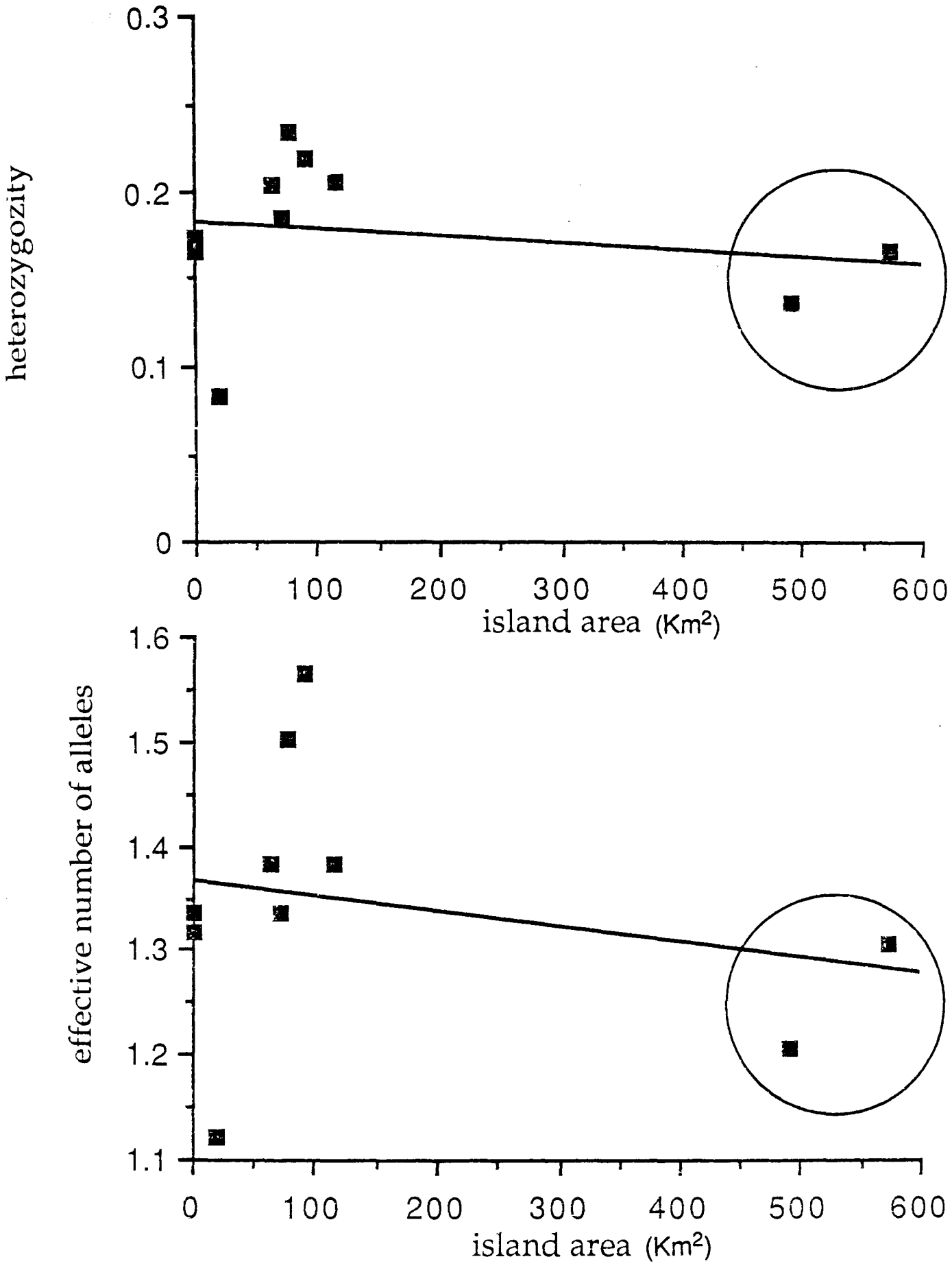
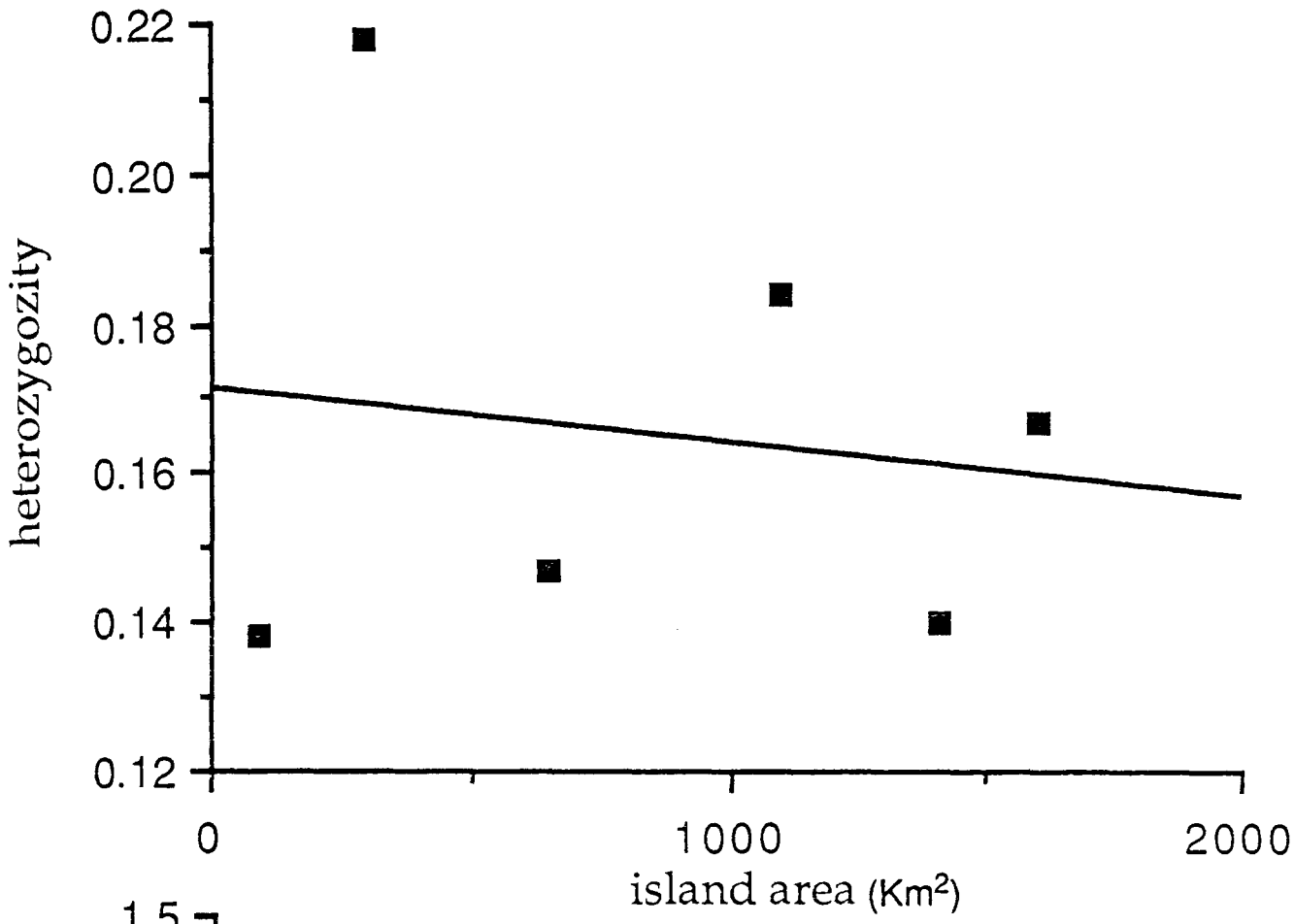


figure 2.4.2a

The relationship between heterozygozity, effective number of alleles and island area in British populations of Maniola jurtina. The fitted regression lines are heterozygozity: $y=0.183 - 3.604 \times 10^{-5}x$ and effective number of alleles: $y=1.369 - 1.490 \times 10^{-4}x$.



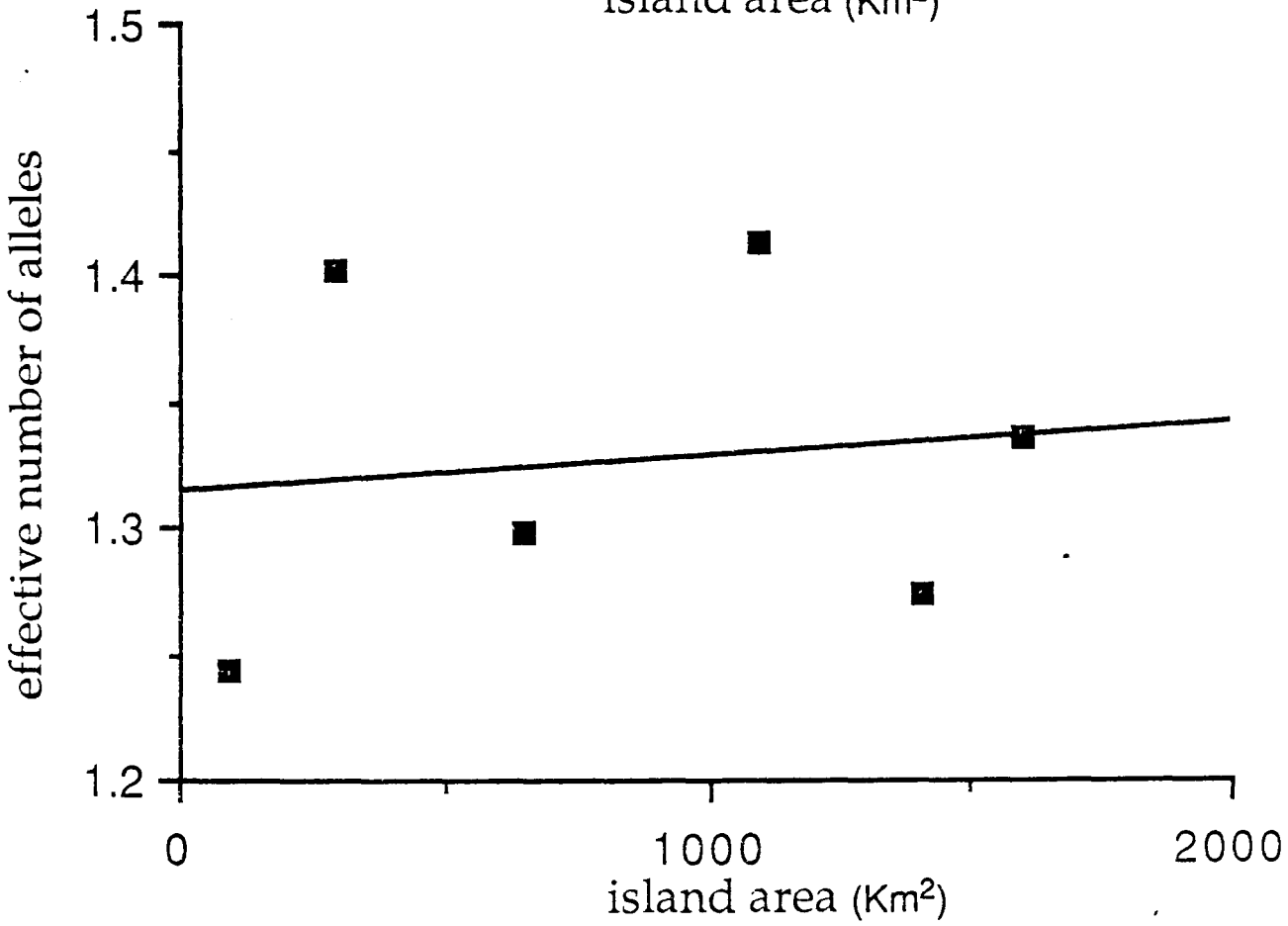


figure 2.4.2b

The relationship between heterozygozity, effective number of alleles and island area in populations of Maniola telmessia. The fitted regression lines are - heterozygozity: $y=0.172 - 7.124 \times 10^{-6}x$ and effective number of alleles: $y=1.316 + 1.397 \times 10^{-5}x$.

and either heterozygosity (r=-0.169 df9) or effective number of alleles (r=-0.239 df9). Nor was there any indication of reduced variation between mainland populations and the islands. Heterozygosity in the islands was spread about the mean for Britain as a whole (table 2.4.2a). There was, no evidence of correlation between areas of the Greek islands and either heterozygosity (r=-0.140) or effective number of alleles (r=0.120) in the populations (figures 2.4.2a and b).

The degree of genetic variation being acquired and maintained by island populations of Maniola could relate to selection in some instances, but it perhaps owes more to fluctuations in population size and colonisation, especially bottlenecking and founder effect. This has implications for any reconstruction of the recent evolutionary biology of the species (section 4.2).

2.4.3 Geographical variation in allele frequencies

As the standard error of the frequencies of most alleles in electrophoretic studies is so large, it is rarely useful to seek trends in the variation of any other than the common allele at each locus. Even so, many papers use data having very high standard errors for these low frequency alleles. In jurtina and telmessia, the alleles GK_{100}, $GOT\text{-}1_{100}$, $IDH\text{-}1_{100}$, $IDH\text{-}2_{100}$, MPI_{100}, $PGI\text{-}2_{100}$, PGM_{100} and PK_{100} are the only ones whose frequencies can be considered useful in this context. At the LAP-2 locus, the common allele in jurtina was $LAP\text{-}2_{100}$ and $LAP\text{-}2_{90}$ in telmessia. At the complex GOT-1 locus, males, females, mean of sexes and non-deviant populations were considered independently.

There was no apparent pattern of geographical variation of the allele frequencies in either jurtina or telmessia at GOT-1. Nor was there any pattern of variation at PK.

Other loci displayed patterns of variation which were rather more interesting, although none were unambiguous. Variation in the frequencies of the common alleles at these loci is fairly considerable and only general observations can be made.

At the GK locus in jurtina, the frequency of GK_{100} was generally higher in Britain than elsewhere, but there was a significant number of exceptions to this. In one Irish and in the Tiree, Barra and Cramond populations, the frequency dropped to less than 0.8, while in continental Europe and north Africa a frequency greater than 0.9 was not uncommon. The frequency of $IDH\text{-}1_{100}$ was generally lower in southern Britain than elsewhere, and was consistently much lower in Ireland. Without exception, $IDH\text{-}2_{100}$ dropped to frequencies below 0.9 in central and northern Scotland.

Within the mosaic of values for the $LAP\text{-}2_{100}$ frequency in jurtina, there were some detectable trends. The more eastern populations were inclined to have lower frequencies than those in the west. Three of the four Irish populations also had low frequencies for this allele. The reverse was the case with MPI_{100}, which had higher frequencies in the east. The frequency of $PGI\text{-}2_{100}$ was lower in Ireland than in many mainland British populations.

Bullini et al. (1975) found the frequency of PGM_{100} to be between 0.52 and 0.67 in Italy and 0.84 in Switzerland. Masetti and Scali (1975a, b, 1977, 1978) calculated a frequency of 0.55 for the same allele in the 'eastern' and greater than 0.70 in the 'western' groups of jurtina populations, based on genitalia form (Thomson 1973b, 1975). The present study utilised a far greater number of jurtina populations and the situation proved to be far more complex than was suggested by these authors. The trend certainly was for higher frequencies in the western part of Europe, although there was considerable variation in the frequency of allele PGM_{100}. One of the five Italian populations, for example, had the allele at a frequency of 0.696, while very low frequencies were found in one French populations (0.523), Alderney (0.579), Cairndow (0.500), Huna (0.417) and Orkney (0.538). Frequencies for the allele in Ireland were very high (0.966, 0.932, 0.905, 0.814).

If any overall patterns of geographical variation in the frequencies of the common alleles at these loci in jurtina are to be found, they are

1. some loci suggest an apparent dichotomous distribution in eastern and western continental Europe (LAP-2, MPI, PGM)

2. British populations have a tendency at some loci to exhibit frequencies which are different from continental European populations (GK, IDH-1, IDH-2)

3. Irish populations have a tendency at some loci to exhibit frequencies different from mainland British populations (GK, IDH-1, LAP-2, PGI-2, PGM).

In telmessia, PGM_{100} shows clinal variation through the six islands studied, decreasing in frequency from Rhodes northwards. Karpathos to the south, however, has a frequency for this allele which is lower than elsewhere. The pattern of variation in GK_{100} was interesting, radiating from a high frequency in Kos (0.909), northwards and southwards. However, evidence for significant variation trends in telmessia, clinal or otherwise, is tenuous.

2.5 Genetic similarities / distances between populations of Maniola jurtina and M. telmessia.

It has become almost standard practice to use Nei's measures of genetic identity I and its transform genetic distance, D, in electrophoretic evolutionary and systematic studies of populations, subspecies and species. Biochemical systematic studies of butterflies in the last ten years or so have used this statistic exclusively (Angevine and Bradshaw 1974, McKechnie et al. 1975, Brussard and Vawter 1975, Brittnacher et al. 1978, Geiger 1978, 1980 (1981), 1982, Courtney 1980, 1982, Tebaldi 1982, Geiger and Rezbanyai 1982, Geiger and Shapiro 1986, Shapiro and Geiger 1986). It became clear, in the course of the present study, that the inter-population genetic similarities of jurtina and telmessia were so close that Nei's coefficient would mask real differences between populations. The disadvantages of using I or D in these situations was recognised by Nei who suggested using Sangvi's G (Sangvi 1953) as the measure of genic differentiation between closely related populations. The in-depth study by Workman and Niswander (1970) on the Papago Indians is a model of the application of G (and Wright's F statistics) in these circumstances. In a modified form (g = 1-G/2), Nei's recommendation has been followed here.

The proportion of monomorphic loci ranges from 25% to 75% in invertebrate populations (Ayala and Powell 1972, Selander and Kaufman 1973). These monomorphic loci are almost invariably fixed for the same allele in all conspecific populations. The importance of this is that virtually all of the contribution to genetic divergence between conspecific populations comes from differences in allele frequencies at polymorphic loci. It became apparent in the present study that there was a useful criterion which could be applied in deciding which was the more appropriate statistic to use. If, in a group of populations (species in the case of Maniolini), one or more locus is more or less fixed for an alternative allele, different from that in the populations (species) with which it is being compared, it is more appropriate to use Nei's genetic similarity or distance coefficient. If slight differences at a number of loci account exclusively for the differences between two populations groups or species, it is better

to use Sanghvi's G (or g). As the relative amount of genetic differentiation between the groups being compared is important, not the actual value of the statistic, it follows that the maximum number of loci possible should be used when I or D is being employed. However, highly polymorphic loci contribute most, if not all of the differences between close groups. It follows, then, that, when no locus is fixed for an alternative allele, it is of no value to utilise more than the polymorphic loci. There must be, of course, some certainty that the other loci are monomorphic for the same allele or almost so. Only a few individuals are required for a relatively safe estimate of I or D: it probably is wasteful, in many studies, to use large numbers. It is, conversely, very important that large samples are used in the analysis of close population groups or species[1]. The logic behind this has already been suggested. In the case of I and D, the number of loci at monomorphic (or nearly monomorphic) loci which are fixed for different mobilities contribute most to the similarity or distance value. Differentiation of g increases as more subtle differences in allele frequencies increase. Thus any inherent error in g decreases with the increasing certainty of the allele frequencies. Unfortunately, no statistic is available for the calculation of standard errors from Sanghvi's G. Calculation of χ^2 simultaneously with g has been practiced routinely in this study, but this gives a measure of the significance level of the difference between groups, not a measure of the error inherent in the value of g. Most major population groups, even in close populations, will show a significant or highly significant level of genic differentiation.

Ten polymorphic loci were utilised extensively in this survey in the analysis of jurtina and telmessia populations and for a study of the telmessia group of species. - CK, GK, GOT-1, IDH-1, IDH-2, LAP-2, MPI, PGI-2, PGM and PK. Because of the unusual nature of the GOT-1 locus, and the apparently random distribution of sex-associated heterozygote deficient populations, it was not used either in continental European population analysis or that of telmessia. This was, in some ways, unfortunate as the locus must be one of the most dynamic of those assayed. Until the evolutionary implications of the allozyme variation are better understood, data from GOT-1 exhibiting the deviant frequencies, cannot contribute to the similarity or distance

[1] For discussions on the effects of small sample sizes on genetic distance and heterozygozity estimates see Nei (1978), Gorman and Renzi (1979) and Thorpe (1982).

figures. The locus was used in the study of British populations, but allele frequencies were calculated as means of those from males and females. This was to circumvent the possibility that there was sex linkage or some other sex-associated allozyme frequency heterogeneity at this locus in 'normal' populations which was not detected (section 2.1.3). This procedure was followed throughout Maniola in the analysis of relationships of species within the genus.

The value of using an appropriate measure of genetic similarity or distance is matched by the importance of utilising analytical techniques which will reflect information in the original data as accurately as possible, while clarifying relationships within and between groups. Just as Nei's I has been used in biosystematic work, average linkage clustering (UPGMA) has almost invariably been chosen for further analyses. In this study, UPGMA, single and complete linkage clustering, as well as nonmetric multidimensional scaling (NMMS) have been applied to the original data matrices (section 1.3.6). The application of NMMS and other multivariate ordination techniques to the analysis of butterfly populations was pioneered by Dennis et al. (1984, 1986) in his study of eyespot variation in Coenonympha tullia. Its use in this type of work is very new.

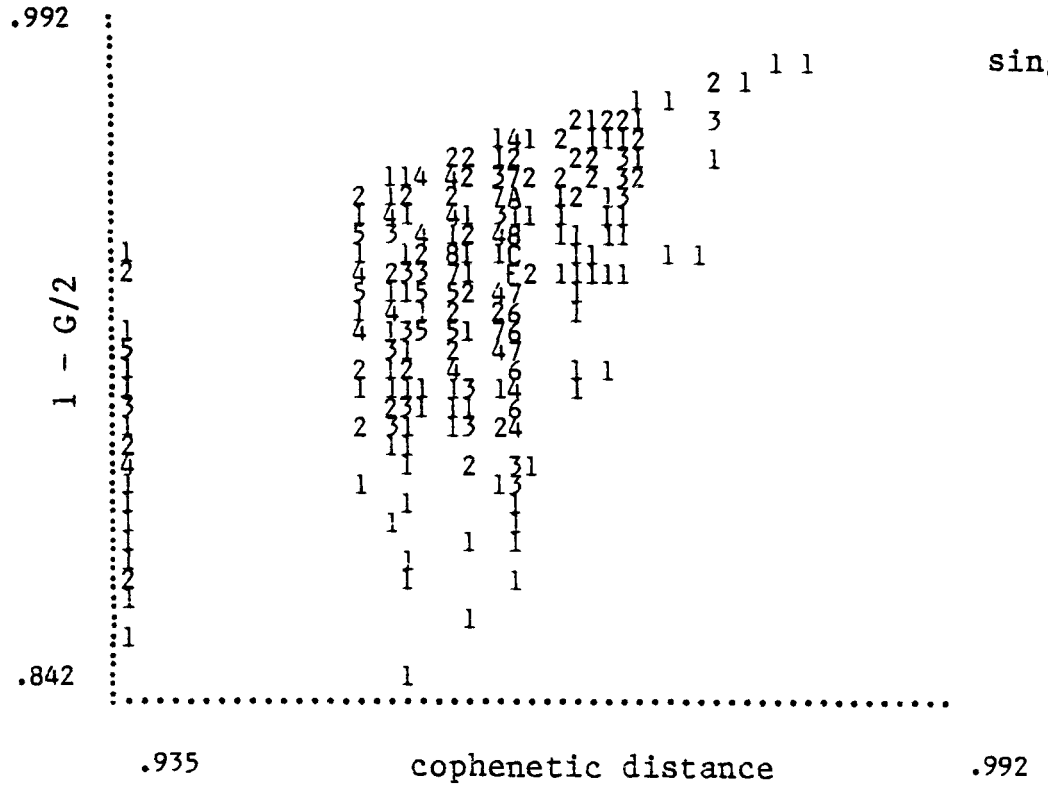
Every analytical technique which attempts to represent in two or three dimensional space the relationships between populations and population groups from data which are inherently multi-dimensional, is, by its very nature only an approximation to the truth. How well a dendrogram or ordination matches the original data can be tested in various ways. Sokal and Rohlf (1962) developed the method of cophenetic correlations. This method assigns a cophenetic value to the similarity between any pair of populations implied by a given dendrogram or plot and generates a matrix of these values for any set of populations. The cophenetic value C_{jk} between any two populations j and k is the maximum similarity (or minimum dissimilarity) between the two populations implied by the dendrogram or, in the case of an ordination, the actual distances (in a specified metric) in the ordination. A product-moment correlation coefficient (r_p) can then be computed between the elements of the original data matrix and the cophenetic values. This, in turn, can be converted to a stress coefficient by using a modification of Gower's formula (Gower 1966), viz

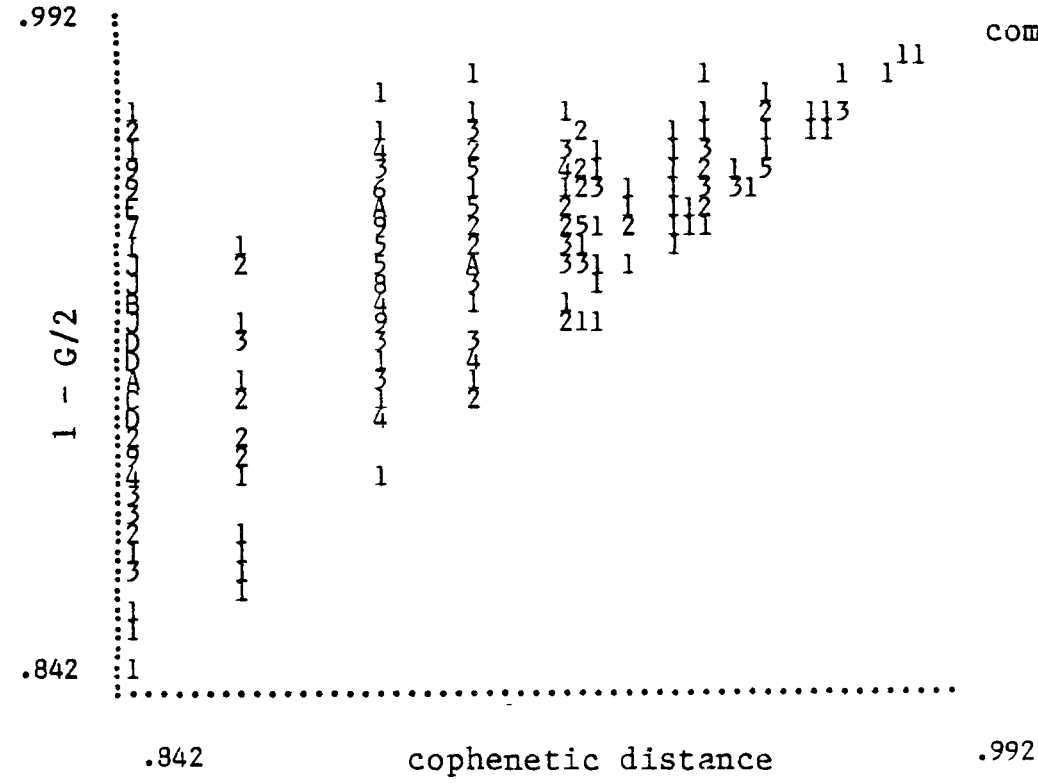
$$\text{stress} = 1-C_{jk}^{2}$$

Similarly, the stress (Kruskal 1964a, 1964b) and r^2 values computed by NMMS are measures of the amount of information (as a fraction, in the case of r^2) in the original data which is represented in the final ordination for a particular dimensionality. Thus, the lower the dimensionality, the greater the stress. NMMS has the potential drawback that it utilises rank orders and these might not represent accurately the precise differences between each similarity (or distance) coefficient.

Similarity values plotted against cophenetic values for each plot gives a graphic representation of the degree to which the two data sets match. This was done for each of the similarity matrices and their corresponding cophenetic values for single, complete and average linkage clustering, as well as NMMS. Those for European populations of Maniola jurtina are shown (figures 2.5a - d). It can been seen from these plots that in linkage clustering the more similar the groups (ie the closer g approaches unity) the better the data are represented in the dendrogram. More distant groups are represented correspondingly less well. The scattergram in which original data is plotted against cophenetic values from NMMS shows a quite different pattern, with a more even spread about the mean line. The implications of this were recognised by Rohlf (1968): linkage clustering techniques represent close population and groups very well but main groups much less well while ordinations represent the main clusters better.

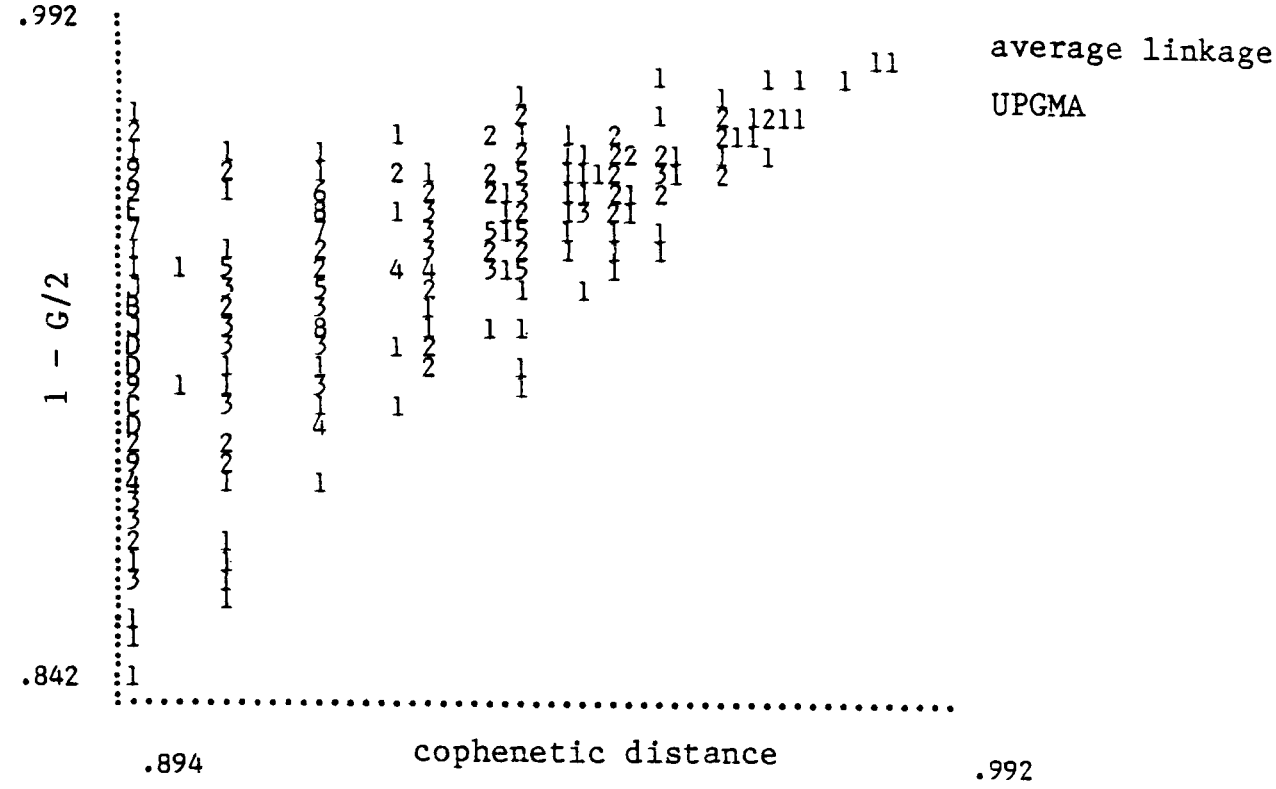
There is a danger of accepting clustering and ordination techniques as definitive and to interpret them as phylogenies, which they are not (Fitch and Margoliash 1967, 1968, Farris 1972). Arguments have raged about whether those using clustering methods require faith in the molecular clock hypothesis and its exact constancy. It has been suggested by the work of Moore (1971) and Colless (1971) that exact constancy is not essential, only sufficient regularity to enable phenetic methods to work. How much constancy is needed is not yet known (Felsenstein 1970). The comments of Felsenstein are relevant.

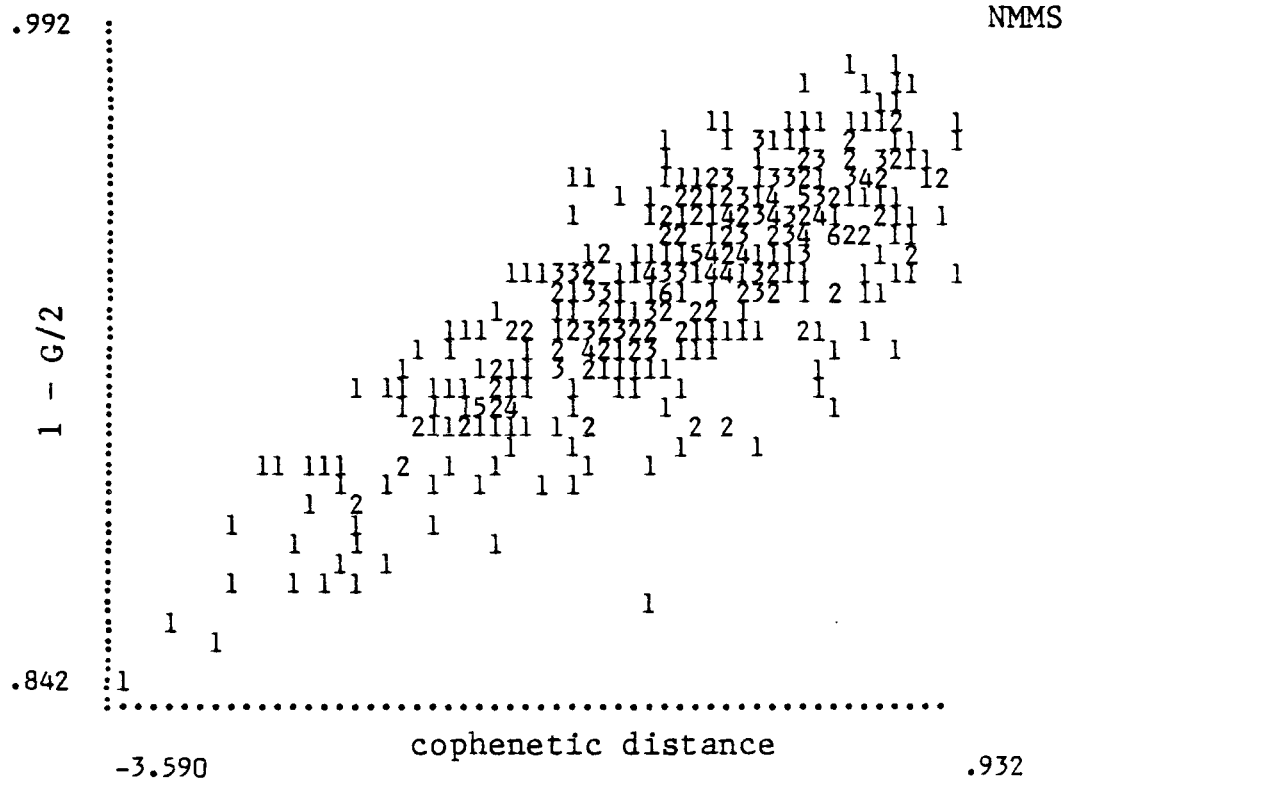




figures 2.5a (above) and 2.5b (below)

Cophenetic distances plotted against genetic similarity (1-G/2).





figures 2.5c (above) and 2.5d (below)

Cophenetic distances plotted against genetic similarity (1-G/2).

table 2.5a

Maximum, minimum and mean genetic similarities ($\bar{I}$) between populations of Maniola jurtina and M. telmessia and subspecies of jurtina. I and g coefficients have been converted using both 99% and 95% polymorphism criteria (section 1.3.6) - 99% above, 95% below.

	maximum		minimum		mean		SE of
	g	I	g	I	g	I	mean I
jurtina	0.926	0.929	0.997	0.9995	0.970	0.984	0.001
populations*	0.942	0.944	0.999	0.9996	0.990	0.988	0.001
telmessia	0.920	0.944	0.992	0.998	0.969	0.983	0.005
populations	0.937	0.956	0.994	0.999	0.976	0.987	0.005
jurtina	0.902	0.902	0.991	0.997	0.963	0.975	0.006
subspecies	0.923	0.923	0.993	0.998	0.970	0.980	0.006

* Morocco, Spain and Sardinia not included in calculations

> '... In focusing our attention on the biological assumption underlying each method, a statistical approach will promote a realisation that there can be no such thing as an all-purpose method for inferring phylogenies.'

It must be remembered that the errors in the original data can be large. It must also be emphasised that this sort of analysis is an approximation. In the present study the linkage clustering methods and NMMS have been used to complement each other and the fit of the original data to the cophenetic values have been calculated in an attempt to reach objective conclusions and to obviate some of the problems encountered by other workers.

Multi-loci studies have led to the conclusion that there is a high degree of similarity between conspecific populations throughout the range of a species (Avise and Selander 1972, Johnson 1974, Avise 1974) suggesting that at least 85% to 90% of the genetic information is contained within a single populations. The levels of mean genic similarity between populations of Maniola jurtina ($\bar{I}$=0.984-0.988) and telmessia ($\bar{I}$=0.983-0.987) are obviously very similar and close to those reported elsewhere for butterflies (table 2.5a). Brittnacher et al. (1978) found a mean level of similarity ($\bar{I}$) of 0.977 in Speyeria and Turner (1979), working on Heliconius species, gives a figure of 0.963. The mean genetic identity between populations of Pieris species given by Geiger (1982), $\bar{I}$=0.990, is also comparable, and very similar to that reported by Courtney (1980), $\bar{I}$=0.997, although Courtney's interpretation of 'populations', 'subspecies' and 'semispecies' is less than easy to follow. (Other problems in utilising Courtney's data have already been mentioned.)

2.5.1 Maniola jurtina in Britain

The similarity matrix (and F_{ST}) for British populations of jurtina (figure 1.3.1b) is shown in table 2.5.1a.

Linkage clustering analysis suggested a number of interesting relationships. All three methods agree on the grouping of Barra and Tiree, all central Scottish populations, as well as Orkney, Huna and Wester Hardmuir populations. There is also agreement on the isolation of Cramond as distinct, especially in the single linkage method. Both UPGMA and complete linkage place three of the four Irish populations together as a group, very distinct from the rest of Britain, and relate Rosebery (Ireland) with the Scillonian jurtina. Single linkage groups all four Irish populations as a clearly defined unit. UPGMA and complete linkage groups Portpatrick, Tiptree and Benderloch populations, but single linkage relates the latter to those of central Scotland.

Major groupings are relatively unambiguous with only a few differences between the computations by the three clustering methods (figures 2.5.1a - c). There is, throughout, a separation of the British populations into three main groups 1.Ireland, 2. Scotland, northern and eastern England and 3. west-central and south-western England. Only complete linkage groups the Isle of Man and St Bees populations with those of west-central England. UPGMA makes a distinction between central Scottish and other north British groups.

The differences between the product-moment correlation co-efficient of the clustering techniques are negligible.

single linkage	r_p=0.753 df495
complete linkage	r_p=0.718 df495
UPGMA	r_p=0.745 df495
NMMS two-dimensional	r_p=0.928 df495

The stress for a two-dimensional solution using NMMS was excessive (0.180 r^2=0.878), although r_p was much better than for any of the linkage clustering methods. The three-dimensional solution was rather better (stress 0.132 r^2=0.919). This loosely corresponded with the linkage clustering analysis in basic configuration, but a number of very distinct, widely separated co-ordinates made identification of clusters rather difficult and left many points unclear. However,

table 2.5.1a

Genetic similarity values, g (1-G/2) above diagonal and F_{ST} below diagonal for populations of Maniola jurtina in Britain. For convenience, figures are given as $X10^3$.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 Orkney	-	950	879	890	902	906	934	925	923	916	942	918	911	890
2 Huna	032	-	894	899	927	917	928	923	926	916	933	923	920	896
3 Barra	070	085	-	960	919	894	954	941	937	922	920	940	937	899
4 Tiree	053	053	015	-	932	913	954	951	950	935	932	950	955	911
5 Seil	028	036	044	034	-	944	951	950	948	957	943	961	951	892
6 N.Erradale	114	060	057	026	009	-	927	906	907	907	915	917	903	898
7 Benderloch	045	059	031	024	022	050	-	971	966	963	970	966	959	930
8 Torrie F	038	043	026	021	026	044	012	-	981	974	977	981	977	919
9 Dunblane	039	033	028	014	022	030	012	006	-	982	982	977	981	928
10 Sheriff	036	043	034	019	023	028	015	008	006	-	979	978	971	922
11 Auchter	031	043	042	030	023	055	009	009	008	007	-	976	969	937
12 Glen Farg	032	037	033	025	023	042	017	006	008	004	009	-	976	917
13 St Andrews	034	034	020	011	021	033	014	008	003	012	014	012	-	918
14 Cramond	071	052	053	028	030	090	047	046	024	038	035	045	032	-
15 Kilsyth	033	034	024	019	021	041	009	008	003	010	012	011	004	033
16 Portpatrick	041	042	027	028	021	044	018	019	012	017	026	013	016	050
17 St Bees	048	046	042	042	024	037	041	035	027	027	038	021	032	063
18 Wilmslow	035	041	037	035	020	046	019	017	019	023	022	014	020	054
19 Stroud	034	038	034	036	019	043	020	016	016	022	020	011	019	055
20 Tiptree	030	038	033	034	015	037	011	011	014	018	013	010	016	048
21 Whiddon	039	040	039	037	016	026	033	029	019	024	032	019	025	055
22 Sennen	034	032	028	028	013	024	021	018	014	019	023	012	015	042
23 Allenwood	113	112	088	095	076	095	078	078	137	084	105	068	091	171
24 Portrush	066	072	057	063	021	059	046	048	062	049	058	038	057	102
25 Rosebery	067	052	052	048	053	056	047	040	046	046	050	032	037	070
26 Kinvara	064	070	067	070	047	066	047	042	071	049	052	034	059	112
27 Ruthvoes	035	037	034	032	011	023	022	020	018	021	026	014	019	058
28 Strathconon	032	042	061	036	034	099	027	015	011	016	018	013	020	067
29 Wester Hard	073	084	178	071	070	069	084	025	026	031	050	013	046	259
30 Cairndow	240	033	100	040	029	043	104	078	049	064	109	078	054	049
31 Scilly	061	075	061	064	042	210	039	036	026	041	029	034	043	060
32 Man	038	036	042	039	028	036	022	021	026	025	028	018	020	055

table 2.5.1a (continued)

	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1 Orkney	937	924	915	910	914	931	919	903	862	909	925	895	896	933	957	931	917	921
2 Huna	931	937	914	932	937	935	928	928	850	910	935	897	919	936	938	935	929	933
3 Barra	943	943	914	927	927	924	915	926	850	891	916	872	915	925	879	882	902	929
4 Tiree	952	934	916	928	938	937	918	921	846	883	915	875	922	937	879	910	889	920
5 Seil	952	958	945	939	946	967	934	944	868	958	914	908	942	946	901	926	911	933
6 N.Erradale	920	904	896	899	911	918	909	906	828	896	884	870	903	926	899	938	894	901
7 Benderloch	975	971	945	957	960	969	951	954	881	926	935	918	954	962	917	911	949	960
8 Torrie F	980	969	947	958	960	969	939	948	886	919	935	924	945	963	932	899	929	963
9 Dunblane	991	970	950	953	961	965	944	951	875	920	938	918	942	955	928	915	928	957
10 Sheriff	979	965	948	941	954	967	936	941	896	920	919	920	942	952	928	899	922	944
11 Auchter	976	965	944	956	963	969	943	951	875	920	929	922	945	965	946	905	938	956
12 Glen Farg	977	968	964	964	968	972	947	956	879	918	929	920	951	967	927	905	924	958
13 St Andrews	979	962	947	955	964	960	934	945	856	897	918	900	941	951	904	903	915	954
14 Cramond	922	914	911	913	922	913	915	927	849	887	902	890	915	901	896	904	911	918
15 Kilsyth	–	974	958	950	960	971	948	948	880	928	939	919	944	960	930	922	935	963
16 Portpatrick	006	–	969	967	973	974	970	973	904	937	948	935	965	956	928	892	952	972
17 St Bees	022	013	–	952	959	961	965	960	907	938	940	939	949	931	917	903	932	964
18 Wilmslow	016	011	015	–	985	959	965	977	905	933	951	937	961	945	917	886	955	971
19 Stroud	013	009	013	003	–	971	970	975	909	941	948	948	971	944	929	894	952	972
20 Tiptree	011	011	021	006	003	–	959	959	907	944	945	946	967	961	938	906	943	957
21 Whiddon	016	009	005	014	009	015	–	977	923	950	954	949	974	939	922	901	961	967
22 Sennen	013	008	009	005	003	007	006	–	909	939	949	941	971	941	917	892	954	966
23 Allenwood	098	062	053	042	049	054	057	048	–	946	923	967	919	862	896	828	902	907
24 Portrush	049	033	024	017	019	023	025	020	013	–	960	973	933	901	938	900	933	943
25 Rosebery	038	026	022	012	016	026	025	014	050	025	–	944	927	917	933	918	947	956
26 Kinvara	053	039	034	019	022	023	034	026	016	006	030	–	948	900	935	864	930	940
27 Ruthvoes	016	011	013	008	005	007	006	003	045	019	023	024	–	950	908	873	946	955
28 Strathconon	015	020	038	023	025	022	036	029	092	063	043	057	033	–	936	905	929	940
29 Wester Hard	042	047	041	033	043	039	084	054	101	070	047	054	075	018	–	909	919	926
30 Cairndow	076	088	093	074	081	076	097	047	179	132	072	170	060	176	090	–	889	897
31 Scilly	028	029	040	029	024	023	034	031	109	052	051	055	040	046	159	393	–	953
32 Man	016	010	015	011	006	011	010	008	056	022	022	025	006	030	090	075	032	–

figure 2.5.1a

Single linkage clustering: Maniola jurtina - British populations

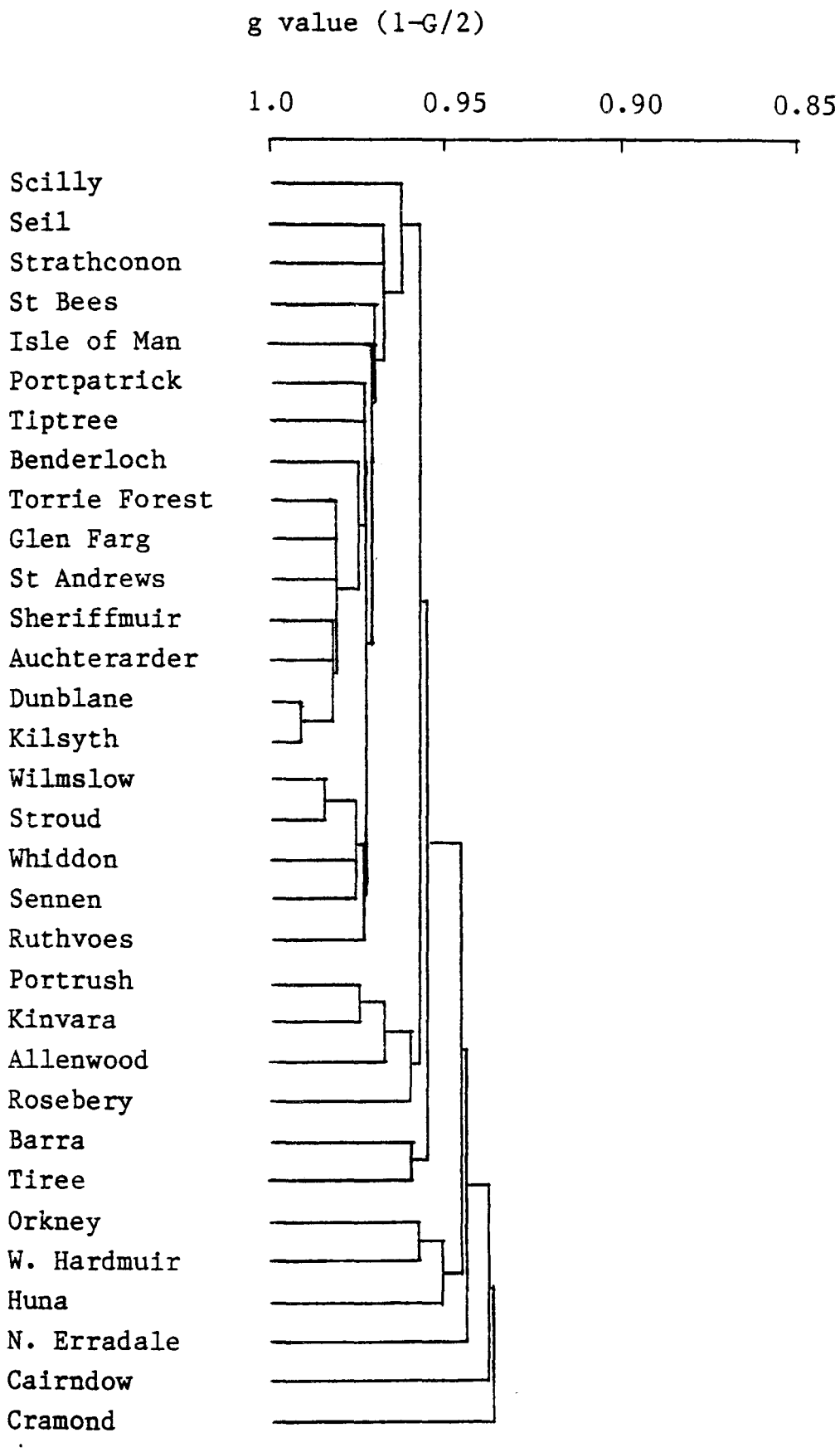


figure 2.5.1b

Complete linkage clustering: Maniola jurtina - British populations

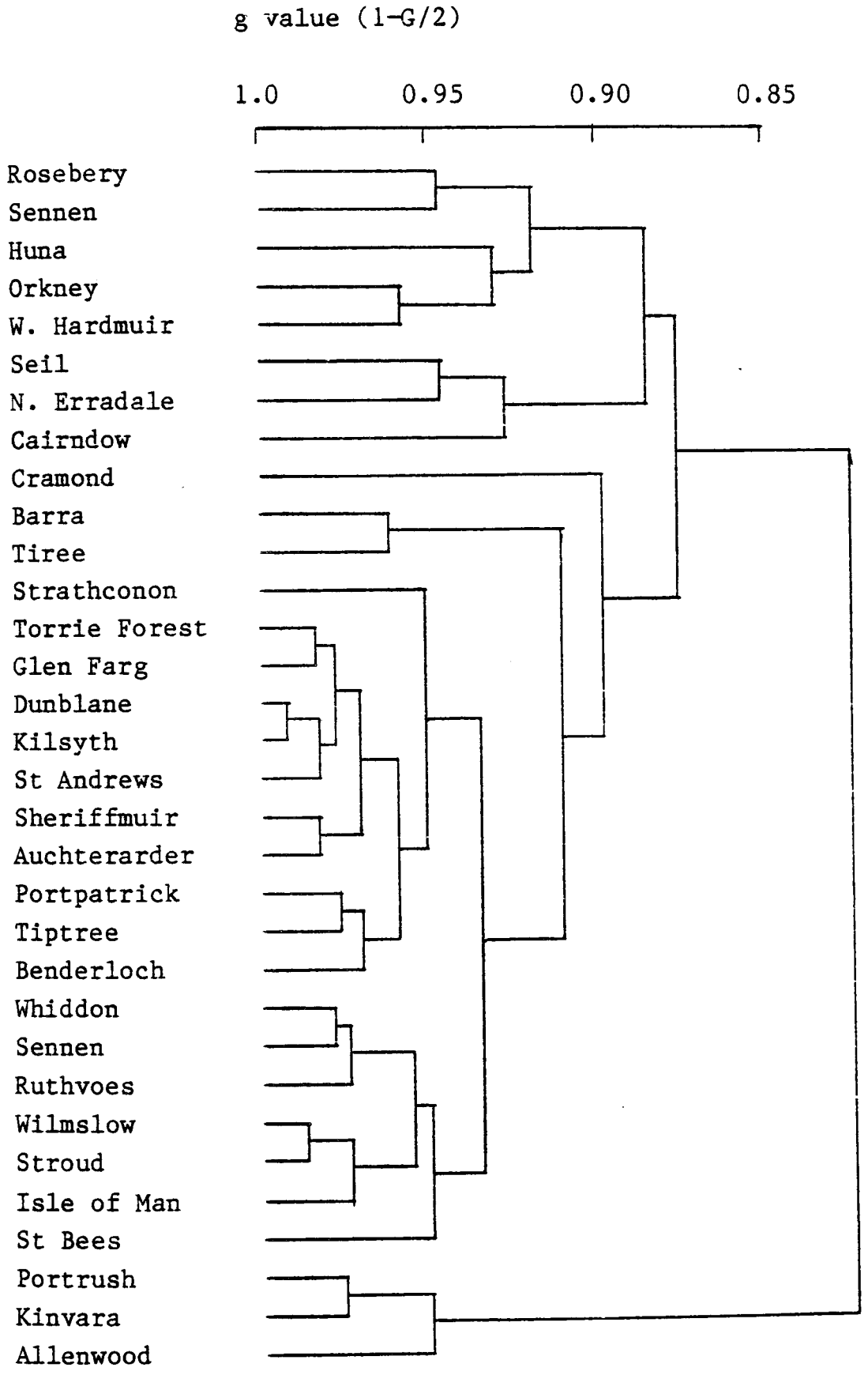
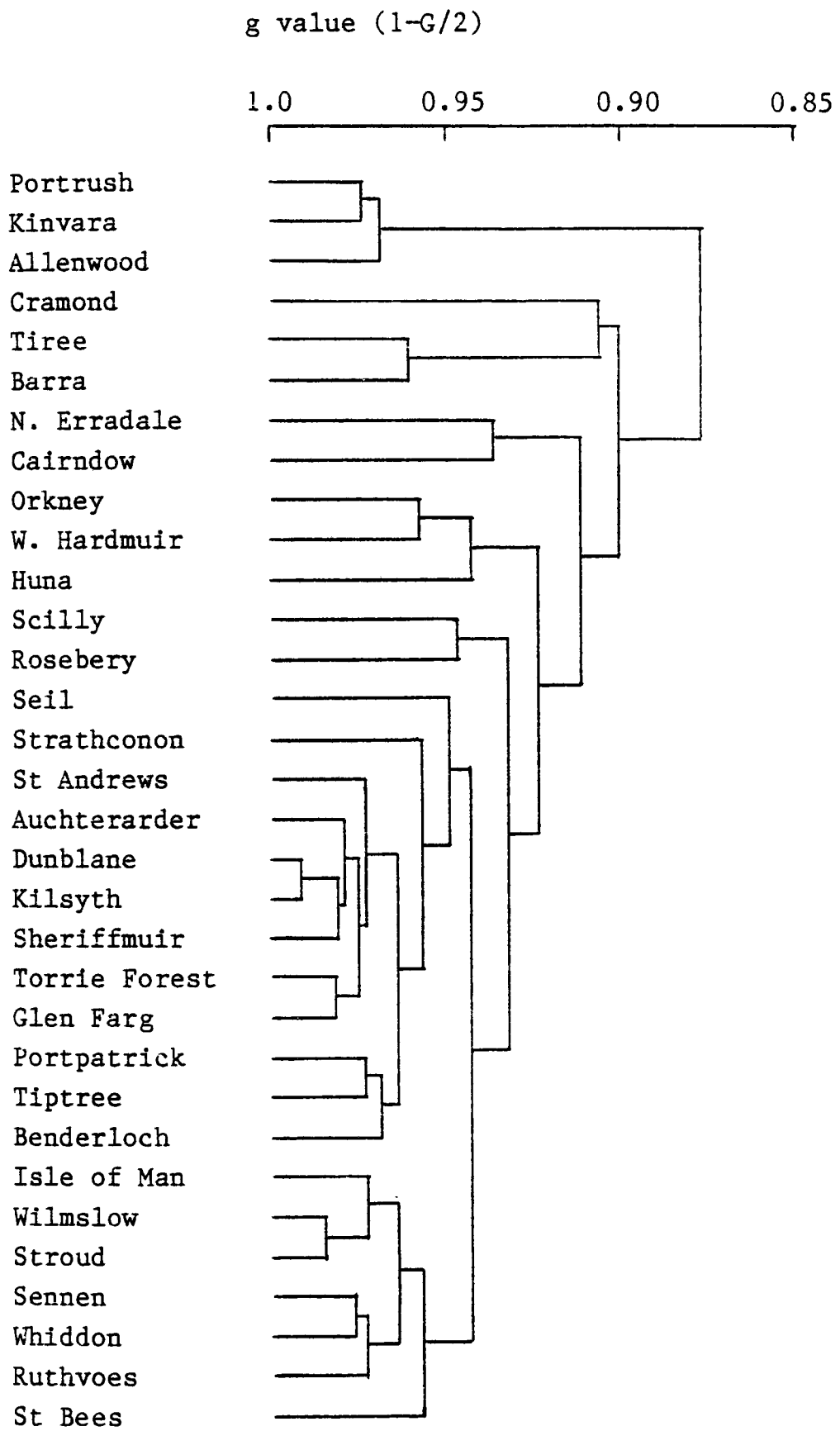


figure 2.5.1c

Average linkage clustering (UPGMA): Maniola jurtina - British populations



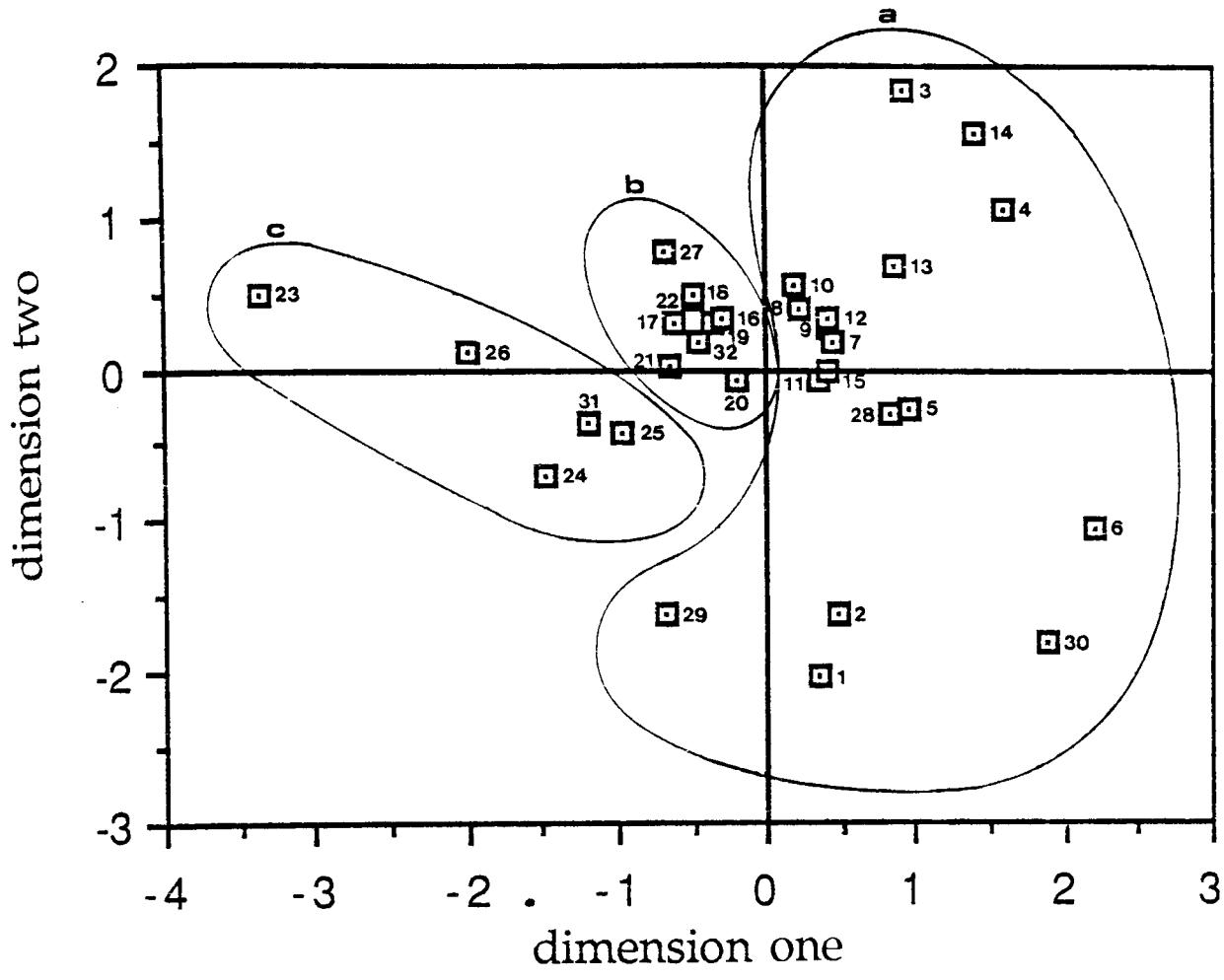


figure 2.5.1d

Nonmetric multidimensional scaling plot of genetic similarity coefficients (1-G/2) from British populations of Maniola jurtina.

Envelopes

a north and central Scotland

b south Scotland, mainland England and the Isle of Man

c Ireland and the Isles of Scilly

Populations

1	Orkney	12	Glen Farg	23	Allenwood
2	Huna	13	St Andrews	24	Portrush
3	Barra	14	Cramond	25	Rosebery
4	Tiree	15	Kilsyth	26	Kinvara
5	Seil	16	Portpatrick	27	Ruthvoes
6	North Erradale	17	St Bees	28	Strathconon
7	Benderloch	18	Wilmslow	29	Wester Hardmuir
8	Torrie Forest	19	Stroud	30	Cairndow
9	Dunblane	20	Tiptree	31	Isles of Scilly
10	Sheriffmuir	21	Whiddon Down	32	Isle of Man
11	Auchterarder	22	Sennen Cove		

the distinct Orkney, Huna, Cramond and Irish populations were well represented (figure 2.5.1d).

Taking account of the analysis by linkage clustering, it was possible to identify significant groups of populations 1. the Irish populations, together with that from the Isles of Scilly, 2. the English populations together with Portpatrick and 3. the central Scottish populations.

The anomalous location in the diagrams of Wester Hardmuir and Cairndow was possibly due to sampling error (Wester Hardmuir n=5, Cairndow n=3).

The distinct nature of Cramond jurtina was recognised on morphological grounds some time ago (Thomson, unpublished data). Samples in 1973 revealed that 33.3% of all males and 30% of all females on the island were of the form erymanthoides Strand (having a 'split' or twin apical eyespot on the forewing) or a related eyespot-aberrant morph (eg addenda Mousley, with supernumery ocelli below the apical eyespot). When the population was sampled again in 1984, the proportion of erymanthoides and similar forms was 50% in the males and 31% in the females. These phenotypes must be maintained at this extremely high frequency by balancing selection, or by some other mechanism which treats the morphs as advantageous or, at least, selectively neutral. Electrophoretic analysis confirmed that this small island hosts a very unusual population of jurtina: an uncommon genotype, $PGM_{78/100}$, was found at a frequency of 0.386. The frequency of this genotype in British jurtina insularis was less than 0.4. Other aspects of the Cramond population will be discussed in section 4.1.1.

The suggested interpretation of these analyses is that there are three main groups of jurtina populations in Britain and Ireland. The most distinct is undoubtedly the Irish jurtina (iernes Graves), which possibly relates closely to jurtina cassiteridum Graves in the Isles of Scilly (see section 4.2). Populations in the Western Isles of Scotland and the the far north, rather distinct in themselves, form a group which is somewhat separate from, but distantly related to those on the rest of mainland Britain which, in turn, can be considered as

two population subgroups, one in central Scotland and the other in southern Scotland and England.

2.5.2 Maniola jurtina in Europe

Table 2.5.2a gives the similarity matrix (and F_{ST}) for jurtina populations in continental Europe. The Moroccan populations were not included in the linkage clustering or NMMS ordinations as the number of polymorphic loci used was less than in other populations. This could distort the data.

The product-moment correlation coefficients were relatively low compared with those computed from other data sets.

single linkage	r_p=0.574 df464
complete linkage	r_p=0.527 df464
UPGMA	r_p=0.553 df464
NMMS two-dimensional	r_p=0.789 df464
NMMS three-dimensional	r_p=0.876 df464

It should be noted that r_p for the three-dimensional solution by NMMS was considerably higher than the others. Stress calculated for the two-dimensional solution was also very high (0.248 r^2=0.776), but much improved in the three-dimensional solution (0.166 r^2=0.828).

Average clustering did not suggest an overall consensus of relationships between the populations (figures 2.5.2a - c). Both complete linkage and UPGMA indicated two very distinct groups corresponding with the 'eastern' and 'western' populations based on genitalia form. Complete linkage indicated another group comprising the western and eastern populations within the 'western' group, but this was not suggested elsewhere. Single linkage clustering was somewhat at odds with the other methods in indicating no major groups of populations which corresponded closely with the relationships suggested by the other methods, including NMMS. Surprisingly, no major clusters were apparent in the NMMS plot. However, the 'eastern' and 'western' groups

table 2.5.2a

Genetic similarity values, g (1-G/2) above diagonal and F_{ST} below diagonal for populations of Maniola jurtina in Europe, excluding Britain. For convenience figures are given as $X10^3$. * inadequate data for clustering procedures.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 Feldthurnes	-	953	916	937	923	948	937	927	942	941	960	949	920	874	947	951	948	954
2 Fiastra	041	-	953	942	961	974	935	936	917	945	979	968	955	888	974	921	928	926
3 Marzabotto	099	023	-	902	933	946	911	897	868	911	953	933	934	841	962	884	891	881
4 Vienna	057	032	053	-	955	933	929	928	935	955	941	952	920	910	942	920	919	925
5 Crete	072	027	042	019	-	937	909	906	904	930	960	947	942	901	948	905	901	912
6 Loipersbach	066	009	027	045	042	-	915	927	906	933	959	964	951	869	967	910	913	909
7 Guernsey	166	046	051	071	065	076	-	930	954	957	934	936	919	878	917	947	927	943
8 Alderney	041	009	057	051	052	017	060	-	940	957	938	936	900	861	935	937	909	931
9 Morocco*	061	043	122	038	061	118	105	036	-	950	924	943	914	907	911	963	937	963
10 Malta	036	017	050	027	031	033	029	017	019	-	953	949	930	884	938	944	992	948
11 S.Leonardo	041	010	036	032	025	014	076	021	046	017	-	969	945	905	968	937	934	945
12 Ponti Lamb	063	017	055	035	028	025	108	028	065	036	019	-	961	924	966	949	943	946
13 Trabzon	063	023	052	040	041	040	098	028	032	013	025	024	-	855	956	896	875	901
14 Trikala*	429	140	155	175	055	190	125	197	391	106	079	186	226	-	873	869	891	889
15 Sopron Um	042	003	030	030	032	007	074	013	050	024	012	014	024	186	-	911	907	915
16 Altenahr	046	021	045	028	028	024	046	020	041	018	018	017	024	141	021	-	947	964
17 Verbier	051	021	025	023	034	027	058	048	079	037	021	037	043	179	023	032	-	956
18 Sare	038	018	046	011	021	029	074	035	039	020	014	024	032	149	019	019	015	-
19 Sorede	065	048	082	070	045	064	062	046	083	028	021	066	072	117	062	030	065	054
20 Concoules	036	026	069	013	022	039	100	034	017	012	017	027	015	086	031	016	038	008
21 Guernanville	065	031	043	031	009	038	056	050	063	026	016	029	036	045	037	023	034	022
22 Belveaux	029	018	057	024	029	023	060	017	038	024	019	021	045	126	022	012	030	019
23 Gottland	026	063	147	062	095	122	101	058	040	037	058	112	087	495	068	068	064	055
24 Gignod	048	017	061	047	032	019	145	029	045	032	015	005	021	102	012	020	044	026
25 Vergon	051	026	036	027	026	031	041	033	028	014	020	018	012	129	028	009	030	019
26 Fountain	056	019	036	033	020	027	061	025	040	018	013	012	016	086	021	013	035	026
27 Digne	073	046	049	037	040	055	024	052	024	025	048	031	023	196	050	024	053	042
28 Celon	025	011	030	040	044	017	023	013	039	012	011	035	028	141	017	021	025	024
29 Cahors	019	016	036	040	064	015	028	016	039	021	023	036	030	362	020	016	025	027
30 Nant	055	020	053	064	050	022	160	026	058	027	024	018	018	237	017	033	051	045
31 Spain+Sard	269	040	049	099	086	065	059	040	142	052	079	095	094	169	059	067	090	101
32 Mouschau	052	026	060	031	027	040	060	025	027	011	017	027	019	121	030	011	043	021
33 Bern+Heinis	046	017	056	026	038	031	065	017	046	022	023	045	049	183	022	023	038	024
34 Raron*	054	050	044	057	071	058	072	095	134	043	056	114	083	180	063	071	034	056
35 Trieste*	046	012	035	024	034	020	061	030	055	025	015	026	034	235	010	030	010	008

table 2.5.2a (continued)

	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1 Feldthunes	943	944	919	949	967	927	921	926	897	946	958	921	884	927	955	932	945
2 Fiastra	910	943	896	920	929	963	914	943	896	948	943	933	914	910	958	911	938
3 Marzabotto	877	885	842	883	898	931	873	902	854	927	906	891	877	862	913	904	908
4 Vienna	901	954	904	933	933	940	935	928	919	929	926	906	882	918	934	878	944
5 Crete	898	942	902	921	907	954	904	933	908	925	907	899	849	904	934	883	906
6 Loipersbach	906	930	879	926	917	955	919	922	888	946	933	925	910	927	965	918	941
7 Guernsey	927	953	914	921	945	912	933	943	941	946	953	922	921	919	932	897	921
8 Alderney	924	926	903	929	919	921	934	950	923	953	955	931	935	920	927	893	921
9 Morocco*	947	951	935	935	948	922	959	949	955	931	944	937	918	956	959	887	936
10 Malta	925	968	918	937	941	950	963	961	952	973	955	937	916	945	941	908	933
11 S.Leonardo	941	949	920	930	939	957	925	955	899	955	947	938	898	916	949	915	940
12 Ponti Lamb	944	946	928	931	924	981	955	958	931	936	936	965	917	935	957	896	962
13 Trabzon	920	945	863	898	917	940	891	924	890	918	891	885	864	898	937	879	898
14 Trikala*	887	895	901	850	855	895	885	886	847	864	855	887	854	871	887	826	894
15 Sopron Um	915	930	868	926	921	953	900	924	871	943	921	907	891	897	950	898	928
16 Altenahr	950	955	954	946	947	916	944	954	942	941	964	939	890	932	928	891	922
17 Verbier	900	940	936	920	918	918	930	935	906	935	955	941	893	910	938	940	931
18 Sare	944	971	949	940	951	922	936	958	928	943	956	936	892	931	935	907	926
19 Sorede	-	943	932	930	953	892	915	939	898	931	925	898	864	916	922	896	906
20 Concoules	029	-	940	951	944	946	976	966	958	956	950	934	904	967	961	902	912
21 Guernanville	026	018	-	908	908	892	933	945	932	910	933	951	869	912	891	850	910
22 Belveaux	029	018	031	-	940	909	925	929	909	941	941	899	860	928	940	903	905
23 Gottland	087	038	089	053	-	896	914	920	905	942	945	886	874	920	935	911	941
24 Gignod	041	031	026	032	083	-	929	939	921	940	919	927	891	931	942	893	929
25 Vergon	034	007	019	027	065	023	-	957	956	955	948	945	916	963	932	896	921
26 Fountain	030	016	016	027	080	013	008	-	954	952	955	944	903	930	931	891	913
27 Digne	065	017	036	045	078	059	013	022	-	935	942	927	888	944	901	872	894
28 Celon	029	028	035	019	033	037	025	022	045	-	968	927	917	949	955	937	925
29 Cahors	070	036	059	017	032	042	024	028	046	007	-	951	931	939	938	925	935
30 Nant	071	044	041	052	094	016	029	021	047	030	039	-	931	917	909	878	937
31 Spain+Sard	108	121	086	076	212	135	064	053	056	038	047	147	-	908	901	859	906
32 Mouschau	022	006	019	021	068	020	008	013	025	023	029	033	083	-	944	892	909
33 Bern+Heinis	050	027	044	011	053	055	034	033	045	017	010	064	088	032	-	936	916
34 Raron*	074	073	067	063	085	135	059	077	071	036	037	135	113	078	050	-	881
35 Trieste*	078	020	036	024	074	029	031	033	054	016	019	036	071	037	025	060	-

figure 2.5.2a

Single linkage clustering: Maniola jurtina - European populations

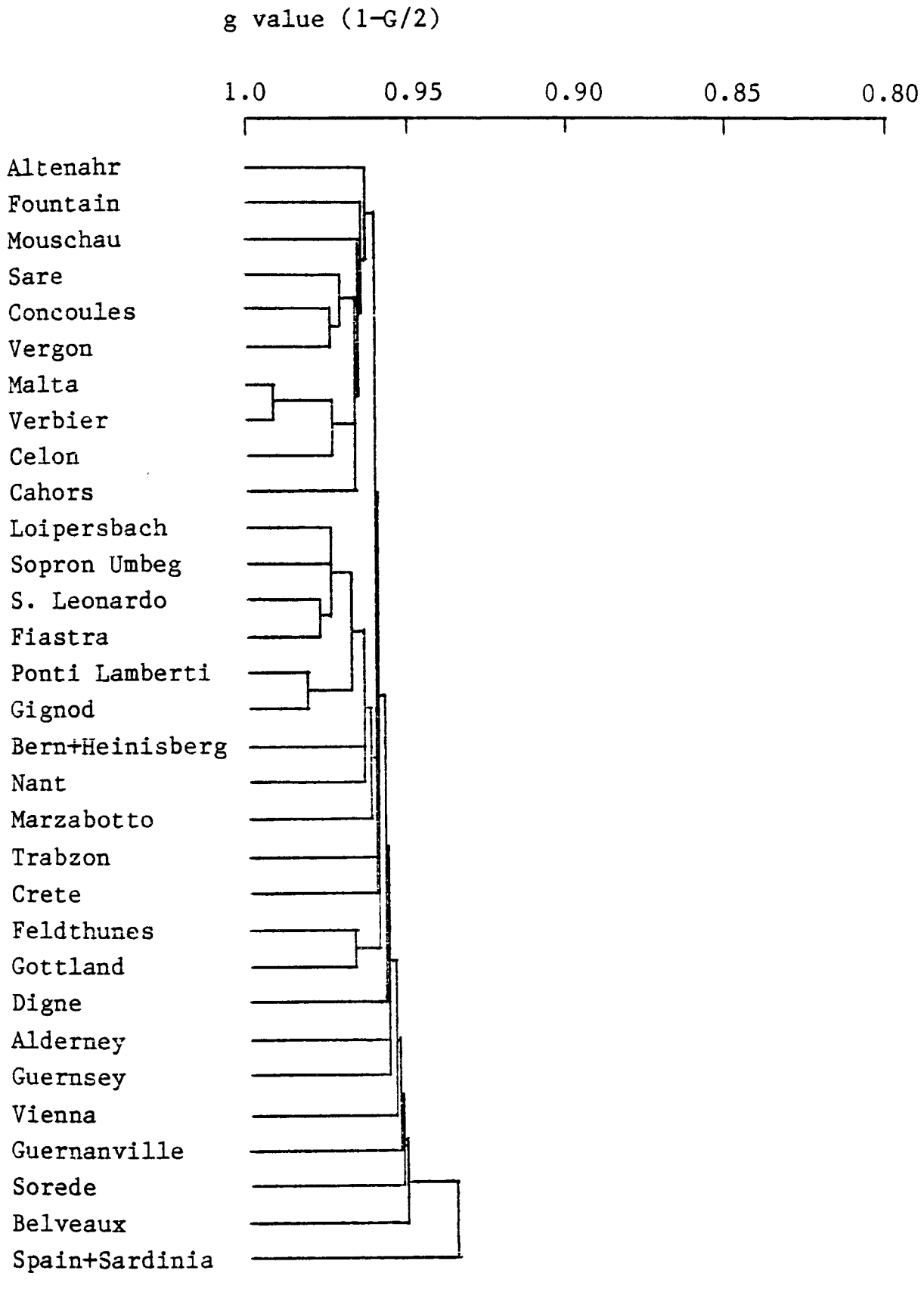


figure 2.5.2b

Complete linkage clustering: Maniola jurtina - European populations

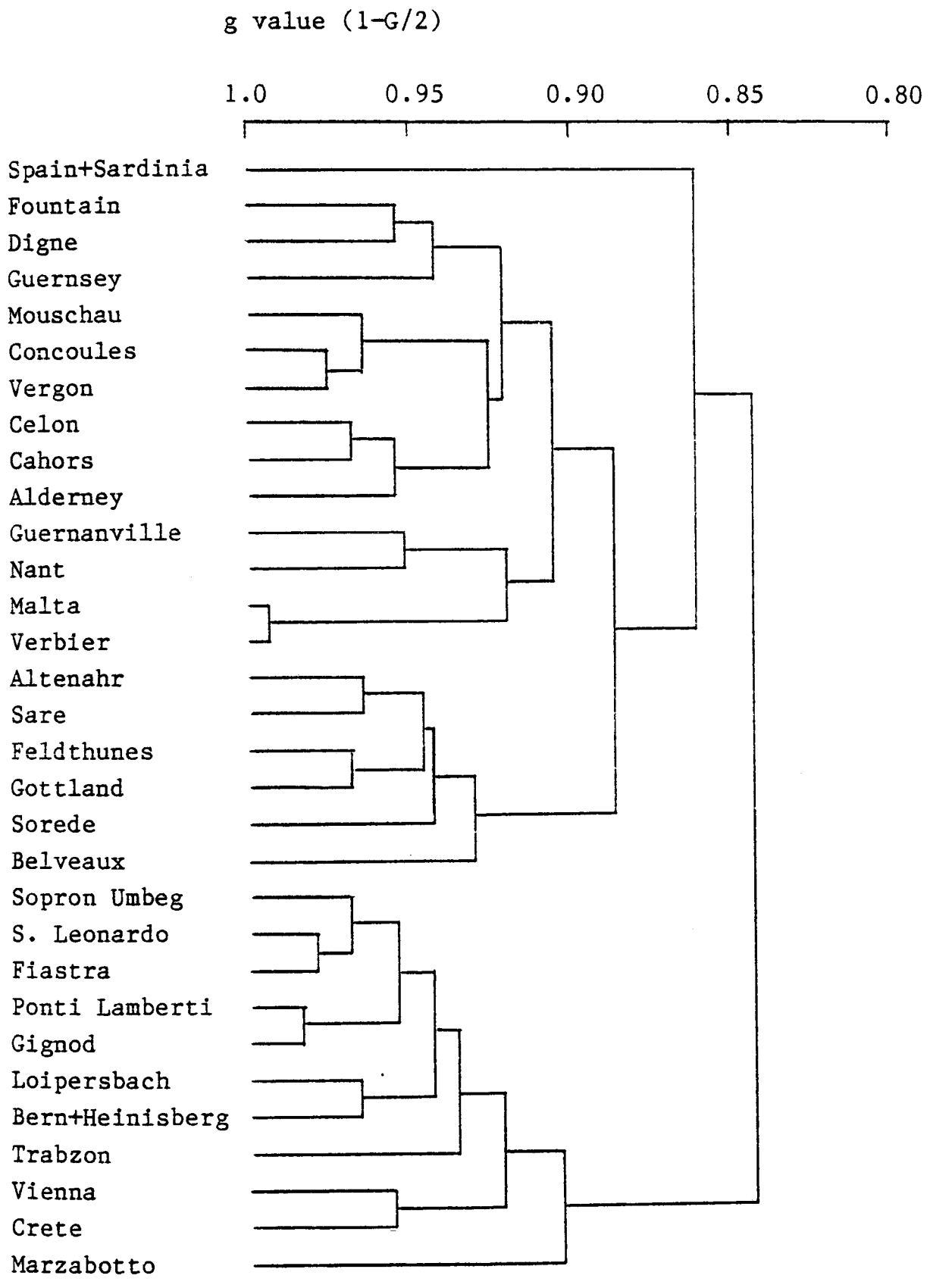
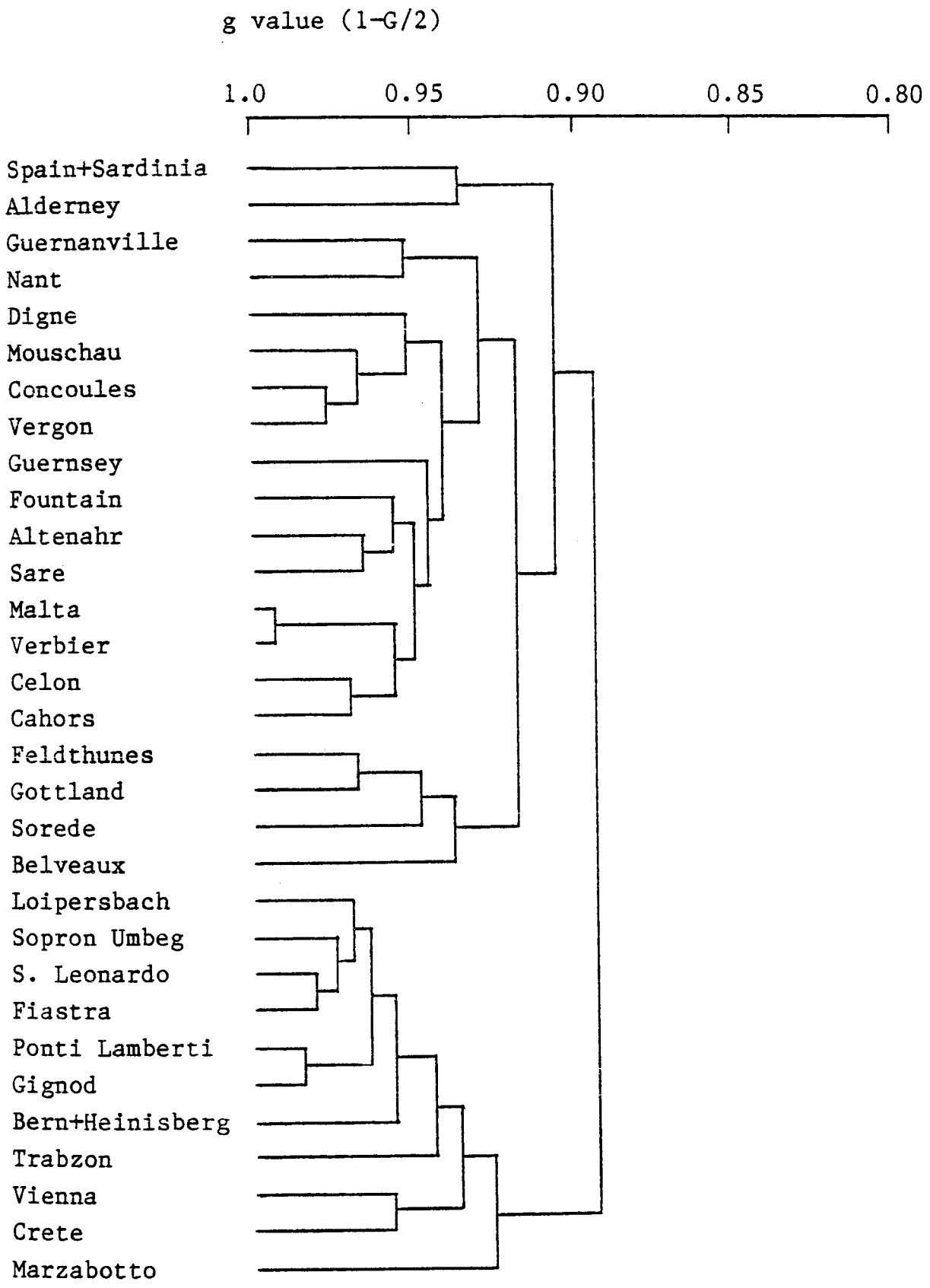


figure 2.5.2c

Average linkage clustering (UPGMA): Maniola jurtina - European populations



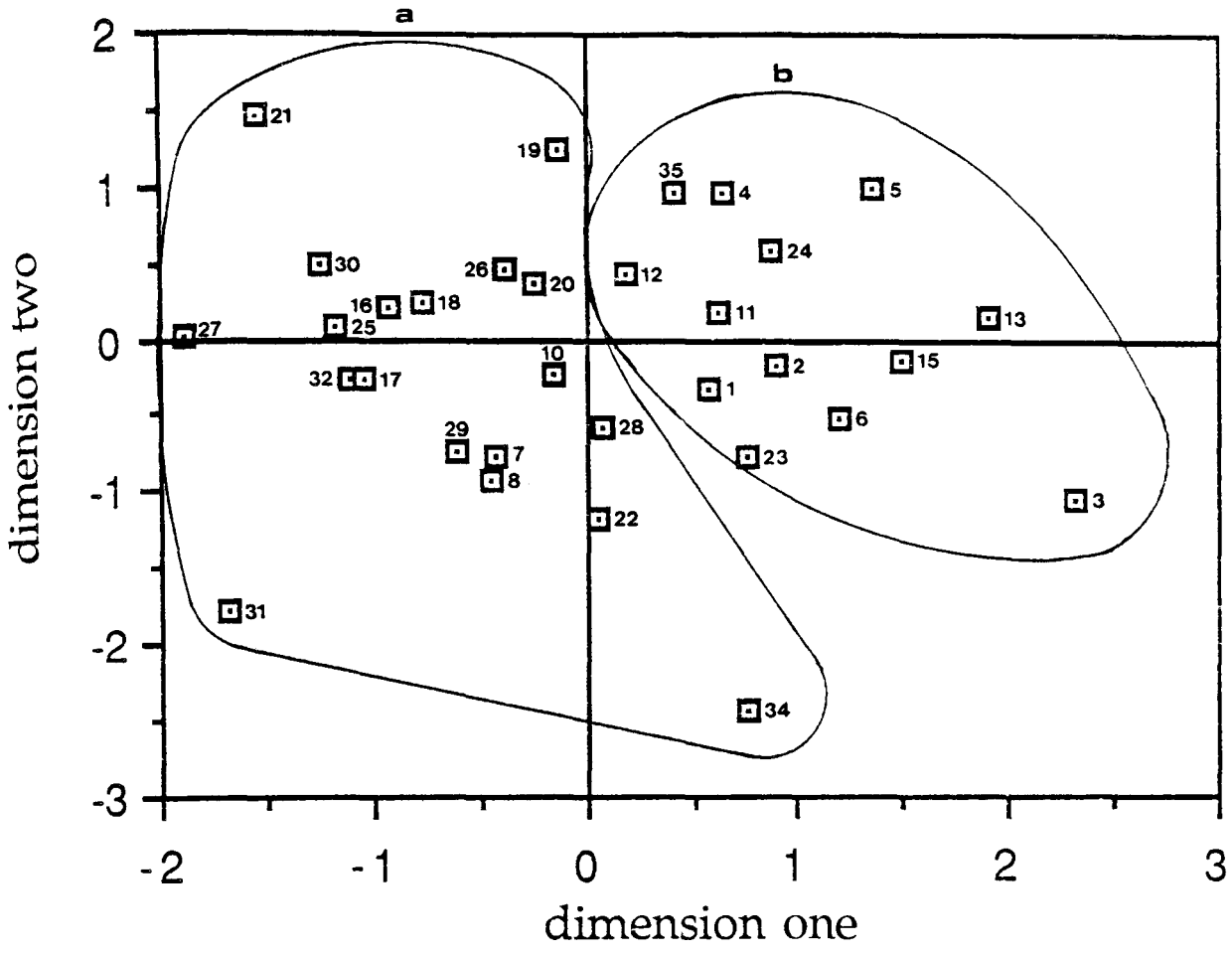


figure 2.5.2d

Nonmetric multidimensional scaling plot of genetic similarity coefficients (1-G/2) from European populations of Maniola jurtina.

Envelopes

a 'Western' genitalia group

b 'Eastern' genitalia group

Populations

1	Feldthunes	13	Trabzon	25	Vergon
2	Fiastra	15	Sopron Umbeg	26	Fountain de V
3	Marzabotto	16	Altenahr	27	Digne
4	Vienna	17	Verbier	28	Celon
5	Crete	18	Sare	29	Cahors
6	Loipersbach	19	Sorede	30	Nante
7	Guernsey	20	Concoules	31	Spain + Sardinia
8	Alderney	21	Guernanville	32	Mouschau
10	Malta	22	Belveaux	34	Raron
11	San Leonardo	23	Gottland	35	Trieste
12	Ponti Lamberti	24	Gignod		

table 2.5.2b

Allele frequencies at the PGM locus in Gottland and Feldthunes populations of Maniola jurtina (standard errors in parenthesis).

allele	Gottland	Feldthunes	jurtina
54	0	0	0.001 (<.001)
60	0	0	<.001 (<.001)
78	0	0	0.031 (0.002)
84	0	0	<.001 (<.001)
88	0	0	0.140 (0.004)
96	0	0	<.001 (<.001)
100	0.936 (0.021)	0.991 (0.009)	0.694 (0.005)
103	0	0	0.001 (<.001)
113	0.064 (0.021)	0.009 (0.009)	0.099 (0.003)
121	0	0	0.031 (0.002)
128	0	0	0.003 (0.001)
145	0	0	<.001 (<.001)

retained their separate identities in the ordination. It is interesting that both Vienna and Bern+Heinisberg populations related better with the 'eastern' group, although the position of Gottland was ambiguous (figure 2.5.2d). The implications of these groups is discussed in sections 4.1.2 and 4.2.

Several other matters are raised by linkage clustering and ordination analysis.

M. jurtina hispulla from Spain and Sardinia emerged as a very clear entity, associating with Alderney in both complete and average linkage clustering and, to a lesser extent, in NMMS. The only method to relate Alderney to Guernsey was NMMS.

Two populations, Gottland and Feldthunes, associated closely in all linkage methods as well as NMMS. Their average similarity was very high (g=0.969). This is not what would be expected considering their relative geographical locations. An examination of allozyme data for these populations showed that, in some other respects, they were very similar. At the PGM locus, only two alleles were found in both populations, PGM_{100} and PGM_{113}, the latter at a very low frequency and, in the Feldthunes population, the locus was, virtually, monomorphic.

table 2.5.2c

Gottland and Feldthunes populations of Maniola jurtina - mean heterozygosity, mean effective number of alleles and total actual number of alleles at the nine loci CK, GK, IDH-1, IDH-2, LAP-2, MPI, PGI-2, PGM and PK (standard errors in parenthesis). Alleles with a frequency <0.01 have been excluded from the computations.

	Gottland	Feldthunes	jurtina
heterozygosity	0.133 (0.055)	0.198 (0.065)	0.250 (0.052)
effective no alleles	1.229 (0.103)	1.180 (0.061)	1.412 (0.020)
actual number alleles	23	28	34

This contrasted with a very high degree of polymorphism at this locus in jurtina as a whole (table 2.5.2b).

The allozyme data from these populations were examined across the nine commonly polymorphic loci (CK, GK, IDH-1, IDH-2, LAP-2, MPI, PGI-2, PGM and PK) and compared with data for the species from the same loci. Rare alleles with a frequency of less than 0.01 were not included in the calculations. Heterozygosity and effective number of alleles were low for jurtina and the actual number of alleles was significantly lower than the average for the species (table 2.5.2c). Gottland is a Swedish island in the Baltic and, being almost at the northern limit of the butterfly's range, jurtina there could well suffer substantial periodic fluctuations in numbers. The jurtina sample from Feldthunes, near Brixen in the Tyrol, was taken in a remote and isolated valley (Magyar pers. comm.). Both populations could be experiencing a high level of selection at these locations. Alternatively, the allelic depauperation could be the consequence of bottlenecking or founder effect (section 4.2).

table 2.5.3a

Genetic similarity values, g (1-G/2) above diagonal and F_{ST} below diagonal for subspecies of Maniola jurtina. For convenience, figures are given as $X10^3$.

		1	2	3	4	5	6	7	8	9	10	11
1	jurtina	-	772	913	909	879	864	859	820	819	877	877
2	hispulla	267	-	886	858	895	909	882	877	907	898	846
3	hyperhispulla	060	058	-	973	979	964	957	906	901	961	936
4	miscens	057	064	011	-	974	946	952	901	880	939	932
5	occidentalis	106	041	009	016	-	971	968	929	921	965	934
6	insularis	456	027	021	040	012	-	978	942	941	964	914
7	splendida	279	054	013	022	010	006	-	928	920	948	902
8	iernes	168	069	036	042	022	039	040	-	935	913	857
9	cassiteridum	168	073	053	064	034	021	035	037	-	923	865
10	janira	147	071	019	029	009	015	010	040	043	-	956
11	corfiothispulla	089	088	032	016	028	091	049	082	132	046	-

2.5.3 Subspecies of Maniola jurtina

The relationships within jurtina were examined further by considering the major subspecies groups (as defined by existing taxonomies). By this means it was possible to minimise distortion in the primary pattern of relationships caused by the inclusion of more or less aberrant populations. It also enabled the inclusion of the nominate subspecies jurtina from north Africa in these calculations as more material was available.

Product-moment correlation values from similarity and cophenetic matrices were satisfactory for single and average clustering methods, but poor for complete linkage. The NMMS stress coefficient for a two-dimensional solution was very low (0.066 r^2=0.982).

single linkage	r_p=0.809 df54
complete linkage	r_p=0.663 df54
UPGMA	r_p=0.802 df54
NMMS two-dimensional	r_p=0.979 df54

Single linkage clustering suggested a monophyletic line from hispulla and jurtina, through cassiteridum, iernes, corfiothispulla and janira to a group comprising insularis, hyperhispulla and occidentalis and miscens. Complete and average linkage clustering separated hispulla, jurtina, iernes and cassiteridum, the remaining populations branching to two groups which represented the 'eastern' and 'western' forms. Major clusters in the NMMS ordination were rather open, although the general configuration was similar to that indicated by the other methods. Perhaps the most definite and, at the same time the most interesting indication to emerge is the association between iernes and cassiteridum and that group's significant lack of relationship with other British populations (figures 2.5.3a - d). The implications of these cluster analyses and ordinations can be summarised.

1. Maniola jurtina jurtina (north Africa) and M. j. hispulla (Spain) are both very distinct from each other and from other jurtina subspecies.

2. M. j. iernes (Ireland) and M. j. cassiteridum (Isles of Scilly) form a group which is very distinct from the other British subspecies, almost as distinct from these subspecies as they are from hispulla and jurtina.

3. The 'eastern' and 'western' subspecies form two fairly distinct entities.

4. The British M. j. insularis and M. j. splendida are very closely related to each other.

5. The curious relationship suggested between M. j. hyperhispulla (Malta) and the French subspecies miscens and occidentalis is anomalous.

The zoogeographical and phylogenetic significance of these relationships will be discussed in section 4.2.

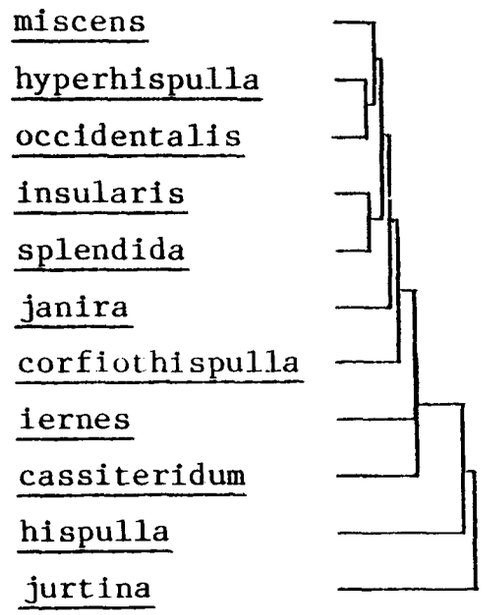
figures 2.5.3a - c

Single, complete and average linkage clustering (UPGMA): Maniola jurtina subspecies

a) single

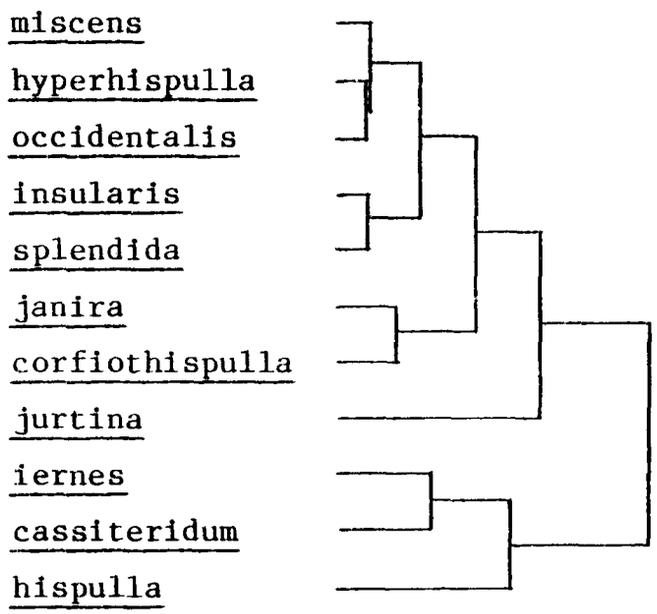
g value (1-G/2)

1.0 0.95 0.90 0.85 0.80



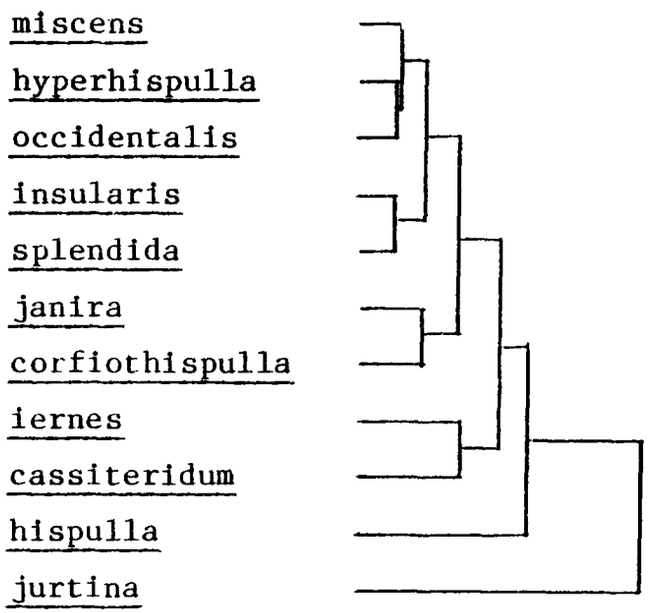
b) complete

1.0 0.95 0.90 0.85 0.80



c) average

1.0 0.95 0.90 0.85 0.80



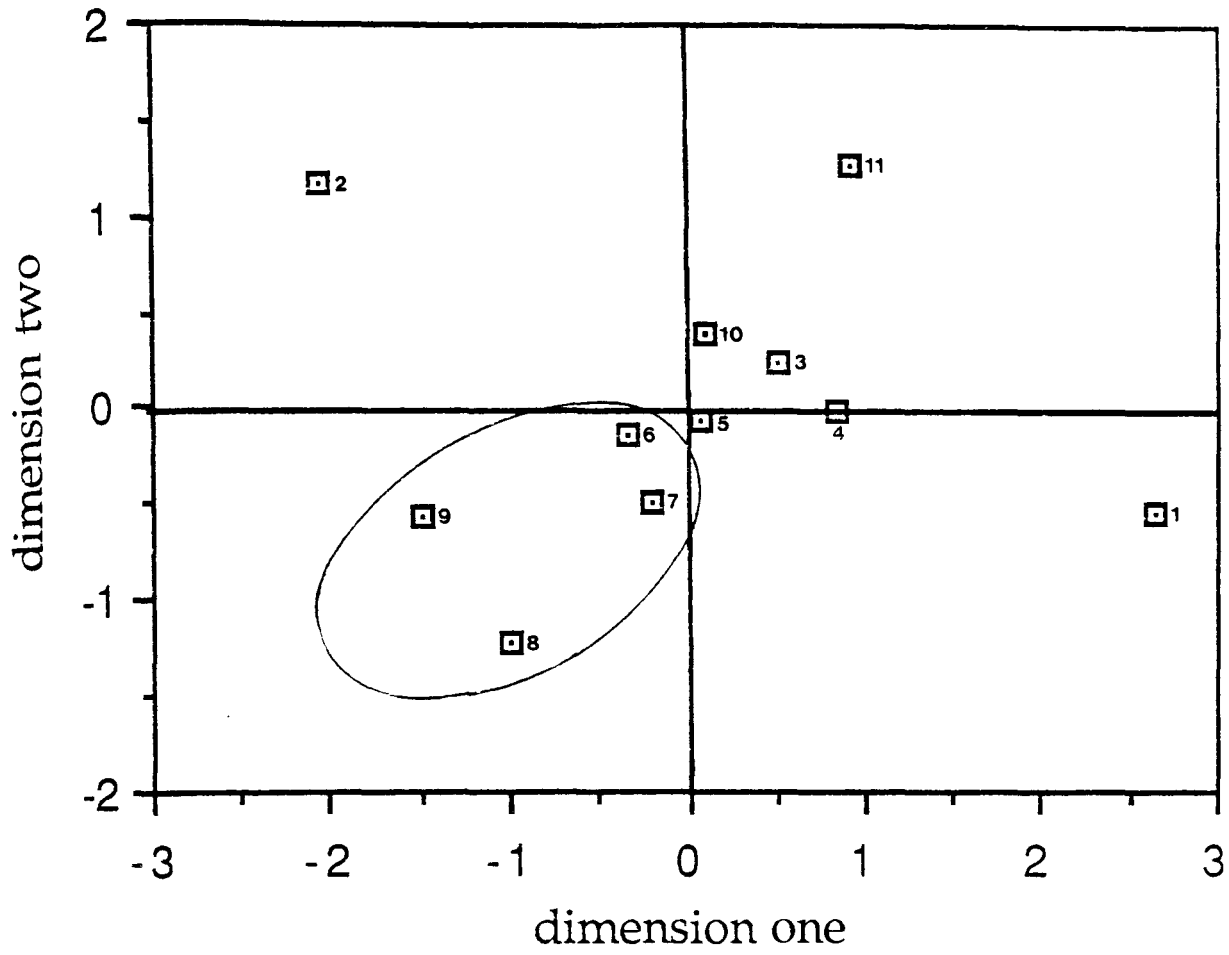


figure 2.5.3d

Nonmetric multidimensional scaling plot of genetic similarity coefficients (1-G/2) from subspecies of Maniola jurtina. Envelope encloses the Atlantic races insularis, splendida, cassiteridum and iernes.

Populations

1 jurtina
2 hispulla
3 hyperhispulla
4 miscens
5 occidentalis
6 insularis
7 splendida
8 iernes
9 cassiteridum
10 janira
11 corfiothispulla

table 2.5.4a

Allele frequencies at variable loci in species of the genus Maniola - standard errors in parenthesis below.

Locus	allele	jurtina	megala	chia	telmessia	cypricola	nurag
ALD	73	0.003	0.250	0.200	-	-	-
		(0.003)	(0.125)	(0.126)	-	-	-
	100	0.997	0.750	0.800	1.000	1.000	1.000
		(0.003)	(0.125)	(0.125)	(0)	(0)	(0)
CK	33	0.002	-	-	0.002	-	-
		(0.001)	-	-	(0.002)	-	-
	87	0.005	-	-	0.007	-	-
		(0.001)	-	-	(0.003)	-	-
	100	0.987	1.000	1.000	0.990	1.000	1.000
		(0.001)	(0)	(0)	(0.004)	(0)	(0)
	140	0.006	-	-	-	-	-
		(0.001)	-	-	-	-	-
DIA-1	100	0.982	1.000	1.000	0.947	1.000	1.000
		(0.012)	(0)	(0)	(0.026)	(0)	(0)
	141	0.072	-	-	0.053	-	-
		(0.012)	-	-	(0.026)	-	-
DIA-2	100	0.975	1.000	1.000	1.000	1.000	1.000
		(0.008)	(0)	(0)	(0)	(0)	(0)
	113	0.025	-	-	-	-	-
		(0.008)	-	-	-	-	-
D(-)3GPDH	90	-	-	-	-	0.273	-
		-	-	-	-	(0.095)	-
	93	0.002	-	-	1.000	0.727	1.000
		(0.002)	-	-	(0)	(0.095)	(0)
	100	0.998	1.000	-	-	-	-
		(0.002)	(0)	-	-	-	-
	112	-	-	1.000	-	-	-
		-	-	(0)	-	-	-
EST-1	100	0.998	1.000	1.000	1.000	1.000	1.000
		(0.002)	(0)	(0)	(0)	(0)	(0)
	121	0.002	-	-	-	-	-
		(0.002)	-	-	-	-	-
EST-A	100	0.991	1.000	1.000	1.000	1.000	1.000
		(0.005)	(0)	(0)	(0)	(0)	(0)
	129	0.009	-	-	-	-	-
		(0.005)	-	-	-	-	-
EST-C	87	0.002	-	-	0.050	-	-
		(0.001)	-	-	(0.013)	-	-
	100	0.994	1.000	1.000	0.935	1.000	1.000
		(0.002)	(0)	(0)	(0.015)	(0)	(0)
	115	0.004	-	-	0.015	-	-
		(0.001)	-	-	(0.008)	-	-
GK	86	0.001	-	-	0.002	-	-
		(0.001)	-	-	(0.002)	-	-
	100	0.848	0.844	0.895	0.890	1.000	0.932
		(0.004)	(0.064)	(0.050)	(0.012)	(0)	(0.038)

table 2.5.4a (continued)

GK	109	0.151	0.156	0.105	0.108	-	0.068
		(0.004)	(0.064)	(0.050)	(0.012)	-	(0.038)
	120	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
GOT-1	67	0.004	-	-	0.010	-	-
		(0.003)	-	-	(0.003)	-	-
	76	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	84	0.060	-	0.078	0.020	-	0.012
		(0.003)	-	(0.044)	(0.005)	-	(0.012)
	93	0.004	-	-	0.008	-	-
		(0.001)	-	-	(0.003)	-	-
	100	0.861	0.934	0.897	0.751	0.861	0.690
		(0.005)	(0.043)	(0.050)	(0.019)	(0.038)	(0.074)
	107	0.005	-	-	0.011	0.011	-
		(0.001)	-	-	(0.004)	(0.011)	-
	114	0.026	0.066	0.025	0.199	0.128	0.298
		(0.002)	(0.043)	(0.025)	(0.018)	(0.028)	(0.075)
	121	<.001	-	-	0.002	-	-
		(<.001)	-	-	(0.002)	-	-
	130	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
IDH-1	21	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	44	0.060	-	-	-	-	-
		(0.003)	-	-	-	-	-
	100	0.939	1.000	1.000	0.998	0.911	1.000
		(0.003)	(0)	(0)	(0.002)	(0.038)	(0)
	124	-	-	-	0.002	0.154	-
		-	-	-	(0.002)	(0.030)	-
	146	0.001	-	-	-	0.136	-
		(<.001)	-	-	-	(0.025)	-
IDH-2	57	0.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	76	0.070	-	-	0.002	-	-
		(0.003)	-	-	(0.002)	-	-
	80	0.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	88	0.001	-	0.026	-	-	-
		(<.001)	-	(0.026)	-	-	-
	100	0.924	1.000	0.974	0.994	1.000	1.000
		(0.003)	(0)	(0.026)	(0.003)	(0)	(0)
	112	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	118	-	-	-	0.004	-	-
		-	-	-	(0.003)	-	-
LAP-1	58	-	-	-	0.006	0.150	-
		-	-	-	(0.004)	(0.080)	-
	69	0.001	0.094	0.053	0.981	0.800	1.000
		(0.001)	(0.052)	(0.036)	(0.007)	(0.089)	(0)
	100	0.997	0.906	0.868	0.014	0.050	-
		(0.001)	(0.052)	(0.055)	(0.006)	(0.049)	-
	127	0.001	-	-	-	-	-
		(0.001)	-	-	-	-	-

table 2.5.4a (continued)

LAP-1	141	0.001	-	0.079	-	-	-
		(0.001)	-	(0.044)	-	-	-
LAP-2	74	<.001	-	-	0.017	0.027	-
		(<.001)	-	-	(0.005)	(0.019)	-
	80	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	90	0.035	0.219	0.079	0.616	0.851	0.614
		(0.002)	(0.073)	(0.044)	(0.020)	(0.041)	(0.073)
	95	0.043	-	0.026	0.045	0.027	0.091
		(0.002)	-	(0.026)	(0.009)	(0.019)	(0.043)
	100	0.827	0.781	0.632	0.297	0.095	0.295
		(0.004)	(0.073)	(0.078)	(0.019)	(0.034)	(0.069)
	107	0.011	-	0.026	0.024	-	-
		(0.001)	-	(0.026)	(0.006)	-	-
	113	0.080	-	0.237	0.002	-	-
		(0.003)	-	(0.069)	(0.002)	-	-
	123	0.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	130	0.003	-	-	-	-	-
		(0.001)	-	-	-	-	-
MDH	47	0.002	-	0.056	-	-	-
		(0.002)	-	(0.054)	-	-	-
	70	0.020	1.000	0.944	0.015	-	-
		(0.007)	(0)	(0.054)	(0.015)	-	-
	82	0.002	-	-	-	-	-
		(0.002)	-	-	-	-	-
	100	0.976	-	-	0.985	0.950	1.000
		(0.007)	-	-	(0.015)	(0.049)	(0)
	143	-	-	-	-	0.050)	-
		-	-	-	-	(0.049)	-
(MES)	70	-	-	-	0.184	0.409	-
		-	-	-	(0.063)	(0.105)	-
	100	1.000	1.000	1.000	0.816	0.591	1.000
		(0)	(0)	(0)	(0.063)	(0.105)	(0)
ME-1	80	-	-	-	0.026	0.046	-
		-	-	-	(0.026)	(0.044)	-
	100	1.000	1.000	1.000	0.974	0.955	1.000
		(0)	(0)	(0)	(0.026)	(0.044)	(0)
MPI	72	0.021	-	-	-	-	-
		(0.002)	-	-	-	-	-
	76	0.074	-	-	-	-	-
		(0.003)	-	-	-	-	-
	84	0.056	-	-	0.019	0.013	-
		(0.003)	-	-	(0.007)	(0.012)	-
	96	0.104	-	0.053	0.026	0.288	-
		(0.004)	-	(0.036)	(0.008)	(0.051)	-
	100	0.660	0.813	0.605	0.460	0.675	1.000
		(0.006)	(0.069)	(0.079)	(0.024)	(0.052)	(0)
	105	0.085	0.187	0.342	0.427	0.013	-
		(0.004)	(0.069)	(0.077)	(0.024)	(0.012)	-
	109	0.001	-	-	0.069	0.013	-
		(<.001)	-	-	(0.012)	(0.012)	-
PGI-2	22	0.056	0.094	0.026	0.064	0.012	-
		(0.003)	(0.052)	(0.026)	(0.010)	(0.012)	-

table 2.5.4a (continued)

PGI-2	40	0.001	-	0.026	-	-	-
		(0.001)	-	(0.026)	-	-	-
	60	0.013	-	-	0.039	0.024	0.045
		(0.001)	-	-	(0.008)	(0.017)	(0.031)
	100	0.845	0.906	0.789	0.855	0.841	0.750
		(0.004)	(0.052)	(0.066)	(0.014)	(0.040)	(0.065)
	119	0.009	-	0.026	0.002	-	-
		(0.001)	-	(0.026)	(0.002)	-	-
	141	0.001	-	-	0.027	0.024	-
		(<.001)	-	-	(0.007)	(0.017)	-
	165	0.063	-	-	0.003	0.085	0.205
		(0.003)	-	0.132	(0.002)	(0.031)	(0.061)
	178	0.002	-	(0.055)	0.010	-	-
		(0.001)	-	-	(0.004)	-	-
	219	0.001	-	-	-	0.012	-
		(0.001)	-	-	-	(0.012)	-
	333	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
PGM	54	0.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	60	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	78	0.031	-	0.053	0.037	0.012	0.045
		(0.002)	-	(0.036)	(0.008)	(0.012)	(0.031)
	84	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	88	0.140	0.219	0.263	0.065	0.063	0.318
		(0.004)	(0.073)	(0.071)	(0.010)	(0.027)	(0.070)
	96	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	100	0.694	0.562	0.684	0.796	0.775	0.500
		(0.005)	(0.088)	(0.075)	(0.016)	(0.047)	(0.075)
	103	0.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
	113	0.099	-	-	0.102	0.150	0.136
		(0.003)	-	-	(0.012)	(0.040)	(0.052)
	121	0.031	0.219	-	-	-	-
		(0.002)	(0.073)	-	-	-	-
	128	0.003	-	-	-	-	-
		(0.001)	-	-	-	-	-
	145	<.001	-	-	-	-	-
		(<.001)	-	-	-	-	-
PK	58	0.036	-	-	0.019	-	-
		(0.002)	-	-	(0.008)	-	-
	100	0.950	1.000	1.000	0.987	0.957	1.000
		(0.002)	(0)	(0)	(0.008)	(0.024)	(0)
	139	0.014	-	-	0.003	0.043	-
		(0.001)	-	-	(0.003)	(0.024)	-
SOD-2	100	0.915	1.000	0.944	0.957	0.833	0.591
		(0.012)	(0)	(0.054)	(0.030)	(0.108)	(0.074)
	300	-	-	-	0.044	0.083	-
		-	-	-	(0.030)	(0.080)	-
	400	0.085	-	0.056	-	0.083	0.409
		(0.012)	-	(0.054)	-	(0.080)	(0.074)

table 2.5.4b

Genetic similarity values, g (1-G/2) above diagonal and F_{ST} below diagonal for populations of Maniola telmessia, cypricola, nurag and chia. For convenience, figures are given as $X10^3$.

	1	2	3	4	5	6	7	8	9	10	11
1 Faliraki	-	974	952	965	969	881	971	959	926	919	926
2 Filerimos	006	-	953	977	980	889	967	955	927	929	929
3 Apolakkia	051	043	-	942	965	868	940	930	884	866	881
4 Kos	024	013	048	-	971	882	964	958	927	925	923
5 Simi	014	011	017	009	-	880	958	948	912	900	909
6 Karpathos	129	092	156	085	102	-	930	946	937	872	814
7 Samos	023	014	051	025	028	068	-	982	947	951	922
8 Lesbos	034	030	045	036	039	042	008	-	948	927	896
9 Cyprus	035	029	167	032	059	051	019	019	-	939	866
10 Sardinia	119	068	211	069	083	144	070	082	056	-	907
11 Chios	074	043	130	047	070	167	067	080	090	104	-

2.5.4 Inter-specific relationships in the genus Maniola

Preliminary electrophoretic work suggested that the Maniola species telmessia, cypricola and nurag should be treated together as a group of closely related forms. The D(-)3GPDH locus proved to be 100% diagnostic in differentiating between telmessia, cypricola and nurag when compared with species in the jurtina group. In jurtina, the locus D(-)3GPDH is virtually monomorphic with $D(-)3GPDH_{100}$ being the common allele and $D(-)3GPDH_{93}$ occurring at a frequency of 0.002. In telmessia, cypricola and nurag, the allele $D(-)3GPDH_{100}$ has not been found and $D(-)3GPDH_{90}$ is the most frequent, the locus being monomorphic for that allele in telmessia and nurag and occurring as the common allele in cypricola, with a third allele, $D(-)3GPDH_{90}$ (not found in the other species) at a relatively high frequency.

Both LAP-1 and LAP-2 were highly diagnostic between the species groups. LAP-1 was virtually monomorphic for $LAP\text{-}1_{100}$ in the jurtina group, whereas $LAP\text{-}1_{65}$ was by far the most common allele in the telmessia group. Similarly, $LAP\text{-}2_{100}$ and $LAP\text{-}2_{90}$ were the most frequent alleles in the two groups respectively at the second LAP locus (table 2.5.4a).

Although D(-)3GPDH and LAP-1 contributed greatly to the genetic distances between the jurtina and telmessia groups, the same loci which were used in the inter-population studies of jurtina were used for the purposes of inter-population comparisons in the telmessia group. The similarity and F_{ST} matrix for the telmessia group is given in table 2.5.4b.

The telmessia samples were collected in the remote island of Karpathos, the large island of Rhodes (three populations), Simi, Kos, Samos, Chios and Lesbos. With the exception of Karpathos, this chain of islands lies in close proximity to the Turkish mainland, where the species is also found. The Chios Maniola looked superficially very different from telmessia from the other islands in size, wing markings and genitalia form (see section 4.2 and appendix). Electrophoresis revealed that these populations were indeed quite different and certainly did not fit the pattern of genetic variation which was detected in the other islands. At the LAP-1 and LAP-2 loci the most frequent alleles were $LAP\text{-}1_{100}$ and $LAP\text{-}2_{100}$, as in jurtina. However, at D(-)3GPDH the Chios material was monomorphic for an allele not found in other Maniola species, $D(-)3GPDH_{112}$ (see table 2.5.4a). The Chios form was described as a new species, Maniola chia (Thomson 1987 - appendix) on genetic (electrophoretic) and morphological grounds.

As Maniola chia had initially been considered within the telmessia group, it was treated as such as part of the analysis for which polymorphic loci only were utilised. Interestingly, even using these loci and omitting the truly diagnostic D(-)3GPDH locus, chia still emerged as a distinct taxon in single, complete and average linkage clustering, and as an entirely separate form in NMMS.

The similarity / cophenetic correlation coefficients for the analytical methods used were relatively low for all the linkage clustering techniques. It was, however, high in the NMMS two-dimensional solution. Kruskal's stress was very low (0.063 r^2=0.982).

single linkage	r_p=0.738 df54
complete linkage	r_p=0.706 df54
UPGMA	r_p=0.730 df54
NMMS two-dimensional	r_p=0.974 df54

Complete and average linkage clustering defined two major groups, cypricola (Cyprus), nurag (Sardinia) and Karpathos as one, and telmessia populations (with the distinct chia) as the other. Single linkage suggested an almost monophyletic line from chia through Karpathos, Cyprus, Sardinia, Apolakkia and the rest of the telmessia group. All methods associated the Samos and Lesbos populations (figures 2.5.4a - d).

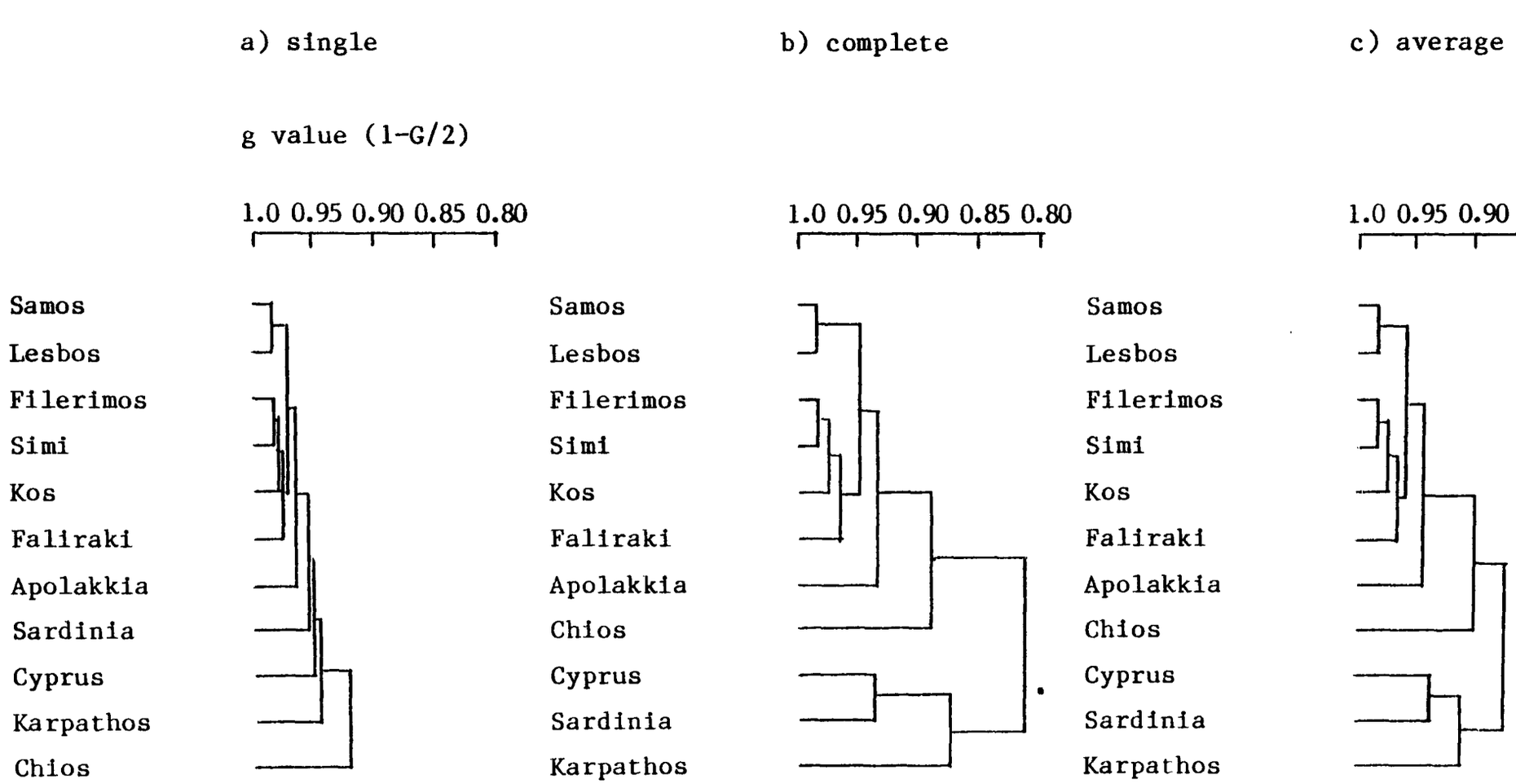
It is suggested by clustering and NMMS that Apolakkia telmessia is a very distinct form. Apolakkia is located in the far south-west of Rhodes, isolated from the northern populations by mountains. The telmessia there could well have become genetically divergent from the other populations in the northern part of the island. This isolation must now be incomplete, however, as telmessia has been found high on Mount Profitis Ilias which rises to 900m (Bretherton 1971).

The distinct nature of Karpathos telmessia, which emerges clearly from all the analyses was unexpected. Linkage clustering and NMMS suggest a relationship between these populations, Cypriot cypricola and Sardinian nurag, but the nature of this association is not clear. It is notable that there is a complete lack of relationship with other populations of the telmessia group. This is revealed most strongly when chia, cypricola and nurag are omitted from the analysis (figure 2.5.4e - g). Morphologically, Karpathos telmessia are very similar to those on the other Dodecanese islands, although they are smaller and there are some slight differences in wing markings which, to date, have not been considered significant. Karpathos already hosts at least one insular endemic butterfly species, Hipparchia christenseni (see Riemis 1986). It is possible that telmessia there represents another.

In its handling of the telmessia group, NMMS is quite unambiguous. It shows the Karpathos telmessia, cypricola, nurag and chia as being quite separate from the other populations, perhaps forming a group distantly related to each other. The Lesbos and Samos telmessia clearly are closely related, which is interesting when the large island of Chios lies between them. The other telmessia populations form a close group, with the Apolakkia population by far the most distinct.

figures 2.5.4a - c

Single, complete and average linkage clustering (UPGMA) - Maniola telmessia group



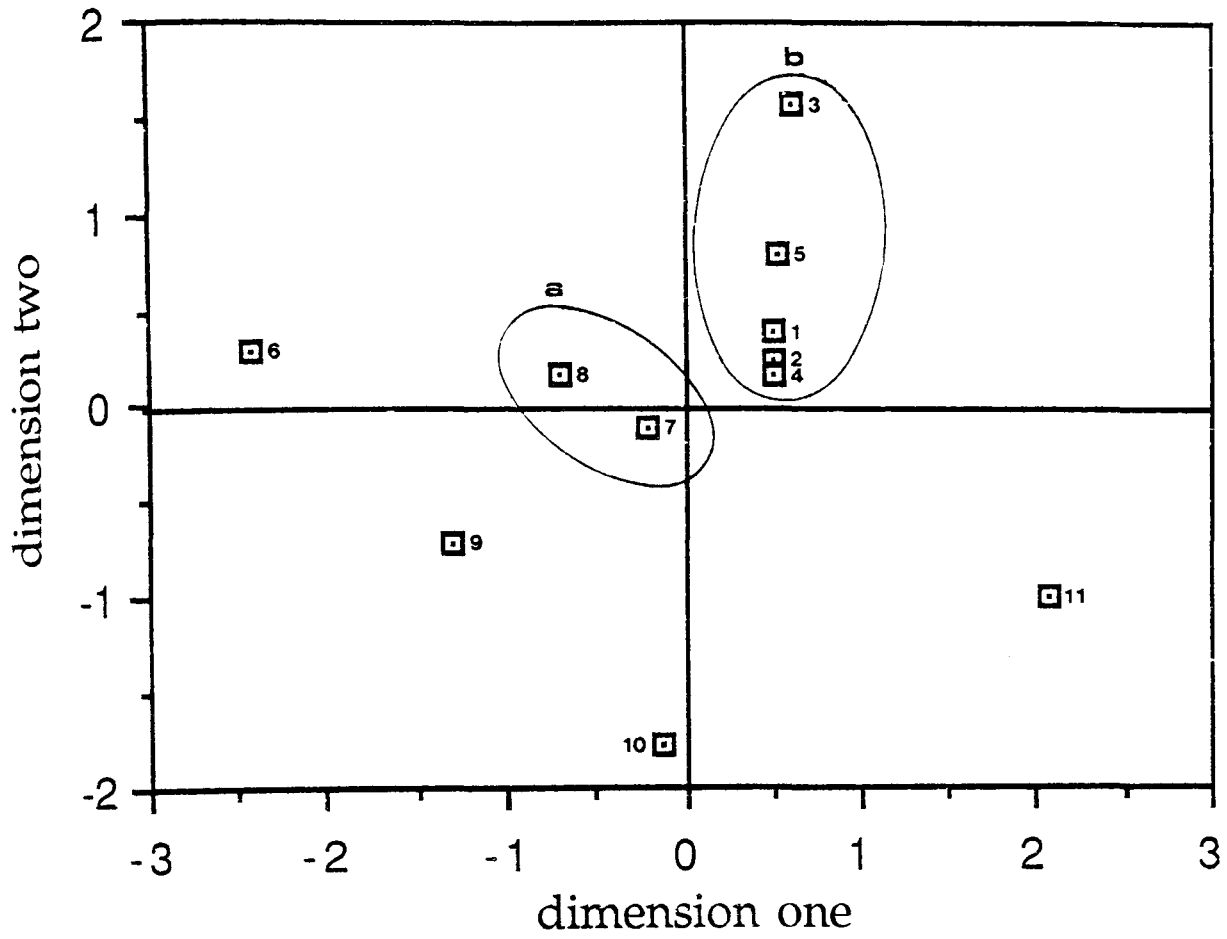


figure 2.5.4d

Nonmetric multidimensional scaling plot of genetic similarity coefficients (1-G/2) from the telmessia group of the genus Maniola.

Envelopes

a Northern Aegean Islands (Lesbos and Samos)

b Dodecanes excluding Karpathos

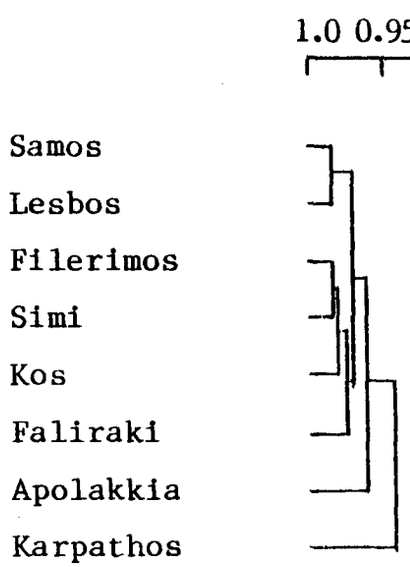
Populations

1 Faliraki
2 Filerimos
3 Apolakkia
4 Kos
5 Simi
6 Karpathos
7 Samos
8 Lesbos
9 Cyprus
10 Sardinia
11 Chios

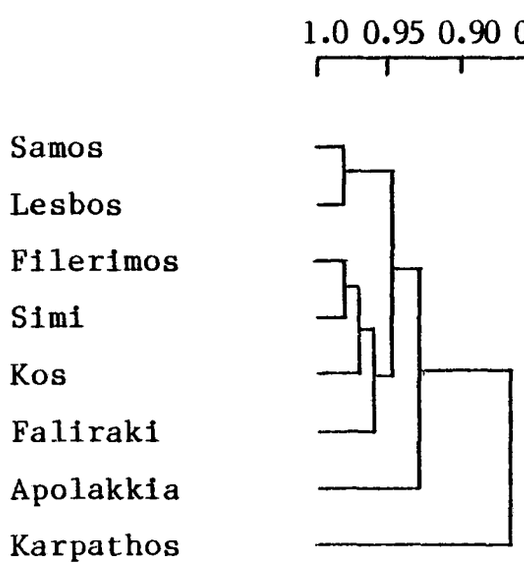
figures 2.5.4e - g

Single, complete and average linkage clustering (UPGMA) - Maniola telmessia populations

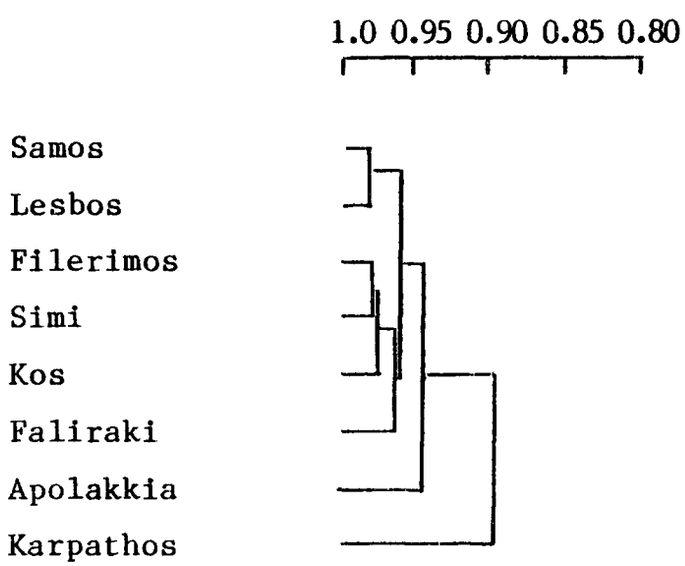
e) single



f) complete



g) average



The relationship of Maniola megala to the other species of the genus and of all species to each other was compared using Nei's coefficient of genetic identity ($\bar{I}$). The genetic similarity between jurtina and megala was very high ($\bar{I}$=0.946 ±0.014). At the loci which were diagnostic in the telmessia group, D(-)3GPDH, LAP-1 and LAP-2, jurtina and megala were very similar (tables 2.5.4c - see also table 2.5.4a). There was also a very high degree of similarity between species of the telmessia group, especially between telmessia and cypricola ($\bar{I}$=0.982). These affinities are discussed in relation to their evolutionary history in section 4.2).

The distinction between the jurtina (jurtina, megala and chia) and telmessia groups (telmessia, cypricola and nurag) can be seen clearly in figures 2.5.5a - c. The three linkage clustering analyses are unanimous in their representation of the Maniola relationships. These correspond well with the ordination (figure 2.5.5d).

2.5.5 Inter-specific relationships in the Maniolini

Throughout the analysis of Maniolini species, three populations of Hyponephele lycaon and three of H. lupina were treated separately and not merged as single taxa: they were highly divergent from each other and merited further study. The three lupina samples represented existing distinct subspecies or geographical isolates, lupina rhamnusia (one populations from Kos, in the Greek Dodecanese) and lupina mauritanica (from north Africa and Cyprus), so a degree of genetic isolation was expected in these forms. The lycaon samples were from three sites in the northern part of Italy, separated by a maximum distance of 300km (between Fiastra, Monti Sibillini and Planet, Val d'Aosta) - see figure 1.3.1a. Two of the samples were collected in localities less than 30km apart (Planet and Coyne, also in the Val d'Aosta). There was no reason to expect the degree of genetic distance which was detected between these lycaon populations.

The mean genetic similarity between the Fiastra, Planet and Coyne lycaon, $\bar{I}$=0.746, was only a little higher than that between other species of the genus and very much lower than that between Maniola populations and species (tables 2.5a and 2.5.5e). At seven loci (CK,

EST-C, GOT-1, IDH-2, ME-2, MPI and PGI-2), the most frequent alleles in the Fiastra and Planet populations were different. In the Coyne sample, at four loci (GOT-1, IDH-2, ME-2 and MPI), the common alleles were identical with that of the Planet lycaon, as expected from their geographical proximity. However, at three loci (IDH-1, PGM and PK), the common alleles in the Coyne lycaon were unique for the species (table 2.5.5a).

The relationship between the lupina populations was similarly distant. At three loci (IDH-1, MDH and MPI), lupina rhamnusia and the north African lupina mauritanica had different common alleles. At one locus, PGI-2, the common allele of lupina mauritanica from Cyprus corresponded with that of rhamnusia. At IDH-2, the most frequent allele in the Cypriot lupina mauritanica was different from that in the other two populations. At IDH-1, LAP-1 and MPI, the situation was unresolved (table 2.5.5b and c). Similar large interpopulation differences were not found in Hyponephele maroccana. Alternative common alleles in geographically close populations less than 3km apart were also found in Cercyonis pegala, but as samples were small and the situation still under investigation, the data are not presented. This level of genic differentiation was not detected within species in other genera, although the differences found between the Fountain de Vaucluse samples of Pyronia bathseba is a possible case (section 2.3.2).

The mobilities of the common alleles at the enzyme loci utilised in all species of Maniolini are given in table 2.5.5c. Table 2.5.5d gives genetic identities and distances for Maniolini.

There was complete unanimity in the configuration of single, complete and average linkage cluster analyses, and that of NMMS (figures 2.5.5a - d).

The product-moment coefficients computed from genetic / cophenetic similarities show that the best fit was obtained from UPGMA clustering and the NMMS three-dimensional solution. Stress for NMMS in two-dimensions was rather high (0.131, r^2=0.927), but much better in the three-dimensional solution (0.048, r^2=0.987).

table 2.5.5a

Allele frequencies at diagnostic loci in north Italian populations of Hyponephele lycaon from Fiastra, Planet and Coyne - standard errors in parenthesis below.

locus	allele	Fiastra	Planet	Coyne
CK	87	-	0.023	-
		-	(0.022)	-
	100	0.792	0.046	-
		(0.083)	(0.031)	-
	140	0.208	0.932	-
		(0.083)	(0.038)	-
EST-C	87	0.042	-	-
		(0.041)	-	-
	92	0.833	-	-
		(0.076)	-	-
	100	-	1.000	-
		-	(0)	-
	109	0.125	-	-
		(0.068)	-	-
GOT-1	86	0.292	-	-
		(0.093)	-	-
	93	0.625	0.184	-
		(0.099)	(0.063)	-
	100	-	0.105	0.333
		-	(0.050)	(0.096)
	114	-	0.579	0.625
		-	(0.080)	(0.099)
	121	-	-	0.042
		-	-	(0.041)
	140	-	0.150	-
		-	(0.050)	-
	150	-	0.026	-
		-	(0.026)	-
IDH-1	86	0.909	0.932	-
		(0.061)	(0.038)	-
	100	-	-	0.333
		-	-	(0.096)
	107	0.091	0.068	-
		(0.061)	(0.038)	-
	114	-	-	0.625
		-	-	(0.099)
	121	-	-	0.042
		-	-	(0.041)
IDH-2	88	1.000	0.046	-
		(0)	(0.031)	-
	100	-	-	0.111
		-	-	(0.074)
	112	-	0.955	0.833
		-	(0.031)	(0.088)
	118	-	-	0.056
		-	-	(0.054)

table 2.5.5a (continued)

MPI	84	0.083	0.150	-
		(0.056)	(0.080)	-
	86	-	0.600	0.750
		-	(0.110)	(0.108)
	96	-	0.100	0.188
		-	(0.067)	(0.098)
	100	0.083	0.150	0.063
		(0.056)	(0.080)	(0.061)
	105	0.625	-	-
		(0.208)	-	-
	109	0.208	-	-
		(0.083)	-	-
PGI-2	64	-	0.068	0.056
		-	(0.038)	(0.054)
	100	0.042	-	-
		(0.041)	-	-
	119	-	0.068	0.111
		-	(0.038)	(0.074)
	141	0.042	0.023	-
		(0.041)	(0.023)	-
	165	-	-	0.056
		-	-	(0.054)
	178	-	0.114	-
		-	(0.048)	-
	186	0.500	-	-
		(0.102)	-	-
	219	0.083	0.727	0.722
		(0.056)	(0.067)	(0.106)
	240	0.333	-	-
		(0.096)	-	-
	333	-	-	0.056
		-	-	(0.054)
PGM	60	-	0.024	-
		-	(0.024)	-
	78	0.083	0.119	-
		(0.056)	(0.050)	-
	88	0.583	0.476	0.167
		(0.101)	(0.077)	(0.088)
	100	0.208	0.333	0.778
		(0.083)	(0.073)	(0.098)
	113	0.125	0.024	0.056
		(0.068)	(0.024)	(0.056)
	121	-	0.024	-
		-	(0.024)	-
PK	139	0.208	0.250	1.000
		(0.083)	(0.065)	(0)
	170	0.042	-	-
		(0.041)	-	-
	183	0.750	0.750	-
		(0.088)	(0.065)	-

table 2.5.5b

Allele frequencies at diagnostic loci in populations of Hyponephele lupina rhamnusia (Kos, Greece) and H. lupina mauritanica (Atlas Mountains, Morocco and Cyprus), standard errors in parenthesis below.

locus	allele	Greece	Morocco	Cyprus
EST-C	80	1.000	-	-
		(0)	-	
	115	-	1.000	-
		-	(0)	-
IDH-1	64	1.000	-	-
		(0)	-	-
	100	-	-	0.200
		-	-	(0.126)
	114	-	-	0.800
		-	-	(0.126)
IDH-2	88	1.000	1.000	-
		(0)	(0)	-
	100	-	-	1.000
		-	-	(0)
LAP-1	58	0.125	-	-
		(0.117)	-	-
	69	0.250	-	-
		(0.153)	-	-
	75	0.625	-	-
		(0.171)	-	-
	100	-	-	1.000
		-	-	(0)
MDH	53	1.000	-	-
		(0)	-	-
	100	-	1.000	-
		-	(0)	-
MPI	86	1.000	-	-
		(0)	-	-
	100	-	-	0.625
		-	-	(0.171)
	105	-	1.000	0.375
		-	(0)	(0.171)
PGI-2	100	-	1.000	0.100
		-	(0)	(0.095)
	165	-	-	0.100
		-	-	(0.095)
	178	0.250	-	0.100
		(0.153)	-	(0.095)
	219	0.625	-	0.700
		(0.171)	-	(0.145)
	333	0.125	-	-
		(0.117)	-	-

table 2.5.5c

Mobilities of common alleles at enzyme loci in Maniolini.

	ADA	AK-1	AK-2	ALD	CK	DIA -1	DIA -2	D(-) 3	EST -1	EST -A	EST -C	GK	GOT -1	IDH -1	IDH -2
Maniola															
jurtina	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
megala	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
chia	100	100	100	100	100	100	100	112	100	100	100	100	100	100	100
telmessia	100	100	100	100	100	100	100	93	100	100	100	100	100	100	100
cypricola	100	100	100	100	100	100	100	93	100	100	100	100	100	100	100
murag	100	100	100	100	100	100	100	93	100	100	100	100	100	100	100
Pyronia															
tithonus	100	100	100	100	100	141	113	81	100	100	100	117	93	42	100
bathseba	100	100	100	100	138	141	113	81	100	-	100	117	100	89	100
cecilia	163	100	100	100	138	141	113	90	100	-	100	100	100	42	100
Aphantopus															
hyperantus	110	100	100	100	140	141	100	66	100	-	100	117	166	100	100
Hyponephele															
lycaon E	-	100	100	100	100	100	100	122	100	100	92	144	86	86	88
lycaon W	100	100	100	100	140	100	100	122	100	-	100	144	114	86	112
l.rhamn[1]	100	100	100	100	100	100	100	100	100	-	-	114	93	64	88
l.maur[2]	100	100	100	100	100	-	-	-	100	100	115	152	114[a]	-	88
maroccana	-	100	100	100	140	-	-	-	100	100	100	144	114	107	88
Cercyonis															
pegala	-	100	100	100	140	100	100	100	100	100	100	162	84	86	94

table 2.5.5c (continued)

	LAP -1	LAP -1	MDH	(MES)ME-1	ME-2	MK	MPI	PGI -2	PGM	PK	SOD -1	SOD -2	αGPDH
Maniola													
jurtina	100	100	100	100	100	100	100	100	100	100	100	100	100
megala	100	100	100	100	100	100	100	100	100	100	100	100	100
chia	100	100	100	100	100	100	100	100	100	100	100	100	100
telmessia	69	90	100	100	100	100	100	100	100	100	100	100	100
cypricola	69	90	100	100	100	100	100	100	100	100	100	100	100
nurag	69	90	100	100	100	100	100	100	100	100	100	100	100
Pyronia													
tithonus	69	90	100	64	100	110	145	76	100	113	100	112	300	100
bathseba	127	85	127	-	100	110	134	128	100	121	139	112	300	100
cecilia	100	61	127	-	100	100	118	86	178	113	100	84	300	100
Aphantopus													
hyperantus	69	90	116	-	100	110	145	122	178	113	139	100	300	100
Hyponephele													
lycaon E	100	90	70	64	100	93	165	105	186	88	183	107	400	89
lycaon W	100	90	70	64	100	100	165	86	219	88	183	107	400	89
l.rhamn[1]	75	90	53	-	-	100	145	86	219	88	100	107	400	89
l.maur[2]	-	90[b]	100	-	-	100	-	105	100	88[c]	183[d]	107	400	89
maroccana	-	90	100	-	-	105	-	86	186	128	183	-	400	89
Cercyonis													
pegala	100	61	137	64	100	93	184	105	240	121	183*	107	400	89

[1] lupina rhamnusia (Greece) [2] lupina mauritanica (Morocco)

[a] with $GOT\text{-}1_{93}$ [b] with $LAP\text{-}2_{85}$ [c] with PGM_{100} [d] with PK_{139}

table 2.5.5d

Genetic identity, I (above diagonal) and distance (below diagonal) for species of Maniolini.

	1	2	3	4	5
1 M.jurtina	-	.910	.901	.913	.994
2 M.telmessia	.094	-	.982	.978	.914
3 M.cypricola	.104	.018	-	.976	.901
4 M.murag	.091	.022	.024	-	.918
5 M.megala	.006	.090	.104	.086	-
6 M.chia	.037	.019	.111	.091	.038
7 P.tithonus	.695	.589	.582	.592	.699
8 P.bathseba	.878	.879	.881	.898	.871
9 P.cecilia	.689	.770	.724	.778	.712
10 A.hyperantus	.858	.709	.699	.716	.835
11 H.lycaon E	.860	.863	.905	.841	.842
12 H.lycaon W	.769	.789	.775	.715	.762
13 H.lupina rh	.936	.935	.911	.843	.913
14 H.lupina mau	.683	.574	.626	.563	.667
15 H.maroccana	.908	.799	.813	.740	.895
16 C.pegala	.817	.965	1.002	.971	.818

	6	7	8	9	10	11	12	13	14	15	16
1 M.jurtina	.964	.499	.416	.502	.424	.423	.463	.392	.505	.403	.442
2 M.telmessia	.913	.555	.415	.463	.492	.422	.454	.392	.563	.450	.381
3 M.cypricola	.895	.559	.415	.485	.497	.404	.461	.402	.535	.444	.367
4 M.murag	.913	.553	.407	.459	.489	.431	.489	.430	.570	.477	.379
5 M.megala	.963	.497	.418	.491	.434	.431	.467	.401	.513	.409	.442
6 M.chia	-	.491	.408	.491	.419	.439	.467	.361	.526	.406	.410
7 P.tithonus	.711	-	.646	.559	.573	.338	.349	.360	.474	.434	.350
8 P.bathseba	.897	.437	-	.565	.505	.229	.277	.238	.348	.330	.282
9 P.cecilia	.712	.582	.572	-	.438	.270	.357	.244	.289	.330	.364
10 A.hyperantus	.869	.557	.683	.825	-	.305	.347	.331	.292	.377	.345
11 H.lycaon E	.823	1.083	1.476	1.308	1.187	-	.746	.598	.727	.656	.614
12 H.lycaon W	.762	1.054	1.285	1.029	1.057	.293	-	.608	.571	.631	.576
13 H.lupina rh	1.020	1.021	1.435	1.411	1.106	.514	.497	-	.672	.543	.400
14 H.lupina mau	.643	.747	1.054	1.242	1.230	.318	.561	.398	-	.637	.488
15 H.maroccana	.901	.835	1.109	1.109	.976	.422	.460	.611	.452	-	.535
16 C.pegala	.891	1.051	1.266	1.010	1.065	.487	.552	.916	.717	.626	-

figure 2.5.5a

Single linkage clustering - Maniolini

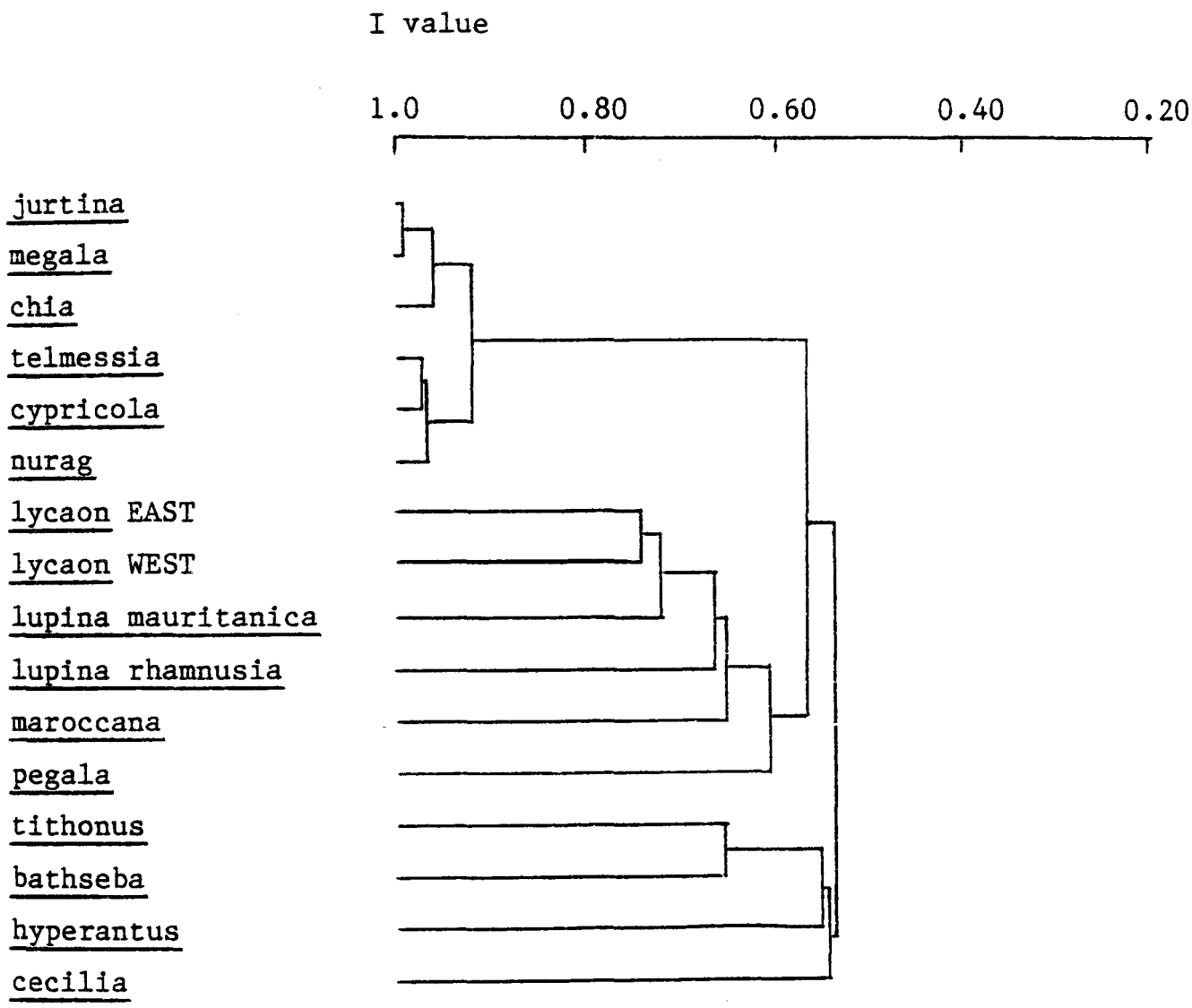


figure 2.5.5b

Complete linkage clustering - Maniolini

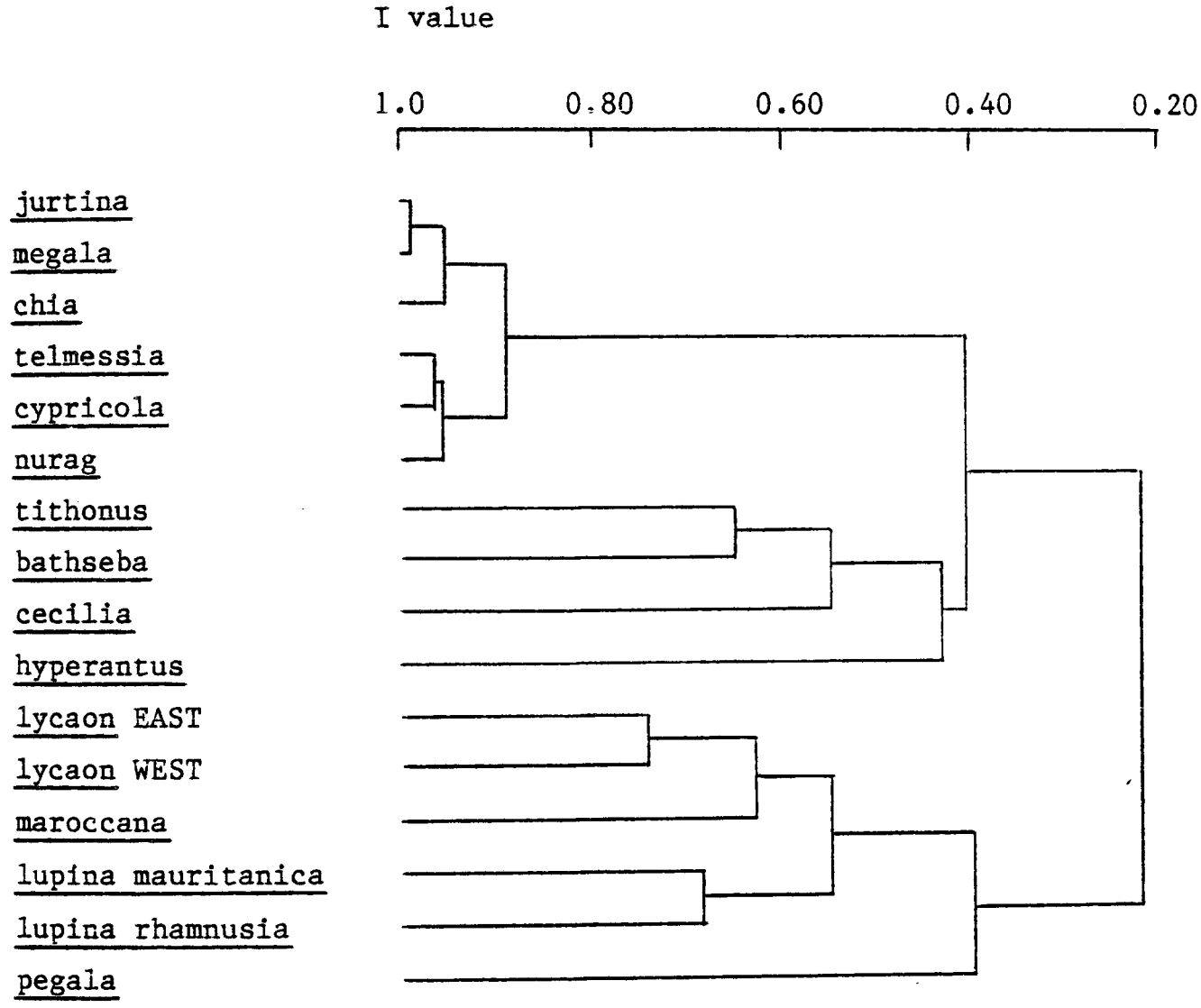
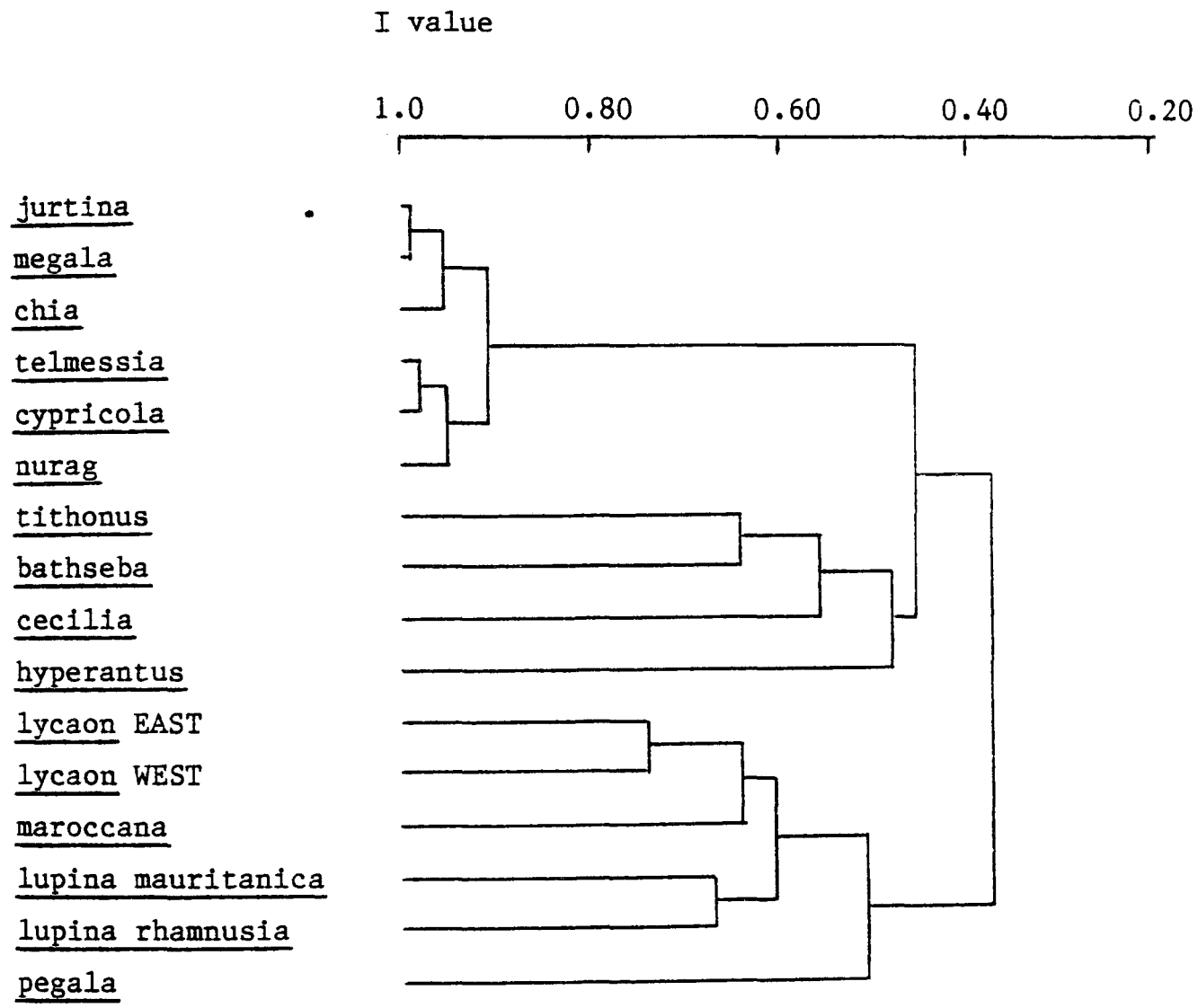


figure 2.5.5c

Average linkage clustering (UPGMA) - Maniolini



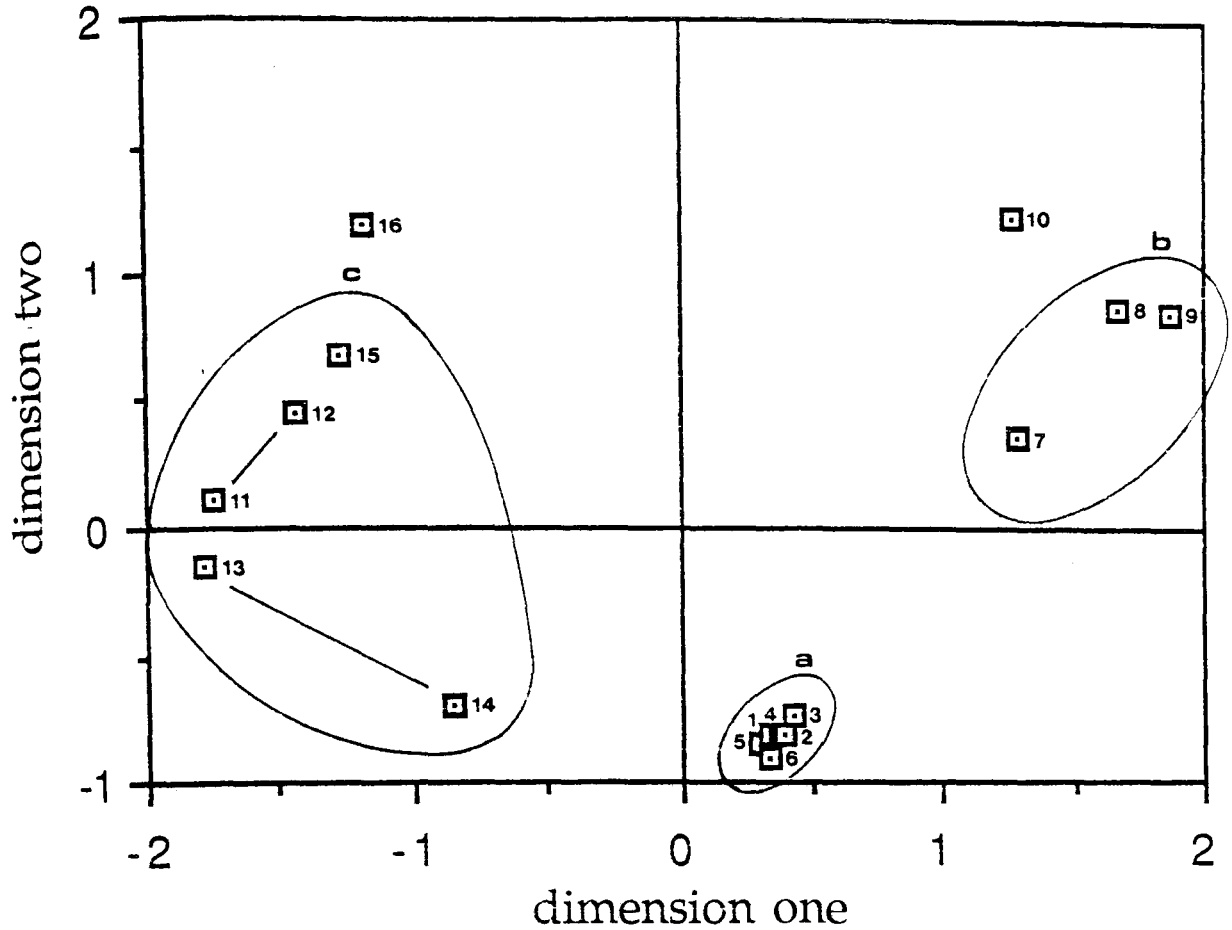


figure 2.5.5d

Nonmetric multidimensional scaling plot of genetic identity coefficients (I) from species of Maniolini and major subgroups of Hyponephele.

Envelopes

a Maniola

b Pyronia

c Hyponephele

Populations

1 Maniola jurtina
2 Maniola telmessia
3 Maniola cypricola
4 Maniola nurag
5 Maniola megala
6 Maniola chia
7 Pyronia tithonus
8 Pyronia bathseba
9 Pyronia cecilia
10 Aphantopus hyperantus
11 Hyponephele lycaon (EAST)
12 Hyponephele lycaon (WEST)
13 Hyponephele lupina rhamnusia
14 Hyponephele lupina mauritanica
15 Hyponephele maroccana
16 Cercyonis pegala

single linkage	r_p=0.888 df119
complete linkage	r_p=0.887 df119
UPGMA	r_p=0.900 df119
NMMS two-dimensional	r_p=0.866 df119
NMMS three-dimensional	r_p=0.910 df119

On electrophoretic evidence, relationships of the Manioline species can be summarised.

1. The Maniolini appear to comprise three distinct groups, a) species of the genus Maniola, b) the species of the genus Pyronia together with Aphantopus hyperantus and c) the species of the genus Hyponephele together with Cercyonis pegala.

2. As has already been suggested, there are two distinct, but close groups of Maniola species, a) jurtina, megala and chia and b) telmessia, cypricola and nurag.

3. The genus Aphantopus relates clearly but not closely with Pyronia.

4. Cercyonis relates clearly but not closely with Hyponephele.

5. The genera Pyronia and Aphantopus relate more closely with Maniola than with the Hyponephele group.

A further observation can be made on the infrastructure of the species relationships. The difference between species of the Maniola group is very much less than between species of Pyronia and Aphantopus. The mean differences between species and genera are given in table 2.5.5e.

Many authors have discussed the observation that intraspecific levels of genetic similarity are very much higher in Lepidoptera that those for most other organisms (see Ferguson 1980). In butterflies, the mean genetic identity figure ($\bar{I}$) given by Brittnacher et al. (1978) for species of Speyeria was 0.833, similar to those of Geiger (1982), Geiger and Scholl (1984), 0.800, and Courtney (1980), 0.819, working independently on the Pieridae. Turner's $\bar{I}$ value for Heliconius,

table 2.5.5e

Minimum, maximum and mean genetic similarities ($\bar{I}$) between species of Maniola, Pyronia and Hyponephele and between Manioline genera.

	minimum	maximum	mean	SE of mean
Maniola species	0.895	0.994	0.936	0.045
Pyronia species	0.559	0.646	0.590	0.161
Hyponephele species	0.543	0.727	0.627	0.170
Maniolini genera	0.381	0.505	0.421	0.016

0.688, (Turner 1979) was rather lower. It can be misleading, however, to interpret these figures as being representative: the uneven treatment of infra-familial taxonomic groups, especially in the Pieridae, does not facilitate statistical comparisons based on subjective assessments of systematic categories. Within the distinct electrophoretically determined groups in Maniolini, there are significant differences in the level of genetic distance between species within each genus (table 2.5.5d). The very close relationships between Maniola species ($\bar{I}$=0.936) corresponds with that seen in some Pieris subgroups. Conversely, the mean similarities between species in Pyronia ($\bar{I}$=0.590) and Hyponephele ($\bar{I}$=0.627) are the lowest so far found between congeneric butterfly species. The phylogenetic and systematic implications of the analysis of Manioline species are discussed in sections 4.2 and 4.3.

3 MORPHOLOGICAL BOUNDARIES

3.1 External adult morphology

Morphological studies were directed towards major trends in geographical variation in Maniola jurtina, relationships within the genus Maniola and the affinities between species in Maniolini. This also included a limited study of photoperiodism and temperature effects on the butterflies which is detailed here.

From the time of the first observations of butterfly variability, and many years before Linnaeus, the differentiation of Lepidoptera was, for a considerable period, based exclusively on superficial external features. In butterflies, these invariably were wing markings and colouration. In 1839, Rambur made the first serious study of genitalia for taxonomic purposes when he used these organs as critical specific characters in a revision of the Hesperiid genus Pyrgus. The relative importance of external wing patterns, genitalia and other morphological characters has been argued on the basis of the entrenched attitudes of numerical taxonomists and cladists on the one hand (see Wiley 1981), and those adhering to the biological species concept (Mayr 1969a, b) on the other. Arguments of systematic prerogatives are seen as irrelevant to the present study.

Manioline taxa and their classification have been based exclusively on external characters, including genitalia. The generic separation of the old genus Epinephele into Maniola, Pyronia and Hyponephele, and the inclusion of Cercyonis in the tribe, was based on the form of the male and female genital armatures (De Lesse 1952a). All species in Maniola were initially described on the basis of wing markings, although confirmation of some was proposed on the evidence of the male genitalia - telmessia (Le Cerf 1912, 1913) and megala (Bernardi and Lagnel 1966). The descriptions of a number of recently discovered species of Asiatic Hyponephele, kocaki (Eckweiler 1978), lycaonoides (Weiss 1978) and leontyi (Stshetkin 1979), have utilised both external markings and genitalia morphology.

In jurtina, geographical variation in wing markings and genitalia form is, apparently, anomalous. Large, bright phenotypes with extensive fulvous areas in the females (and to a lesser extent in the males) are most frequent in south-western Europe, north-west Africa and the Canary Islands (jurtina, hispulla, hyperhispulla and miscens), whereas smaller dark forms predominate in eastern, central and northern Europe (occidentalis, janira, corfiothispulla, insularis and possibly iernes, cassiteridum and splendida). Some authors see a similarity between the bright south-western forms and those in parts of south-eastern Europe, but melanism is greatly reduced in these eastern populations and the phenotypic resemblance is superficial (Thomson 1969, 1973a). The 'eastern' and 'western' genitalia forms (Thomson 1973a, 1975, 1976) comprise major subspecies groups. It is not surprising that numerous views have been expressed on the significance of these geographical forms, the most extreme being that jurtina is not one, but two distinct species (De Lattin 1967).

Clarification of the identity of the geographical forms of jurtina was presented by Thomson (1973b) in an extensive analysis of male genitalia variation. Some of the unpublished data is presented here for the first time.

The relationships of telmessia and cypricola to each other and to jurtina have been disputed for some time (see section 1.1). The most commonly accepted classifications have been based on wing markings and genitalia form. It was considered important to identify their affinities, utilising other characters. It was also considered of value to examine the relationships of nurag to the group, as the genital armature of the male and female suggested an apparently close affinity with telmessia, even although it is geographically isolated from that species by 1000km (Thomson 1976).

De Lesse (1952a) demonstrated that the Nearctic genus Cercyonis should be part of the group which included Maniola, Pyronia and Hyponephele. Without explanation, Higgins (1975) located Aphantopus between Hyponephele and Pyronia. This affinity had not been suggested before. Miller (1968) placed Aphantopus in the Coenonymphini. As electrophoretic analysis confirmed the appropriateness of their posi-

tion within Maniolini, these genera were included in the present morphological studies.

3.1.1 Photoperiodism and temperature effects

A major disadvantage of morphological characters in the study of evolution is their susceptibility to current environmental conditions. The effects of temperature on wing markings of butterflies have been known for some time. Dorfmuster (see Hoegh-Guldberg and Hansen 1977) demonstrated that divergence of butterfly form developed when pupae were kept at increased or decreased temperatures. Merrifield (1898) and Standfuss (1896) determined the critical point for thermal modification and found that it was, in certain Lepidopterous species, at least 12 hours after pupation. Reinhardt (1969) resolved the problem of Araschnia levana's seasonal dimorphism. Hoegh-Guldberg and Hansen (1977) review important papers on thermal effects during the pupal stage. Merrifield showed that melanin production was inhibited in most of the Pieridae and accelerated in the Satyridae in high temperature conditions (Merrifield 1894 - see also his 1890 and 1892 papers).

The marked reduction in fulvous colouration in Maniola jurtina, through increased melanism, and the increase in apical and other eye-spot sizes as a result of increased thermal exposure was demonstrated by Thomson (1973a).

None of these studies considered the possible effects of differential photoperiod regimes. In multivoltine butterflies, it has been shown that photoperiod, as well as temperature, can greatly affect the external morphology of insects (see Beck 1980 for a review). Possibly the best known examples of this are the levana (spring) and prorsa (summer) forms of Araschnia levana which are induced by a facultative diapause controlled by the photo- and thermoperiod regimes. Shapiro (1975, 1977) described photoperiod induction of Pieris napi forms and the role of temperature and photoperiod in coastal Californian populations of the species.

All *Maniola* are univoltine. However, they are very unusual in exhibiting both hibernal and aestival diapause. In *jurtina* aestivation occurs only in southern Europe. As diapause induced phenotype differentiation is not uncommon, the situation with *Maniola jurtina* merited investigation. Terms used for the photoperiod regimes employed are those proposed as standard terminology by Beck (1980). Thus DD means 24D: 0L or continuous darkness and LL is equivalent to 0D:24L or continuous light.

Some 550 ova were obtained from vagile females of *jurtina*, originating from three populations, two females each from Head of Holland, Orkney , Portrush, Co. Antrim and Kinvara, Co. Clare. The choice of parents was entirely arbitrary, except that they were all non-diapausing. A total of 205 ova were laid by the Orkney and 345 by the Irish females. The maximum number of ova laid in one day by one Orkney female was 34, while one Irish female laid 64 in a 24 hour period. The laying period was relatively short, possibly due to adverse weather conditions, and all parents died within seven days. Female *jurtina* can lay continuously for up to 10 weeks in captivity (Thomson unpublished). The ova were divided evenly among eight experimental environments which exposed the insects to cool ($12^{o}C \pm 4^{o}$), warm ($30^{o}C \pm 4^{o}$), LL and DD conditions, together with three controls. The DD photoperiod was maintained using individually darkened containers. Clear rearing tubes, positioned 0.5m from three 40W 'Grolux' fluorescent lamps, were used for the LL regime. Larvae being reared in identical temperatures were kept adjacent to each other, irrespective of the photoperiod. Other light sources could have been used as, within certain limits, the spectral qualities of the sources appears to make little difference (Lees pers. comm.). In addition, three controls were established 1. stock maintained in warm conditions in ambient light and 2. (two batches) stock maintained (outdoors) in ambient temperatures and light.

Larvae were fed on fresh cut cultivated fine grasses (species not determined). Experiments with artificial diets were unsuccessful. It was hoped that larval mortality would be reduced or eliminated by the use of wheatgerm based sterile artificial diets, incorporating bactericides and fungicides (Morton 1979), but *jurtina* larvae refused to eat the compounds.

table 3.1.1a

Photoperiod and temperature effects on the development of Maniola jurtina maintained in experimental conditions.

			ova	larvae			imagines (F_1)	
stock (P_1)	temperature ^oC	photoperiod regime	n	development rate*	high mortality stage - ova or instar	diapause	n	weeks from ova - mean
Ireland	$12^o \pm 4^o$	DD	50	slow	3 (100%)	-	0	-
Ireland	$12^o \pm 4^o$	LL	50	normal	3 - 4	no	2	42
Orkney	$12^o \pm 4^o$	DD	50	slow	3 (100%)	-	0	-
Orkney	$12^o \pm 4^o$	LL	50	normal	3	no	3	40
Ireland	$30^o \pm 4^o$	DD	50	-	ova (100%)	-	0	-
Ireland	$30^o \pm 4^o$	LL	50	fast	3 - 4	no	4	13
Orkney	$30^o \pm 4^o$	DD	50	-	ova (100%)	-	0	-
Orkney	$30^o \pm 4^o$	LL	50	?	2 (100%)	no	0	-
CONTROLS								
Ireland	$30^o \pm 4^o$	ambient	50	fast	3 - 4	yes	2	35
Orkney	ambient	ambient	50	normal	3 - 4	yes	3	40
Ireland	ambient	ambient	50	-	ova (100%)	-	-	-

* rate assessed in relation to control stock reared at ambient temperature and photoperiod.

The outcome of this work is summarised in table 3.1.1a. Certain aspects are difficult to interpret. It is not possible, at the present time, to suggest a reason for the complete failure of the Irish control material, as ova from the same P_1 stock proved fertile in experimental conditions. Nor is it easy to explain the total mortality, by the end of the second larval instar, of the Orkney LL material kept in warm conditions.

It can be seen from the table that there was very strong evidence that continuous light obviates diapause, irrespective of thermal conditions. Possibly, there is a low temperature threshold at which point photoperiod is ineffective, but this cannot be deduced from this experimental situation. Interestingly, Rawlins (1980) found that decreasing solar radiation alone, not temperature, was responsible for triggering the frenetic (pre-roosting) search flight of Papilio polyxenes asterius. It is also likely that continuous darkness is not a requirement for diapause induction and a short day regime (>12D:<12L) could be effective. The sensitive time is possibly late ova to early first instar larva, although nothing in the present study shows that the critical time is anywhere between ova and third instar.

Continuous darkness during the ova and early larva stage produced 100% mortality. Hatching was totally eliminated in cool conditions and mortality was 100% by the end of the third instar at high temperatures. Rapid growth was not induced by increased light in the absence of increased temperature.

Brakefield (1984) suggests that the lack of synchrony in adult emergence is due to the long laying period and a lack of 'true' diapause. The present study shows that diapause (sensu Beck 1980) always occurs, unless artificially modified. The proposition of Slansky (1974), that the low moisture and high fibre content of grasses leads to slow development in the majority of grass and sedge feeders (all Satyridae and most Hesperiidae), is relevant here. He suggests that the hibernation of many of the satyrids and hesperiids as early instar larvae may be an adaptation for the avoidance of nutritionally poor, mature grass plants and the maximum utilisation of the spring flush of succulent growth when moisture and nitrogen levels are high

and fibre content low (Watson 1951). It is most likely that the variable development rate is due to a varying microclimate (especially temperature) and the non-discriminatory utilisation of host plants of different nutritional values.

Unfortunately, the number of F_1 imagines was inadequate for statistical comparison, with the normal phenotypes, of jurtina reared under the various experimental regimes. Only a few general observations can be made. Imagines reared in a LL photoperiod regime and at $12^{o}C$ with no diapause revealed no obvious differences from control phenotypes or source populations samples, in wing markings, colouration or genitalia form. The four adults (all female) reared in LL conditions at $30^{o}C$ displayed increased melanism, similar to that described by Thomson (1973a).

Aestival behaviour in jurtina, cypricola and telmessia created problems in obtaining ova from females collected in May and June, as it was extremely difficult to keep the butterflies alive throughout the summer period. Six females of Maniola cypricola were placed in various photoperiod conditions, two were exposed to continuous light (LL), and two to a short day reducing light regimes (16D: 8L reducing light by 10 minutes in each 24 hour period). Both pairs were kept at $30^{o}C \pm 4^{0}$. Two females were positioned in a sheltered spot outdoors in ambient light and temperature, although it was obvious that this did not replicate the conditions which they would have experienced in Cyprus.

The females in reducing light began laying after 28 days, while those in continuous light produced their first ova after 58 days. Control females did not survive. It was clear from the behaviour the two females in LL, that they were in diapause: they were sluggish and moved only when disturbed, feeding sporadically. The butterflies in reducing light were active much of the time, feeding regularly. It was clear from this that a short day reducing light environment obviated diapause in cypricola. Later similar experiments with Moroccan jurtina, telmessia and megala confirmed the effect of photoperiod in modifying normal behaviour patterns in the butterflies.

It is suggested that diapause, both hibernal in the larvae and aestival in the imagines of Maniola is facultative and not obligatory.

3.1.2 Wing markings

Schwanwitsch (1924) constructed what he called a 'ground plan of wing pattern' in butterflies, with special reference to the Nymphalidae. Later he constructed ancestral plans of all Palaearctic satyrid genera, including the Epinephele, now separated into Maniola, Pyronia and Hyponephele (Schwanwitsch 1928a, 1928b, 1929a, 1929b, 1931, 1935, 1940, 1948). Although much of Schwanwitsch's work is inherently evolutionary, he did not claim for it any genetic basis. Indeed, he made it clear that the principal purpose of his work on the Palaearctic Satyridae was

> 'to find out if the prototype of the wing pattern constructed [by him] in 1924 is a valid scheme to derive from it all the patterns of large systematic groups.'

In achieving this aim he was undoubtedly successful. However, in his concluding paper Schwanwitsch (1948) demonstrated that the pattern of evolution recorded in the patterns of satyrid genera was governed by two well known principles, divergence and parallelism. The value of Schwanwitsch's work is difficult to assess. He has been quoted by numerous authors who have admired his single-minded enthusiasm for his work, but the results of his highly analytical investigations have not found their way into the contemporary biological debate. As is the case in the present study, the value of his work is in the usefulness of his wing topography nomenclature.

The variable nature of the wing markings of Maniola jurtina, both discrete and continuous, has received close attention at infraspecific and infrasubspecific levels (Lempke 1935, Thomson 1969, 1970a, b, 1971b 1973b, 1975). Geographical variation in wing markings is extensive in all species and in Maniola in particular. That in Maniola jurtina has been reviewed in full by Thomson (1969). A large number of races and subspecies has been described on the basis of wing markings alone. Some 30 infra-specific and nearly 90 infrasub-

specific names have been applied to variants of the butterfly. The other species of Maniolini have been treated with only a little less restraint by indiscriminate authors who have described minor variants as forms and slight geographical phenotypic variation as subspecific.

Data from previous work on jurtina variation by Thomson (1973b, 1975) was substantially updated through an analysis of material from populations utilised in electrophoretic work. Four easily recognised characters were used, two in each sex, to develop an overall picture of variation in the species (figure 3.1.2a).

male

1. Presence or absence of a medial line on the underside forewing. This is a reliable indicator of the average degree of melanism in the population.

2. Spotting average on the underside hindwing.

female

3. Fulvous area present on the upperside hindwing.

4. Apical eyespot bipupilled.

The maps (figures 3.1.2b, c, d, e) show that there is little correlation between the geographical distribution of these characters, although a few underlying trends can be noted.

1. All British populations have a medial line on the underside forewing in the male (character 1) at a frequency greater than 66%. This corresponds with the characteristic melanisation of the 'Atlantic' subspecies (Graves 1930).

2. Male underside hindwing spotting averages decreased northwards through Europe.

3. Females with extensive fulvous areas were much more frequent in southern and western than in northern Europe.

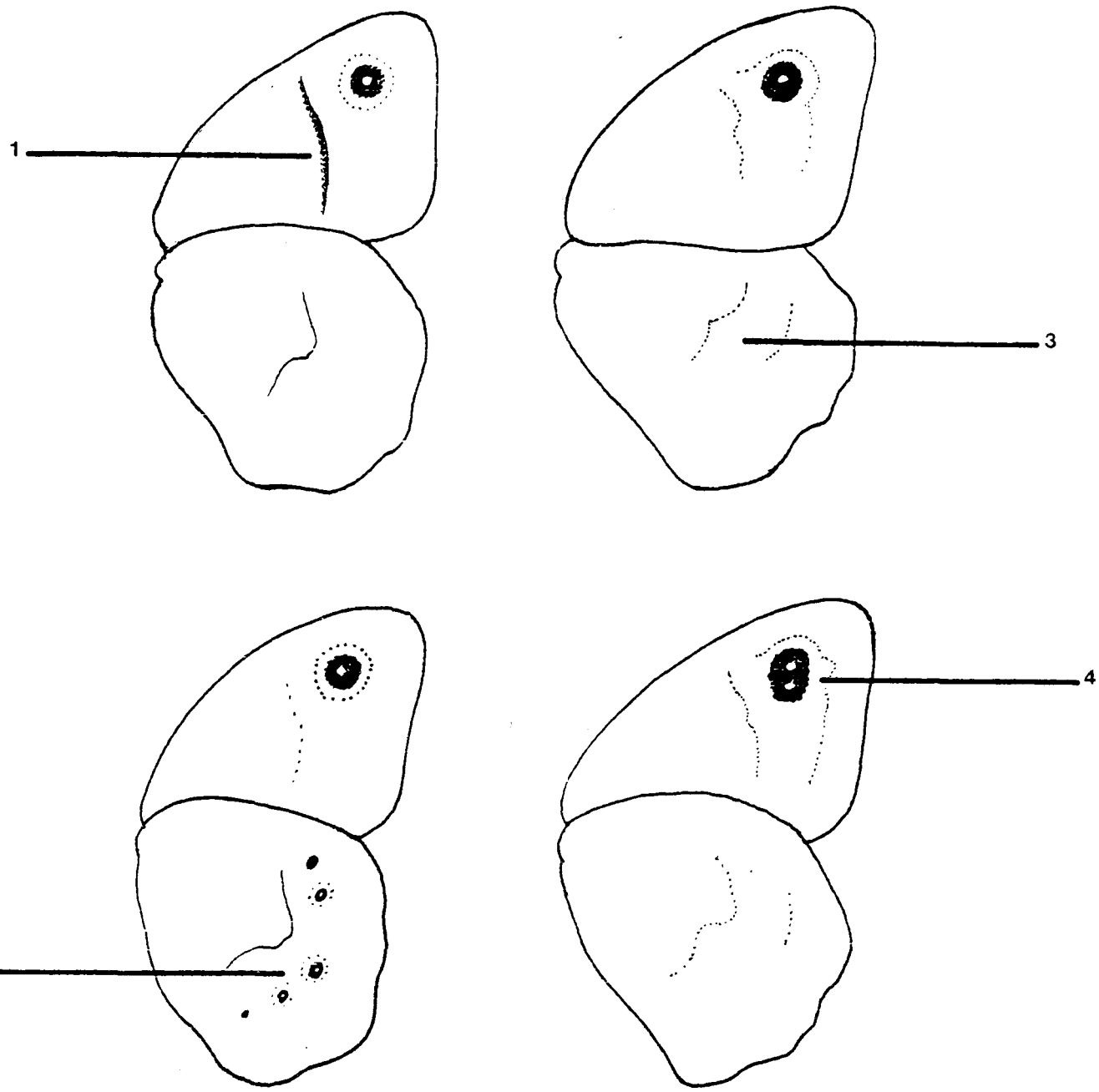
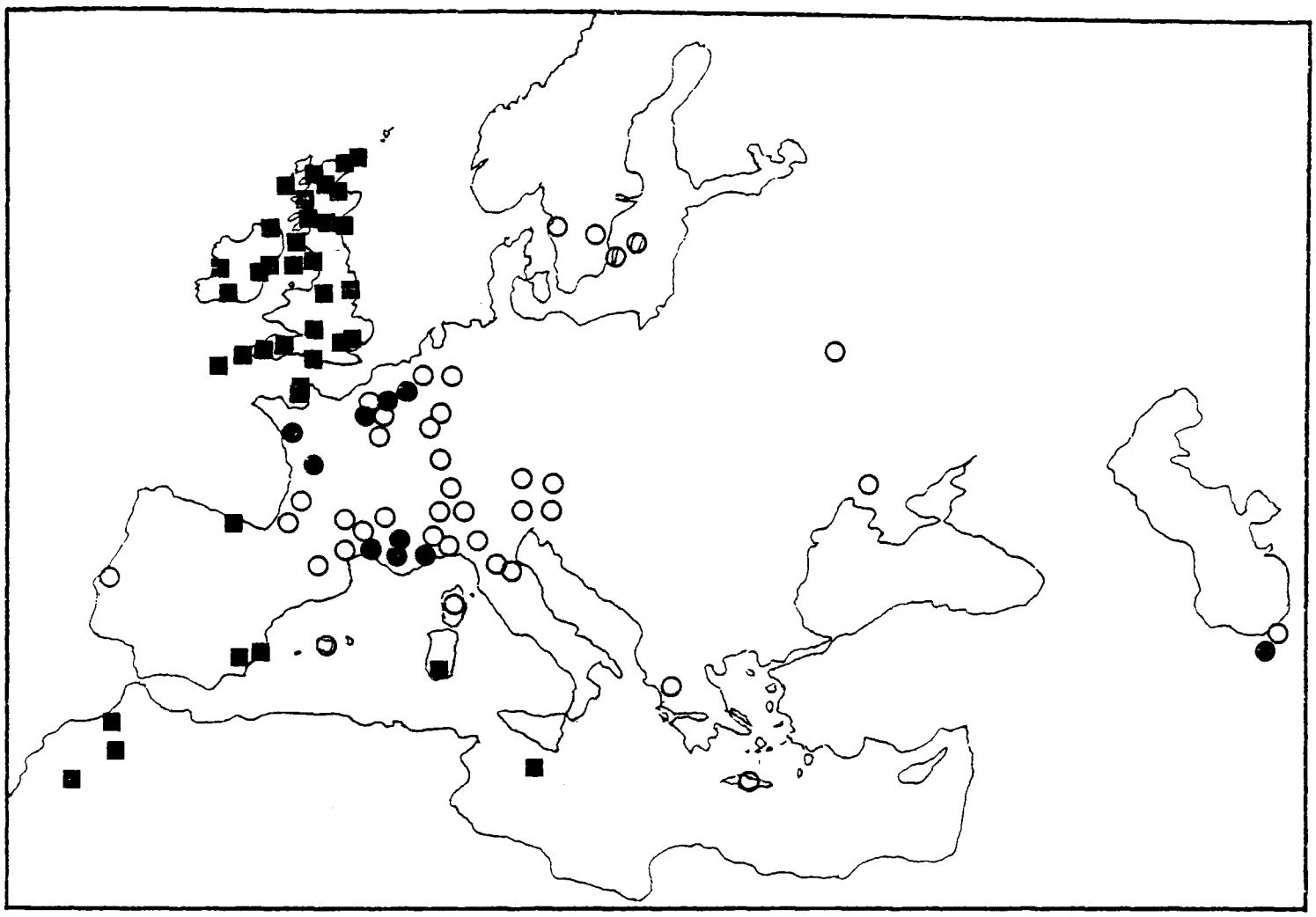


figure 3.1.2a

Maniola jurtina - wing marking characters

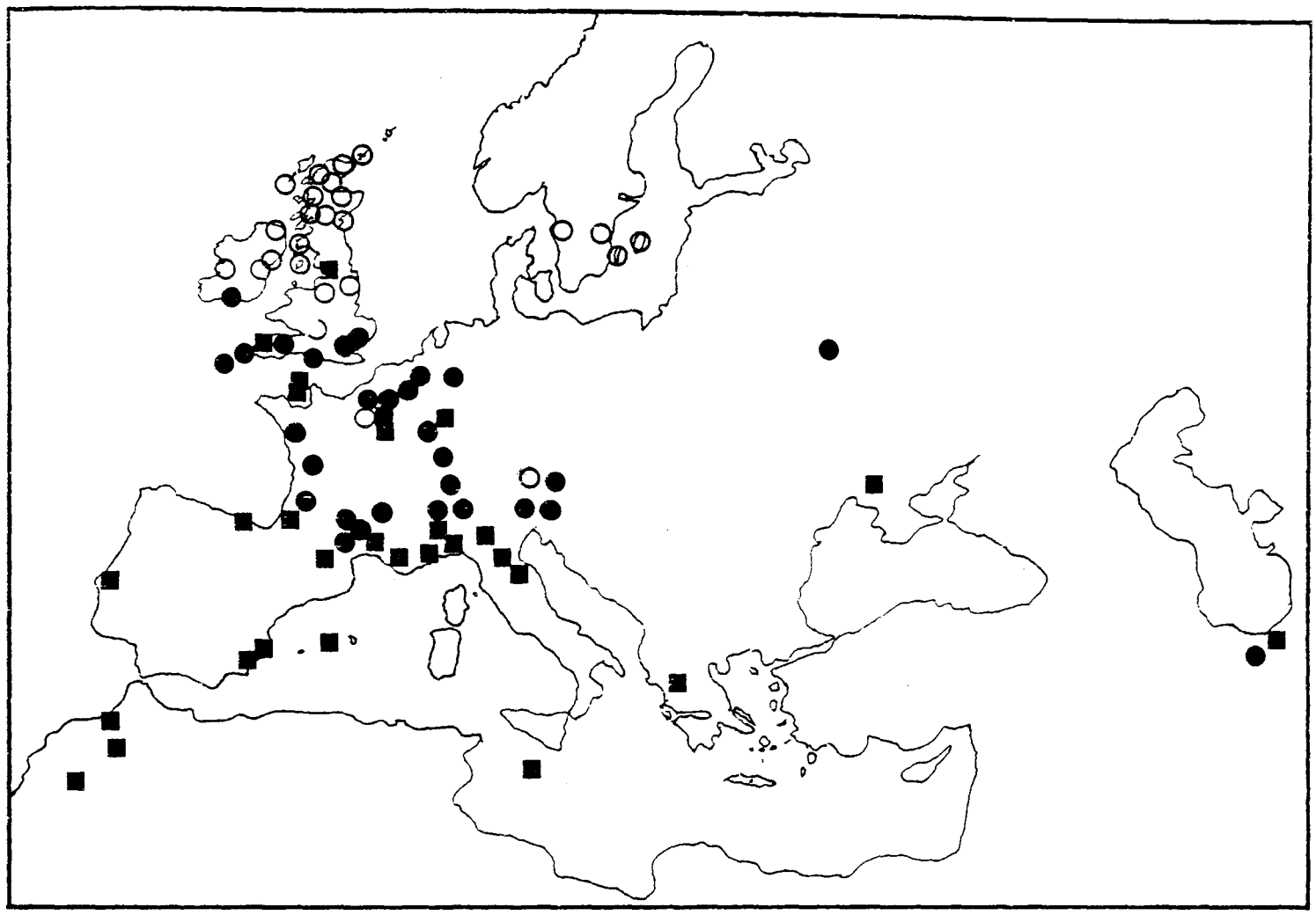
1. male underside forewing medial line
2. male underside hindwing spotting
3. female upperside hindwing fulvous
4. female underside forewing apical eyespot pupillation



○ 0 - 33 ● 34 - 66 ■ 67 - 100

figure 3.1.2b

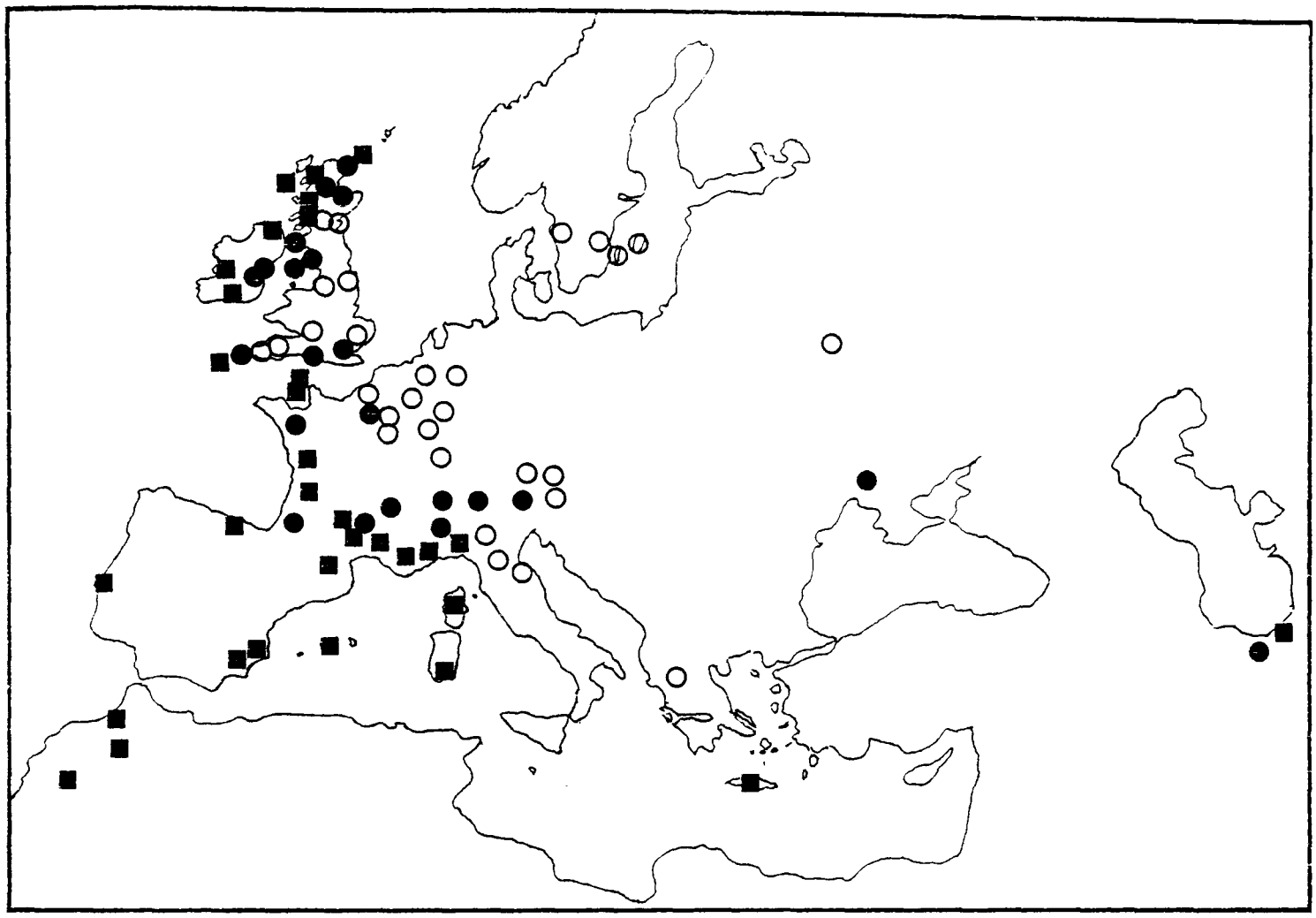
Wing marking characters in Maniola jurtina: percentage males with medial line on underside forewing.



○ 1.5 - 2.1 ● 2.2 - 2.7 ■ 2.8 - 4.1

figure 3.1.2c

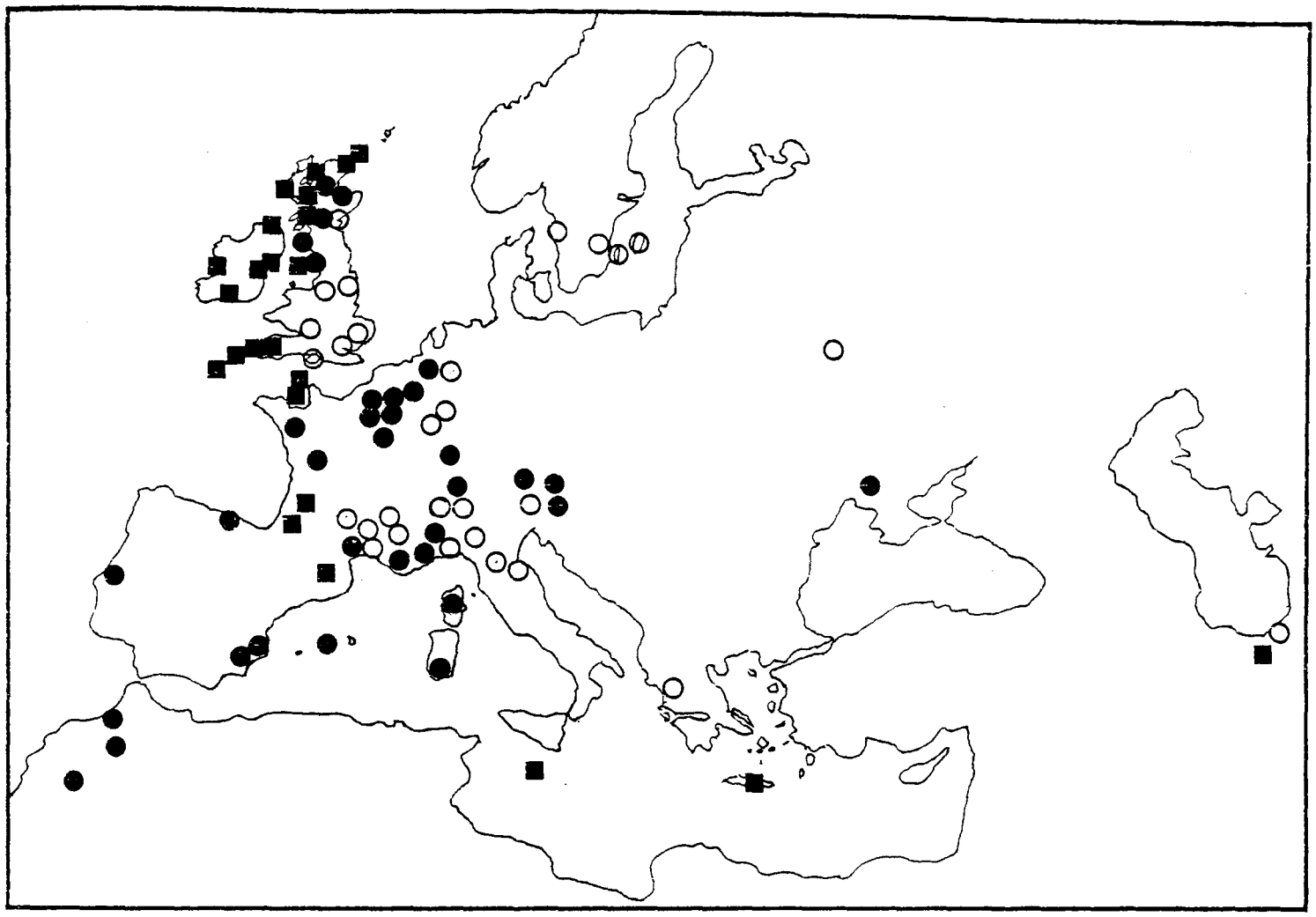
Wing marking characters in Maniola jurtina: male underside hindwing spotting average.



○ 0 - 33 ● 34 - 66 ■ 67 - 100

figure 3.1.2d

Wing marking characters in Maniola jurtina: percentage females with fulvous on upperside hindwing.



○ 0 - 33 ● 34 - 66 ■ 67 - 100

figure 3.1.2e

Wing marking characters in Maniola jurtina: percentage females with underside forewing apical eyespot bipupilled.

It would be appropriate here to discuss recent work on the underside hindwing spotting which was begun by Ford and his colleagues and which is quoted widely in many texts on evolutionary biology. No single group of characters in a butterfly has been studied so extensively and over such a long period. The spots (eyespots or ocelli) which are present on the pale submarginal band on the under surface, are positioned on the intervenosa between veins two and three, three and four, four and five, five and six, six and seven and seven and eight and vary in size and number (zero to six). The spots may be present or absent in any of the six positions. The potential usefulness of such relatively inconspicuous characters, offering possible discrete markers, was first recognised by Ford, Fisher and Dowdeswell when they began their work on Tean, in the Isles of Scilly. This developed into a long term study of evolutionary and ecological genetics by these scientists, and several others who followed (see Dowdeswell 1981). The results of their work may be usefully summarised as follows.

1. Using the quantal distribution of spots (number of spot phenotypes) as genetic markers, it was found that males and females differed markedly and the apparently isolated populations on the island of Tean were statistically similar ($p>.05$) (Dowdeswell and Ford 1952). Spotting on the large islands of the Scilly group differed from that on the smaller islands, the large islands being remarkably stable while on the smaller islands each had its own particular expression. A similar situation was found by Bengtson (1978) on the Swedish islands. Dowdeswell and Ford (1953) suggested that this was due to selective adaptation to the special characteristics of habitats on small islands, while jurtina on large islands could adapt only to the average conditions there. They reject the possibility of bottlenecking as a cause.

2. In the females a so-called 'general European stabilisation' was established. Here, the statistical distribution of spot morphs was very similar in southern England and virtually all mainland Europe north of the Alps and Pyrenees (Dowdeswell and Ford 1953, Dowdeswell 1956, Dowdeswell and McWhirter 1967, Foreman, Ford and McWhirter 1959).

3. It was found that the situation in south-west Cornwall was unique and that there was an abrupt change from the 'east Cornish' to the 'south English' forms (Creed, Dowdeswell, Ford and McWhirter 1959), with evidence of character displacement - the sympatric evolution and reverse cline phenomenon - a situation later described in plants by Antonivics and Bradshaw (1970). The boundary between the groups of populations was found to move (up to 70km) from year to year, as well as to broaden to several kilometers (the 'boundary phenomenon') (Creed, Dowdeswell, Ford and McWhirter 1962).

4. Later studies indicated that there was some evidence of both inter-seasonal and intra-seasonal variation in the spotting stabilisations. There was also clear evidence that annual shifts were associated with drought conditions.

5. An analysis of spot placing (costality index) apparently confirmed that spotting stabilisations and the boundary phenomenon (Creed 1959, 1971, McWhirter and Creed 1971, Ford 1975 - see also Creed, Ford and McWhirter 1964, Dowdeswell and McWhirter 1967, McWhirter 1969, Dowdeswell 1961, Dowdeswell and Ford 1955, Dowdeswell, Ford and McWhirter 1957). Scali (1971a, b), working in Tuscany, found that there was significant shifts in spotting in samples taken of pre- and post-aestivation females.

The significance of the work of Dowdeswell, Ford et al. is discussed in the papers cited and in Dowdeswell (1981). Throughout, their arguments are strongly selectionist. Clarke (1966, 1970 and pers. comm.) was, possibly, the first to suggest that environmental factors might have some bearing on the spotting in the species as a direct effect, rather than through the effects of selection pressures, but it was the work of Brakefield (1979a) which gave evidence that this might, in fact, be the case.

Brakefield's work (see also Brakefield 1974, 1977, 1979a, b and 1984) is, arguably, the most extensive and thorough research on the spotting by a single worker. Between 1973 and 1979 he worked on jurtina in the West Midlands of England and central Scotland, following the general lines of previous workers. His work is important, as he suggests that the spots have an ecological function and are thus more

than quantitative markers of a yet undetected gene assortment. Brakefield, Ford, Dowdeswell, Masetti and Scali demonstrated that jurtina populations exhibit a marked tendency towards higher spotting levels and increased costality (Brakefield 1979a) under conditions of environmental stress, especially drought or range marginality. Brakefield concludes that the spots reflect a balance between the pattern's cryptic qualities and its effectiveness as an escape mechanism (by deflection attacks), presumably from birds. Hence, the forces of selection supposedly produce an increased incidence of higher spotting and costal forms, especially in those individuals which are dispersing from the centre of a colony.

The notion that eyespots in Lepidoptera act as both attraction and deflection marks is not new. Blest (1957) summarises previous findings and describes the results of his own research using birds. Brakefield's uncertainty with regard to the possible effects of environmental factors is interesting. He suggests that higher levels of light and temperature could be a determining selective factor for genotypes. His experiments with jurtina larvae and pupae used small samples and inadequate controls. He assumed that any direct environmental effect on spot patterns would be most likely to occur during the pupal stage. While this can be so, in the case of the effect of temperature on the production of melanin (Merrifield 1890, 1892, 1894, Thomson 1973a), Beck (1980) shows that in Lepidoptera the photoperiod regime in the ovum and first instar larva stage, through a 'switch' mechanism, can have a profound effect on the pigmentation processes in later development stages of the organism. Brakefield did not indicate at which stage his material was subjected to controlled environmental conditions for most of his work, but is significant that the results from the samples collected from Cramond Island, which were introduced to controlled conditions at the young larva stage, differed from those reared from other stock. It could be argued that, if an environmental factor had a direct effect on the spotting, it would show in wild populations. It is surprising that the distribution of the spotting genotypes has not been recognised as exhibiting such a phenomenon. Brakefield points out that the spotting averages are higher in north-west Scotland, north-west England, possibly west Wales, Isle of Man, south-west England and the Isles of Scilly. These are oceanic areas of higher mean temperatures and high

relative humidity. But Brakefield claims that high temperatures induced no increase in the size of the apical eyespot (or any other spot) in his laboratory samples. This is contrary to the finding of Thomson (1973a). Bengtson and Hagen (1977) have shown an increase in melanism in the two-spot ladybird (Adelia bipunctata) with increased oceanicity in western Norway.

The probable effects of environmental variables on the underside hindwing spotting has direct relevance to evaluating their importance in assessing the evolutionary relationships of intra- and infra-specific groups within the genus. Dowdeswell (1981) claimed great phylogenetic significance for the spotting in these butterflies. Indeed he goes as far as to say that

> '... the patterns of variation [ie spotting] which they [the genes] control may well be more significant than some other characters used in distinguishing species ... the genes controlling spotting and its characteristic patterns appear to be trans-specific, trans-generic, trans-familial, and therefore of great antiquity.'

This view cannot be maintained in the light of the work of Frazer and Wilcox (1974, 1975), Scali and Masetti (Scali 1971a, b, 1972, Scali and Masetti 1978). Frazer and Wilcox show that there is no evidence of parallel geographic variation in the incidence and manifestation of the spotting throughout the Maniola species. They found marked variability, in the form of clines and quantal steps, in the species examined. Recent work by Brakefield (pers. comm.) should dismay those who have worked for many years on jurtina spotting. He suggests that almost all of the spotting variation in south-western Britain (and presumably elsewhere) can be explained by June temperatures and that the boundary phenomenon is due partly to annual fluctuations. This is simply confirmation of the detailed suggestions of Thomson (1973a) who demonstrated that thermal conditions can affect spot expression. Sampling times, apparently, have also distorted the data, as early and late flying jurtina exhibit different spotting frequencies. Although it does not necessarily invalidate all such work on spotting in the species, the implications of this on the concepts developed by

ecological geneticists who have worked on jurtina will become clear only after an objective evaluation of the situation.

Work on spotting has been based on a quantitative analysis of variation differences, not on the spots themselves as characters per se. This, and the gradual realisation that temperature is effective after all, is possibly why there is, apparently little agreement between spotting morph frequencies and geographical variation exhibited by other, probably more important, external and biochemical characters.

3.1.3 Ultraviolet and infrared reflectance

The extended spectral receptive vision of insects into the ultraviolet was known last century (Lubbock 1899, see also Eltringham 1933). Patterns differing between closely related species and between sexes were identified in Gonepteryx (Nekrutenko 1964) and Colias (Ferris 1973) and it was suggested that these patterns could be a useful identification aid in some instances (Silberglied and Taylor 1973). Some discussion has arisen on the physiological interpretation of UV reflection as interference structure (Nekrutenko 1965, Hinton 1973a, Ghiradella et al. 1972) or pigmentary (Hinton 1973b, Bowden 1977). No UV reflection has been reported in Satyridae, so it was not surprising that visual and photographic investigations of Maniolini revealed no differential UV reflectance patterns, although the white 'pupil' in Maniola reflected brightly in ultraviolet light.

Infrared photography, which can identify surfaces which absorb or reflect infrared radiation, has been used in numerous biological surveys. Cott (1956) found that green larvae of the genus Protoparce showed red in infrared, while, Gibson et al. (1965) found similar photogaphically detectable pigmentary effects in frogs. The pigments on butterfly wings have not been surveyed photographically in the infrared part of the spectrum.

A preliminary study of Maniola species was made, utilising infrared reflectance photography, to find if patterns of variation, which could be of evolutionary significance, were present in butterflies of the genus. The techniques used were those described in Kodak Publica-

tion number M-28 (1977). Photographs were taken using Kodak High Speed Infrared Film 2481, utilising a Wratten number 15 filter (orange) and two 3400K photoflood lamps for illumination. For controls, Kodak Panatomic X with the same light source was used for photographs taken in the visible spectrum. Panchromatic materials record in the spectral range of 250 to 675nm. Infrared photographic film is sensitive to the approximate wavelengths 250 to 900nm, which includes a large part of the visible range, so there is a high percentage of duplication in the photographic imagery.

In all species, most images in the visible infrared spectra were identical. However, a dark area on the underside forewings was revealed in all males of Maniola species on the infrared images which was not present in the visible spectrum. Its location was directly lateral to the androconial area on the upperside, but was rather more extensive. Optical examination indicated that this area appeared slightly lighter, if anything, than the surrounding background colour in normal light (plate 3.1.3a).

An interesting study by Watt (1967, 1968) on the adaptive signals of pigment polymorphism in Colias butterflies, showed a correlation between melanin pigment and thermoregulation. By the use of implanted thermister probes and spectrophotometry, he claimed that in cold alpine or northern habitats, dark Colias achieve greater reproductive success than light Colias, since their solar heating advantage will lead to greater activity for feeding, mating and oviposition, while the reverse is true in warm lowland habitats, where light Colias will be less susceptible than dark ones to heat stress and resulting forced inactivity. In a later paper (Watt 1969) he considered the role of photoperiodism in melanistic pigmentary determination. Hoffman (1974) studied the environmental control of seasonal variation in Colias eurytheme and the effects of temperature on pteridine pigmentation. Similar work was undertaken by Douglas and Grula (1978) who suggested that melanic forms of Nathalis iole resulted from short day and immaculate forms from long day photoperiods.
Thermoregulatory behaviour has been studied in some depth (see Baker 1978 and Kingsolver 1985a for reviews). Kingsolver (1985a, 1985b) describes two types of basking

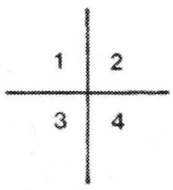
plate 3.1.3a

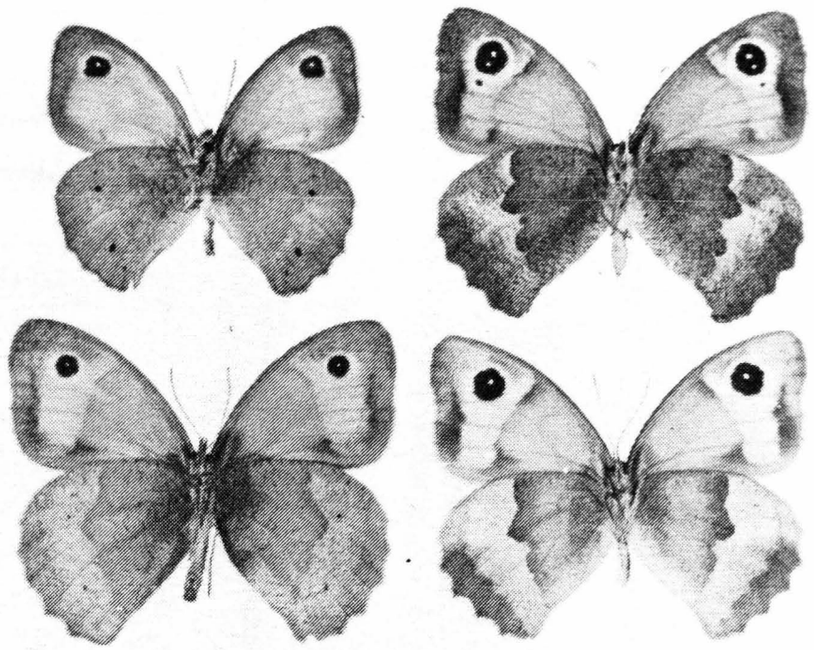
Infrared reflectance patterns on the underside of *Maniola telmessia* (1, 2, 5, 6) and *M. jurtina* (3, 4, 7, 8) - males left, females right.

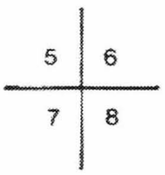
1 - 4 visible pattern recorded on panchromatic film (Kodak Panatomic X)

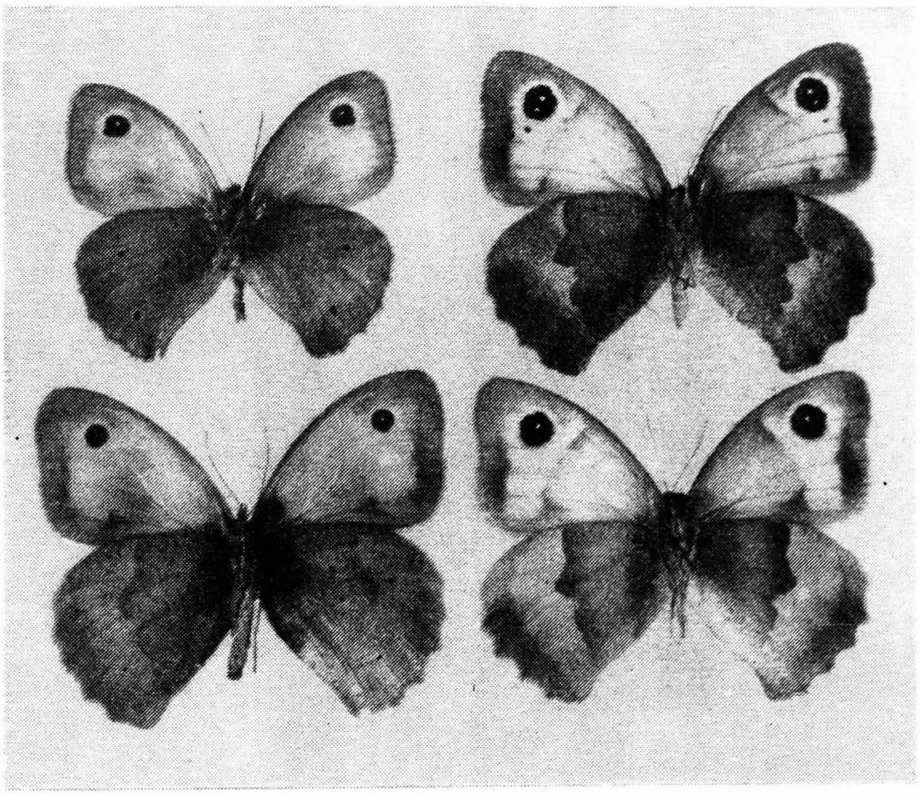
5 - 8 infrared reflectance patterns recorded on infrared film (Kodak High Speed Infrared 2481)

9 lateral basking positions of male *Maniola* butterflies









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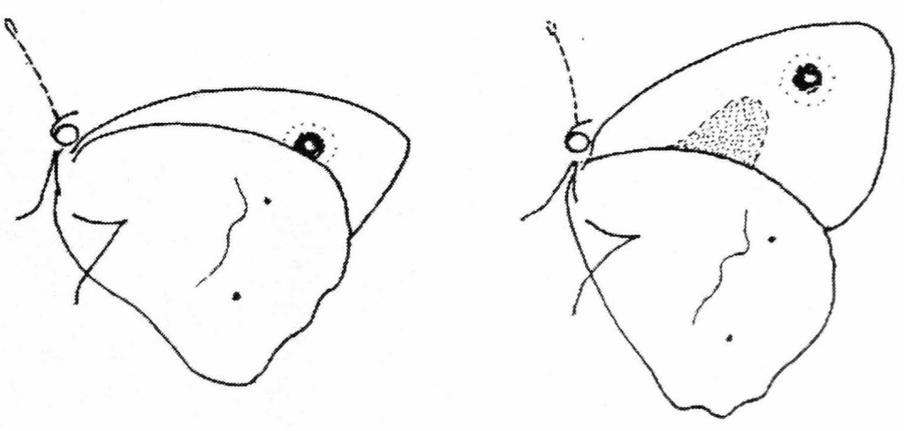


1. absorptive, when wings absorb solar radiation and conduct thermal energy to the body, either by lateral basking with the wings overhead, or by dorsal basking

2. reflectance basking, in which the wings are held over the body in the form of a 'V' and heating is achieved via the upper surface only.

The location of this patch suggests that it could be used as a means of fine control of body temperature, enabling and disabling warming by adjusting the relative position of the hindwing to the forewing (plate 3.1.3a:9), a possibility strengthened by the observation that male Maniola tend to bask laterally with the wings held closed over the body (Brakefield 1984). No intraspecific variation was detected in this feature.

3.1.4 Scale morphology

Scales are the most conspicuous feature of butterflies' wings. They are highly modified integumental structures, fixed by a short pedicle, covering most external surfaces of the insects except the compound eyes and proboscis. Formed of thin lamellae with adjoining trabeculae, scales vary greatly in size and shape, and their shape and infrastructure may be characteristic of different areas of the wings and other parts of the insects' anatomy. Much has been published on Lepidoptera scales, but most authors have dealt with pigmentation, development and physiology (see Mayer 1896, Tower 1903, Nijhout 1981 and numerous other modern works), morphology (Richards 1951, Smith 1968) or pheromones (see Birch 1974). Downey and Allyn (1975) give a good outline of the Lepidoptera scale types and devised a nomenclature which is much needed.

Nothing has been published on the scales of Maniolini, although Thomson (1973b) illustrates the male androconia of Maniola jurtina without comment. In this work, scales from various parts of Maniola wings from both surfaces were examined. As in all Lepidoptera, Maniola scales were found to vary greatly in shape and size from one area of the wings to another (figure 3.1.4a). For comparative studies of geographical and interspecific variation, it was essential

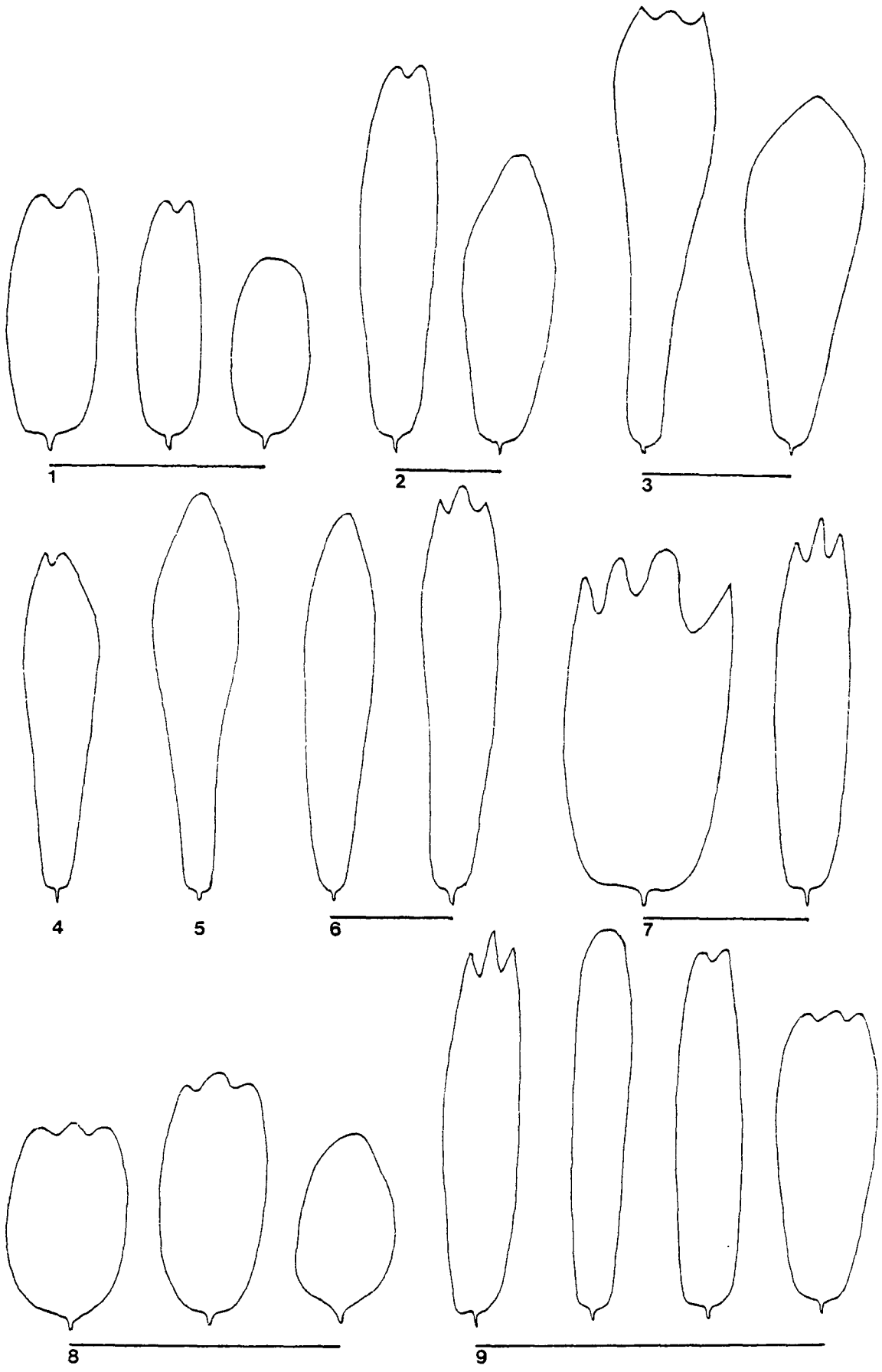


figure 3.1.4a

Maniolini scales.

1 - 6 scales from apical eyespot area: 1. Maniola jurtina (Barra), 2. M. megala (Marmaris, Turkey), 3. M. chia (Nea Moni, Chios), 4. M. telmessia (Beirut, Lebanon), 5. M. cypricola (Episkopi, Cyprus), 6. M. nurag (Nuoro, Sardinia)

7 - 9 scales from Maniola jurtina (Barra): 7. costal area, 8. discal area, 9. submarginal area

that scales were selected from precisely the same part of the wing in each individual. This was a relatively simple matter in Maniola. Scales were removed from the apical eyespot area and from ocellar areas on the underside hindwings. These were examined optically at high magnification (X400) and drawn by projection (section 1.3.6).

Consistent patterns of variation between populations were noted in Maniola jurtina and between Maniola species. Rothschild and Jordan (1895, 1906) referred to scale differences in Papilio, but gave no explanation of his use of the character, referring to scale shape in some species and not in others. In their study of the Psychid moth subfamily Solenobiinae, Sauter (1956) utilised scale shape in species and subspecies identification, recognising that comparisons must be made of scales from the same parts of the insect's wing. This procedure was followed by Suomalainen (1980) who worked on the same group of moths.

In view of other concurrent morphological studies of androconia ultrastructure and larval chaetotaxy, it was decided that analysis of these structures, which are clearly potentially valuable measurable morphological characters, should be a matter for future research.

In some species, including all Maniolini, except a few Asiatic Hyponephele, numerous highly modified scent scales are found in males, forming conspicuous 'sex brands'. The taxonomic and evolutionary significance of these androconia was first recognised by Kellogg (1894), although 'other scales - plumules' were mentioned by Deschamps (1835) who credited their discovery to Baillif, and noted that these specialised scales varied in shape.

In Maniola, Pyronia, Hyponephele, Aphantopus and, to a lesser extent, Cercyonis, the androconia shape was found to be characteristic of each genus (figure 3.1.4b). Within Maniola, there was little detectable difference between species, However, androconia were distinctive in Pyronia and Hyponephele, especially between lycaon and lupina. In his magnificent monograph of the genus Erebia, Warren (1936) discusses the androconial scales and their development in the genus. He describes three stages in their evolution which he called

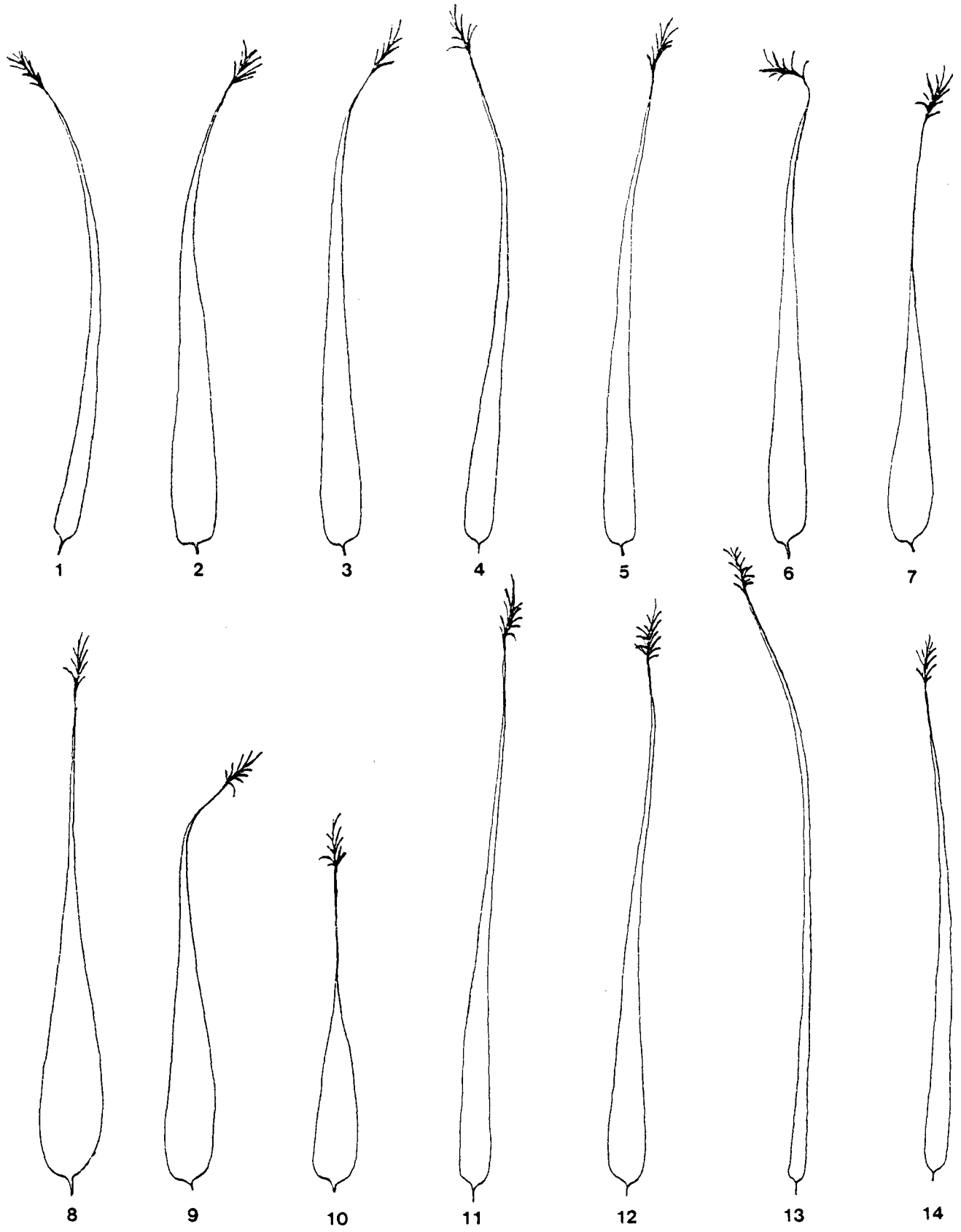


figure 3.1.4b

Maniolini androconia

1. Maniola jurtina, 2. M. megala, 3. M. chia, 4. M. telmessia, 5. M. cypricola, 6. M. nurag, 7. Aphantopus hyperantus, 8. Pyronia tithonus, 9. P. bathseba, 10. P. cecilia, 11. Hyponephele lycaon, 12. H. maroccana, 13. H. lupina, 14. Cercyonis pegala

palaeomorphic, eomorphic and neomorphic, in ascending order of specialisation. Warren later studied the androconia in Pieris (1961, 1963, 1965), but his work on this group has not found favour. Androconia have been found taxonomically useful in many groups (Eliot 1973), including Satyridae (De Lesse 1952b, 1954, Kudrna 1977).

The androconia of Manioline species (several scales from a number of specimens) were examined by scanning electron microscope (section 1.3.3). It was noted that the scale ultrastructure, presumably representing various evolutionary states, varied markedly between genera and, to a lesser extent, between species. There was also considerable variation between the scales from the same butterfly. Some androconial scales, possibly aberrant, were noted which may or may not have some evolutionary significance. One such recurring type was a large, broad scale in Maniola jurtina which might parallel the 'primitive' Pierid androconium (Warren 1936). The importance of these morphs can be deduced only after examination of considerably more material.

Three characteristic scale types were identified from details in their ultrastructure (terminology used from Downey and Allyn 1975) (plate 3.1.4a)

1. scales in which the longitudinal ribs were indistinguishable from the scale body (Cercyonis) or very poorly developed (Hyponephele),

2. scales in which the longitudinal ribs were absent in the central area of the scale, but well developed in the outer parts (Pyronia)

and

3. scales in which the longitudinal ribs were very well developed across the whole scale (Maniola and Aphantopus).

Cross-ribbing in these scales was more regular in some Hyponephele than in other genera, although it gave the appearance more of a perforated surface on the lamella body. It was least regular in the central area of the Pyronia scales. In Maniola the cross-ribbing was vary variable, but usually, though not invariably, very regular.

Aphantopus was, in this respect, very similar to Cercyonis, having what appeared to be irregular perforations rather than cross-ribbing.

It was important that the same surface (the upper or exposed surface) of the androconium was compared. In Hyponephele the lower surface (ie that lying closest to the wing) was usually completely lacking both longitudinal and cross-ribs. In Pyronia, cross-ribbing on the lower surface was quite regular, almost as regular as in an ordinary scale. The surface differences in Maniola androconia, on the other hand, were virtually indistinguishable.

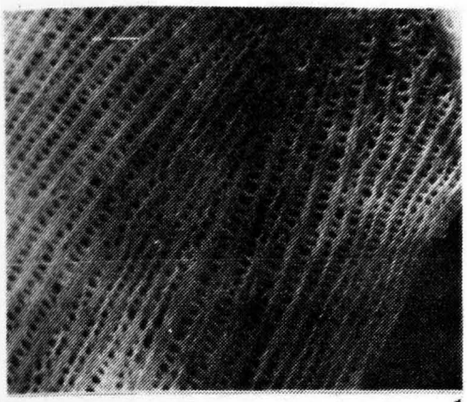
Ultrastructural differences between congeneric species were not marked, although some trends were apparent. It was possible to distinguish the more regular cross-ribbing of H. lycaon from that of lupina. Differences between Maniola species were more obscure. M. chia androconia had longitudinal ribs which were more prominent than any other species examined. It should be noted that the androconia of M. cypricola were particularly variable.

Kellogg (1894) identified two types of scale (in Lepidoptera), the thin 'hair scale' which he considered to be the more generalised, while the more specialised was flattened, symmetrical, broadly expanded, irregularly corrugated and striated (cross-ribbed). Kristensen (1970) examined the ultrastructure of the scale of some primitive moths of the family Micropterygidae and Eriocraniidae and the evolutionary implications of his work are interesting. He suggests that the most primitive scales are formed of a single membrane (lamella) and lack transverse ribs (the cross-ribs of Downey and Allyn). It is generally believed that the androconia represent an evolutionary development of the abundant long, fairly broad 'ordinary' scales. Warren (1936) noted that the 'pigmentation' of ordinary scales consists of a series of dark dots of more or less uniform size and shape, arranged in parallel rows, with perfect regularity, from end to end of the scale. Conversely, the androconial 'pigmentation', he observed, was fundamentally composed of similar black dots of irregular sizes and shapes, which appeared to be disseminated in an orderless manner over the whole area. It must be remembered that Warren's work was 'pre-electron microscope'. It is now clear that what he thought were 'pigmentation dots were, in fact,

plate 3.1.4a

Scanning electron micrographs of Manioline androconia.

1. Maniola jurtina, Parma, Italy, X6200, SEM0405/12
2. Maniola megala, Marmaris, Turkey, X4700, SEM0588/12
3. Maniola chia, Nea Moni, Chios, X6200, SEM0615/2
4. Maniola telmessia, Filerimos, Rhodes, X4600, SEM0623/1
5. Maniola cypricola, Episkopi, Cyprus, X4900, SEM0637/6
6. Maniola nurag, Nuoro, Sardinia, X4600, SEM0628/7
7. Pyronia tithonus, Le Touquet, France, X4600, SEM0625/4
8. Pyronia bathseba, Rabat, Morocco, X2800, SEM0630/10
9. Pyronia cecilia, Valance, France, X2800, SEM0627/6
10. Aphantopus hyperantus, Callander, Perthshire, X7700, SEM0424/1
11. Hyponephele lycaon, La Grave, France, X4900, SEM0638/7
12. Hyponephele maroccana, Oukaimeden, Morocco, X6500, SEM0635/4
13. Hyponephele lupina, Greece, X4700, SEM0632/12
14. Cercyonis pegala, Toronto, Canada, X7000, SEM0432/12
15. Pyronia bathseba, Rabat, Morocco, X4700, SEM0631/11 - unspecialised cloaking scale



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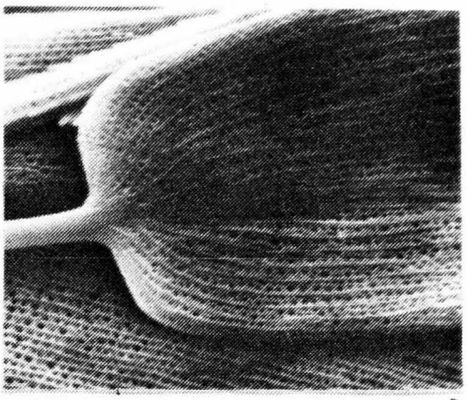

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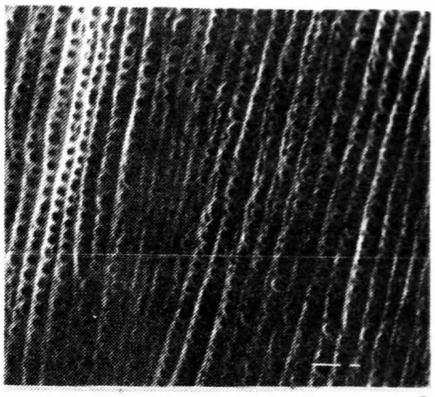

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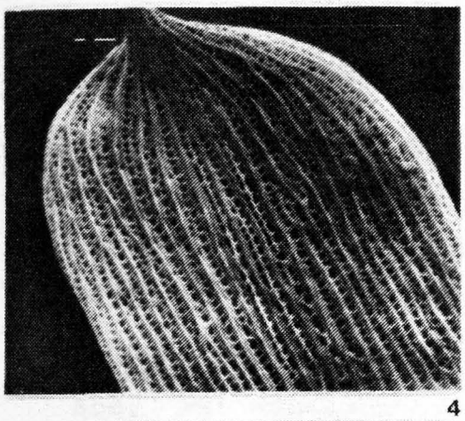

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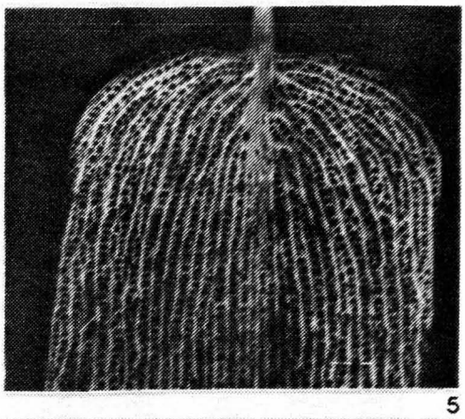

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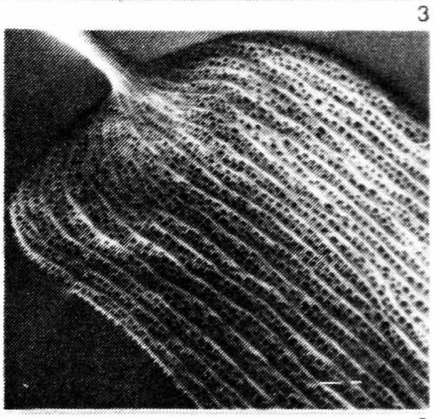

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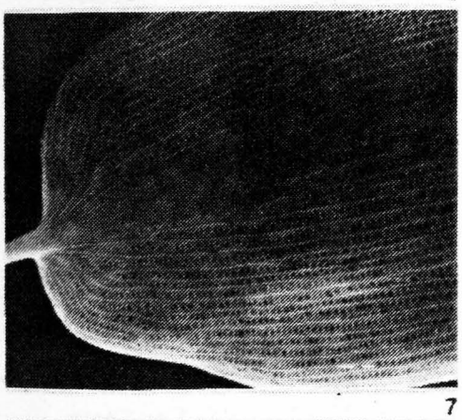

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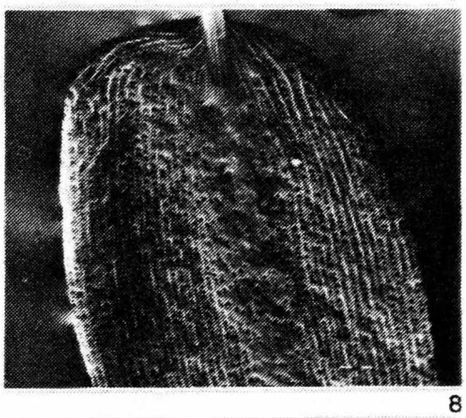

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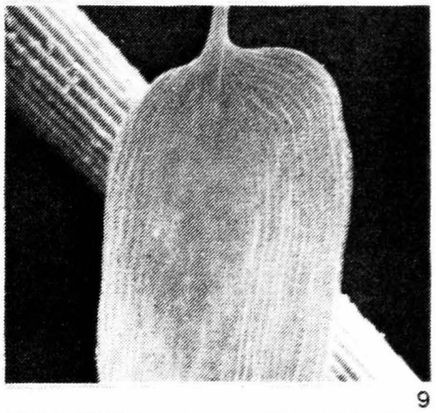

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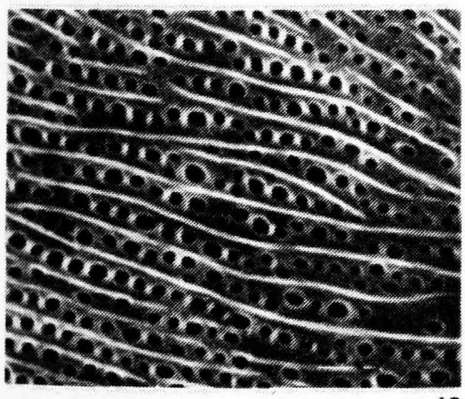

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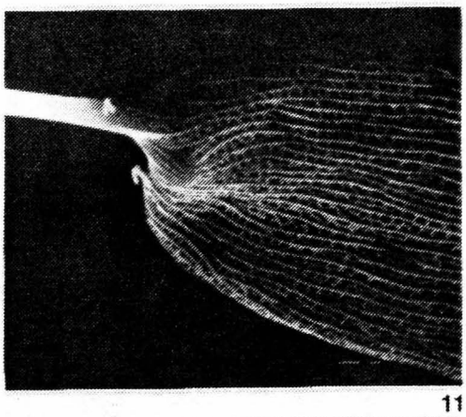

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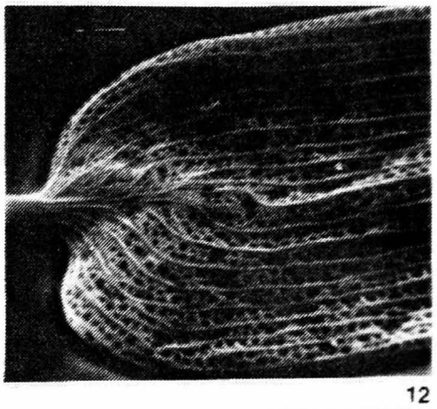

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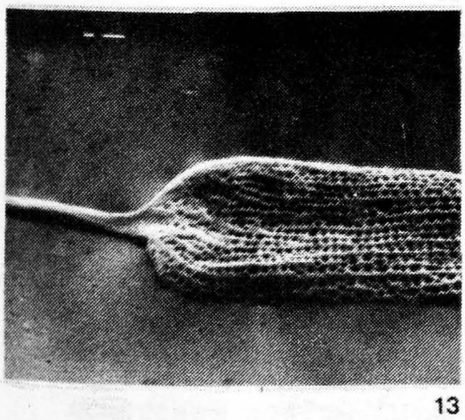

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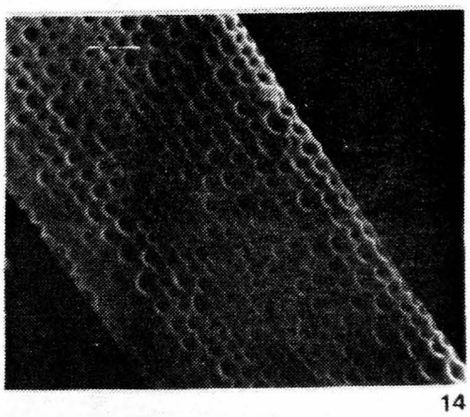

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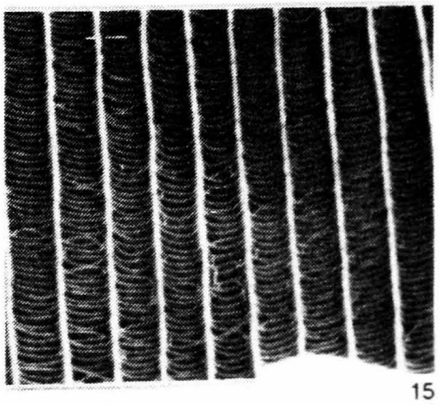

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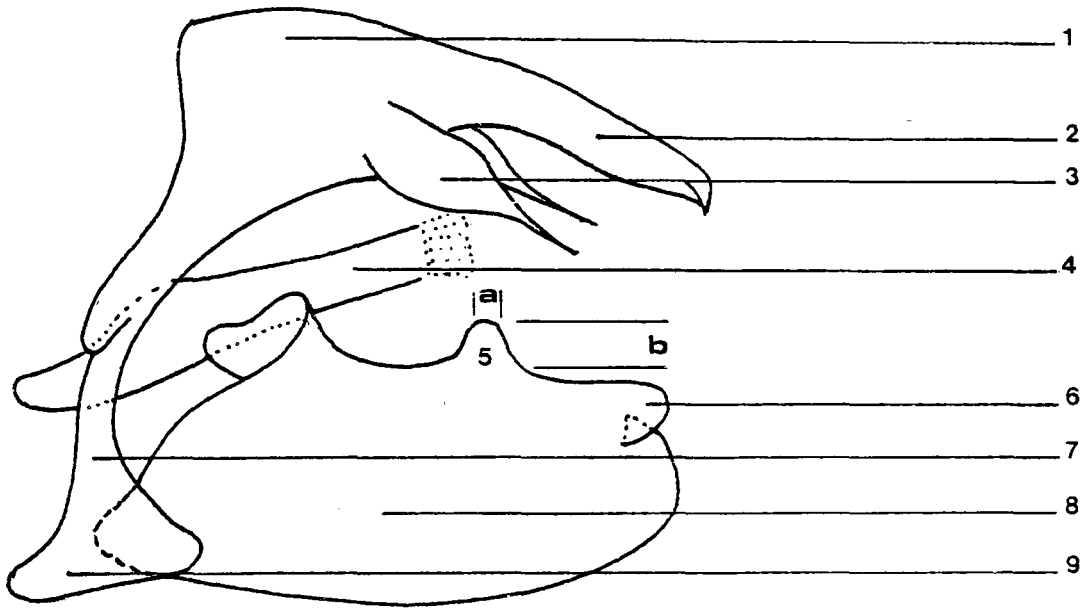
holes or spaces between the cross-ribs (plate 3.1.4a:15), although the cross-ribs themselves may anchor pigment bodies (Downey and Allyn 1975). From this it can be reasoned that most Lepidoptera scales have evolved from a single character state lacking cross-ribs, to a more complex, but highly regular structure. As part of the evolutionary development of androconia, this regularity has been gradually replaced by an apparently random arrangement of holes in a more solid body (plate 3.1.4a:1-14).

It is rarely possible to identify evolutionary direction with any degree of certainly. Characters may be acquired or lost. The ultrastructure of androconia could, in some instances, contribute to the interpretation of possible evolutionary trends. From the above it could be suggested that the Manioline scales which are less regular in cross-ribbing, which have less pronounced longitudinal ribbing and are, in consequence, furthest removed from the ordinary scale structure are the most advanced. In the Manioline group there are some anomalies: the regular Hyponephele scale has, at the same time, the least prominent longitudinal ribs. Taking into account both surfaces of these scales it seems likely that Cercyonis - Hyponephele - Pyronia - Maniola is the evolutionary direction from most to least advanced with Aphantopus in an uncertain position between Pyronia and Hyponephele. This interpretation will be discussed in relation to the evolutionary implications deduced from other morphological and biochemical studies (section 4.2).

3.1.5 Genitalia

Several taxonomic systems for Lepidoptera genitalia have been proposed with little agreement on usage or homology (Pierce 1909, Tuxen 1970, Sibitani et al. 1954, Higgins 1975). The male genitalia in Maniolini is a relatively simple structure, and terminological confusion can arise only in the name given to the gnathos or brachia. The system of Tuxen has been used here.

The male genital armature (figure 3.1.5a), representing highly modified sclerites of the ninth and tenth abdominal segments, comprises a ring formed of a broad dorsal tegumen and ventral saccus,



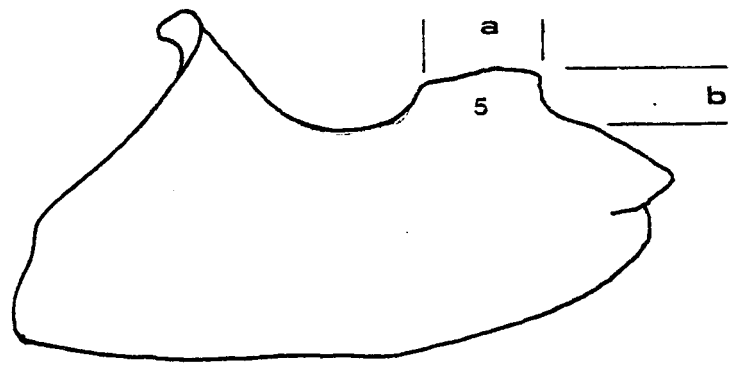


figure 3.1.5a

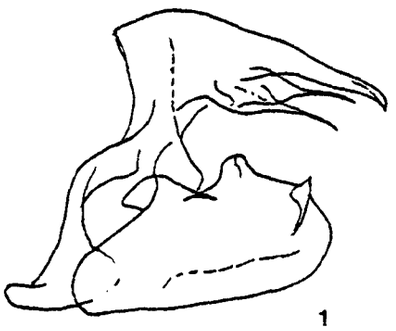
Maniolini male genitalia (Maniola jurtina - 'Western' above, 'Eastern' below): anatomy and taxonomy of the armature and measurement paramters used.

1. tegumen, 2. uncus, 3. gnathos, 4. adeagus, 5. dorsal process, 6. distal process, 7. vinculum, 8. valve, 9. saccus, a. dorsal process width, b. dorsal process length

figure 3.1.5b

Maniolini male genitalia.

1. Maniola jurtina (Morocco - 'Western'), 2. M. jurtina (Turkey - 'Eastern'), 3. M. megala (Turkey), 4. M. chia (Chios, Greece), 5. M. telmessia (Samos, Greece), 6. M. cypricola (Cyprus), 7. M. nurag (Sardinia), 8. Pyronia tithonus (Italy), 9. P. bathseba (Spain), 10. P. cecilia (Spain), 11. Aphantopus hyperantus (England), 12. Hyponephele lycaon (Italy), 13. H. maroccana (Morocco), 14. H. lupina (Spain), 15. Cercyonis pegala (USA)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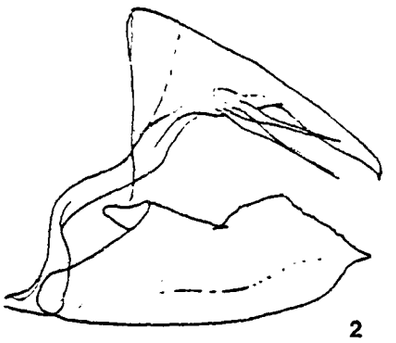
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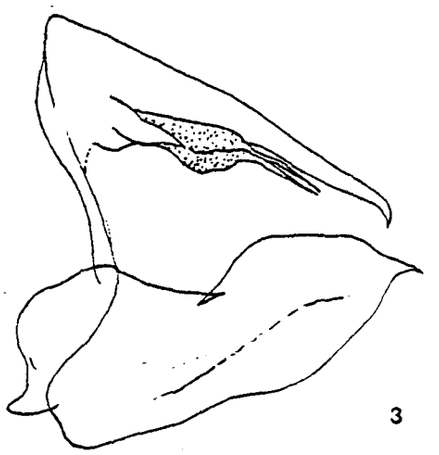
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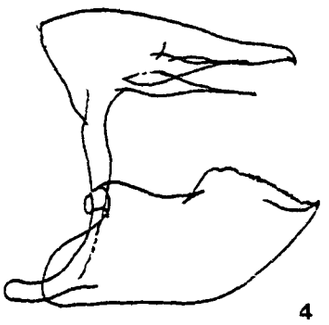
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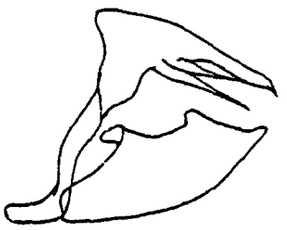
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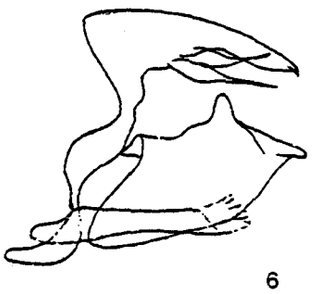
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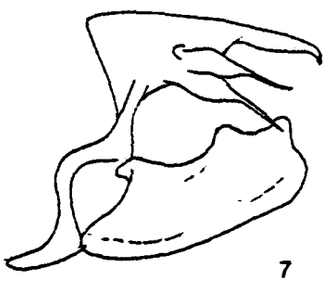
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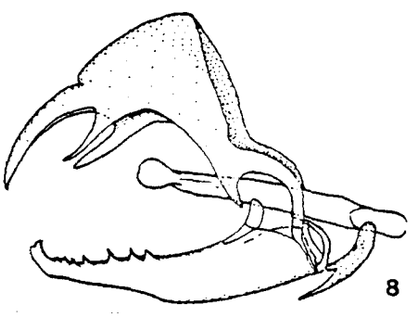
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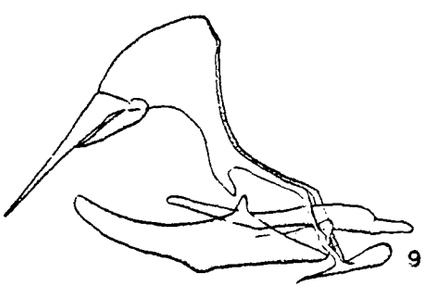
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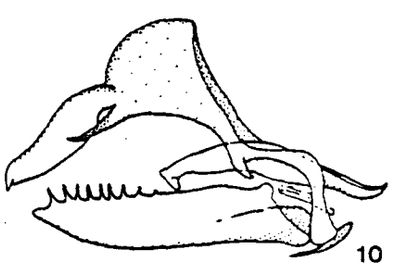
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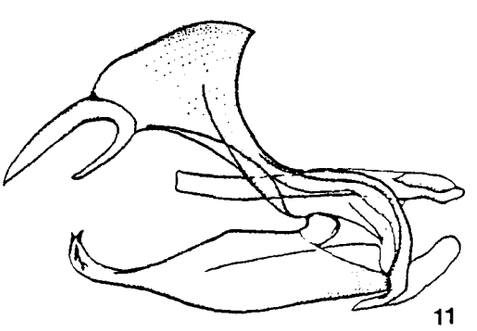
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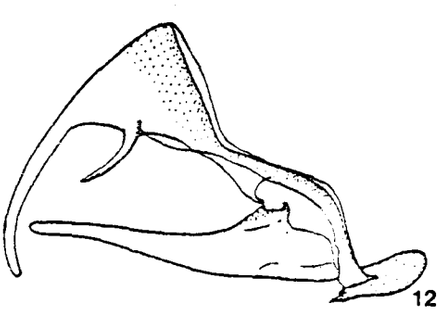
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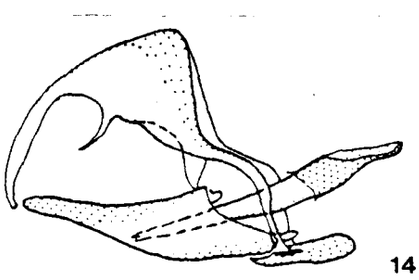
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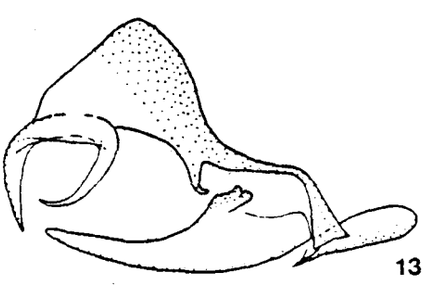
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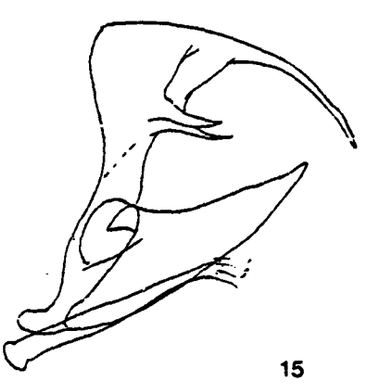
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joined laterally by the vinculum. Anterior to the tegumen are the uncus and paired gnathos (brachia). The valves (or claspers) lie on each side of the adeagus which emerges from a membranous diaphragm. In Maniolini, variation of systematic and, presumably, evolutionary importance occurs in the uncus, gnathos and valves.

Characters of the male genitalia within Maniolini are distinctive (figure 3.1.5b). The length, form and shape of the uncus and gnathos is characteristic to each group, more so at species level, but much more information of evolutionary significance can be deduced from the shape and form of the valve in each genus. In Hyponephele, Cercyonis, Pyronia and Aphantopus the valve is long, narrow and tapering. The dorsal border in Hyponephele is smooth, while in Pyronia (with the possible exception of janiroides, not included in this study) it is toothed. The distal area of the Aphantopus valve broadens and is armed with tooth-like process. The Cercyonis valve is similar to that of Hyponephele. Maniola is conspicuous in being the only genus in which the valve is broad. Its form is quite unlike that of the other genera and far more divergent that would be expected from its relatively close relationship with the other Maniolini.

Intrageneric differences in the male genitalia are also distinct. In western Palaearctic Maniolini it is possible to identify, with some certainty, almost every species in the tribe from genitalia alone (De Lesse 1952a, Higgins 1975, Thomson 1976). Geographical variation is conspicuous in both male and female armatures in Maniola jurtina (Higgins 1975, Thomson 1973b, 1976). Similar patterns of geographical variation have not been noted in other Manioline species, although it is possible that they exist. The present study was concerned with intraspecific variation in the male genitalia of jurtina and with interspecific variation in the genus Maniola.

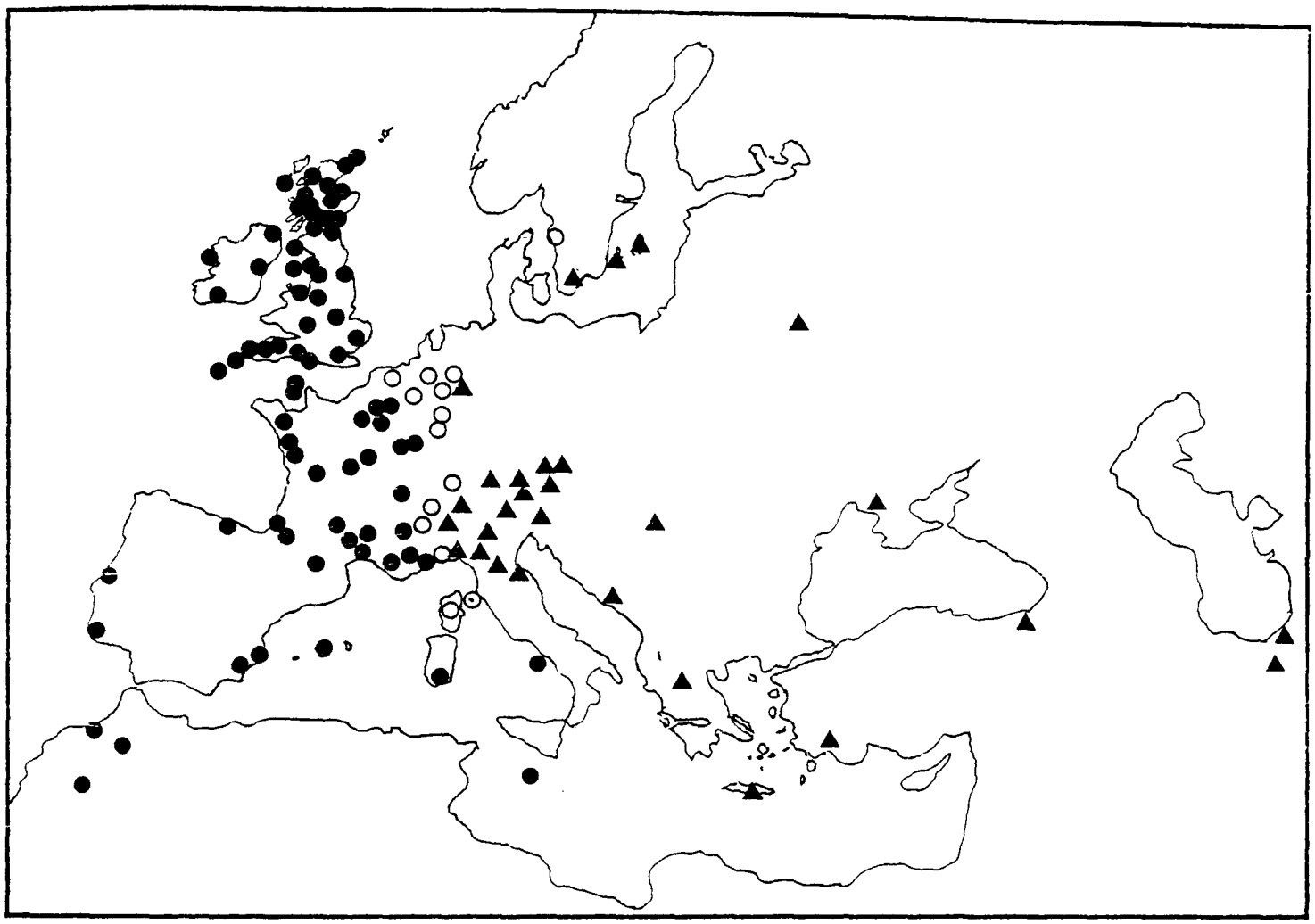
The complex structure of the male genital armature made a choice of measurement parameters difficult, although the shape and size of the valve have been shown to be species specific and to vary geographically in jurtina (Le Cerf 1912, 1913, De Lattin 1967, Mouschamp 1915 Bernardi and Lagnel 1966, Higgins 1975, Thomson 1973b). Sophisticated algorithms for the measurement of shape (Bookstein 1978) were considered, but found to be unnecessarily cumbersome as a means of

analysing morphological differences. Tauber (1970) utilised several measurement parameters which could be usefully applied to future studies. The length and width of the dorsal process of the valve was measured by projection from slide preparations (section 1.3.6 - figure 3.1.5a). Data already published (Thomson 1973b) was checked and greatly extended by utilising the new material collected for this study. Thomson demonstrated that the 'western' group of jurtina populations is characterised by having the dorsal process longer than its breadth. in 'eastern' populations the converse is the case. Data from the 'transitional zone' revealed intermediate and mixed values. Thus, the simple coefficient valve height minus valve breadth gave positive values when the populations were 'western' and negative values when they were 'eastern'.

The data from this analysis are represented in figure 3.1.5c, which clearly shows the distribution of the two major population groups and the 'transitional zone' between. It has been suggested by Thomson that this 'transitional zone' is a zone of secondary intergradation.

The characteristics of this area merited further study. Two transects were identified, one in the southern part of Europe from Santander in northern Spain to Cortina d'Ampezzo in north-eastern Italy (17 populations), and the other from Falais on the north-western French coast to Kiev in the USSR (14 populations). The southern transect thus crossed the 'transitional zone' where the boundary between 'western' and 'eastern' populations was very narrow (see Thomson 1973b, 1975), while the northern series encompassed samples from the broad zone of intermediate and mixed populations. Data for these transect analyses are given in tables 3.1.5a and b and represented graphically in the population range diagrams (figures 3.1.5d and e).

The differences between the transects are obvious and not unexpected. In the south, the transition from west to east is sudden and dramatic. Indeed, only a single population, Col du Tende, could be classified as 'transitional' in the south. In the north the change is gradual. However, it can also be seen from the standard deviation values that there is more variation in populations in 'transitional zone' populations. This could be the consequence of past admixing of individuals from 'western' and 'eastern' populations. If such



▲ 'Eastern' ● 'Western' ○ 'transitional'

figure 3.1.5c

Maniola jurtina: distribution of 'Eastern', 'Western' and 'transitional' male genitalia forms.

table 3.1.5a

Maniola jurtina - male genital valve: dorsal process height minus length in populations forming a southern transect in Europe (see figure 3.1.5d).

population	n	min	max	mean	(SE)	SD
Santander	12	0.510	2.510	1.750	(0.193)	0.670
Sare	12	0.050	2.990	1.550	(0.201)	0.695
Oloron	12	0.000	3.040	1.433	(0.293)	1.011
Sorede	16	0.010	2.760	1.450	(0.250)	1.001
Brassac	20	0.000	2.510	1.470	(0.168)	0.752
Serignan	28	1.890	3.520	2.486	(0.097)	0.514
Fountain de V.	27	0.520	3.020	1.667	(0.155)	0.804
Digne	30	0.490	3.510	1.320	(0.186)	1.017
St Valier	20	0.000	3.490	1.390	(0.205)	0.917
Vergon	30	0.110	3.220	1.330	(0.173)	0.950
Col du Tende	24	-3.020	2.500	0.688	(0.380)	1.860
Marzabotto	25	-4.580	-1.200	-2.800	(0.208)	1.041
Ponti Lamberti	18	-5.120	-1.110	-2.950	(0.323)	1.369
M Sibillini	20	-4.140	-1.110	-3.041	(0.273)	1.220
Bolzano	18	-4.510	-1.020	-2.583	(0.249)	1.057
San Leonardo	19	-4.750	-1.350	-3.151	(0.275)	1.200
Cortina d'Amp	24	-5.530	-1.000	-3.750	(0.299)	1.465

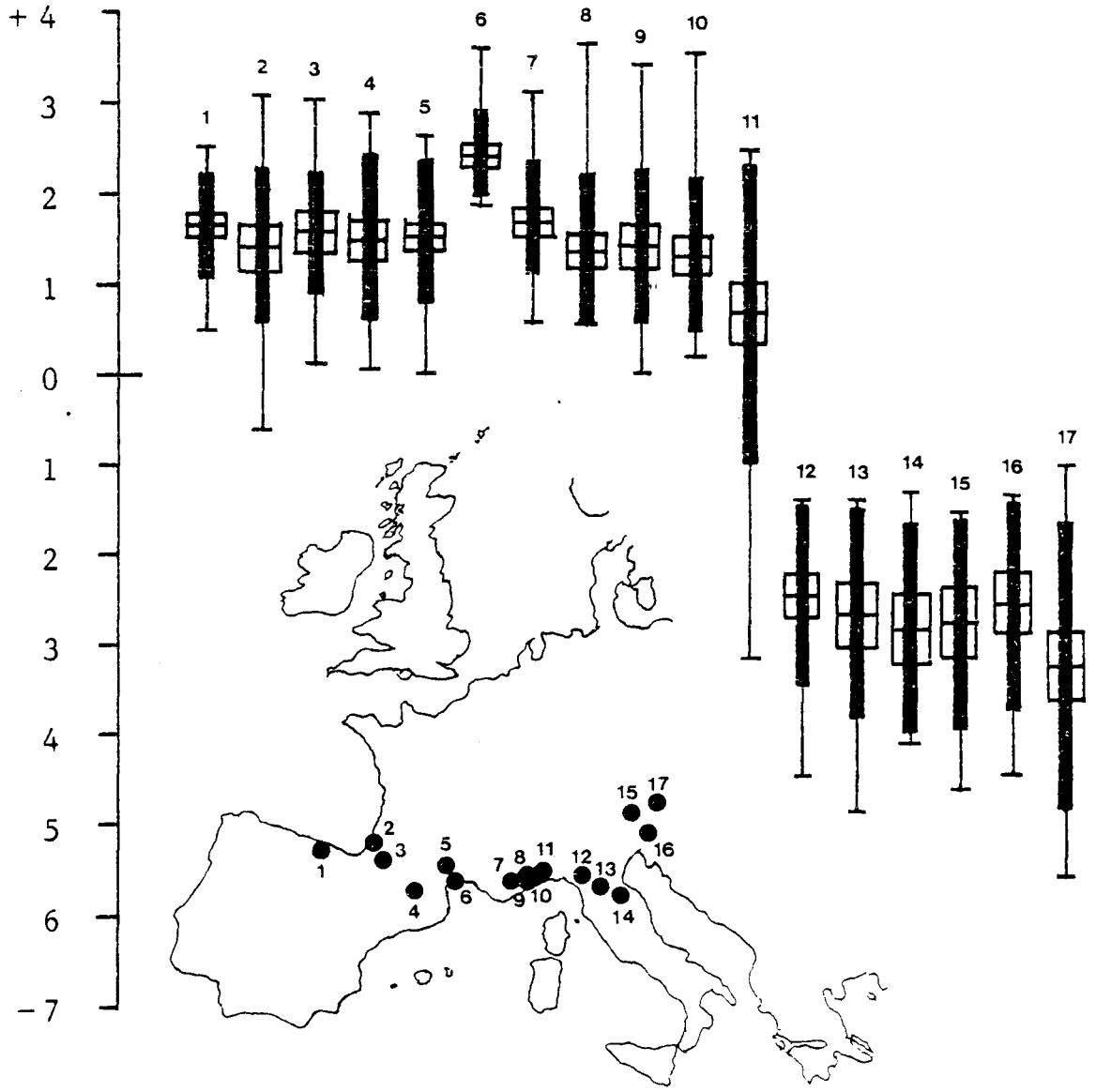


figure 3.1.5d

Population range diagram: Maniola jurtina - male genital valve: dorsal process height minus length in populations forming a southern transect in Europe (see table 3.1.5a). The thin line represents the range, the thick line one standard deviation from the mean, the open box the standard error and the horizontal line the mean.

table 3.1.5b

Maniola jurtina - male genital valve: dorsal process height minus length in populations forming a northern transect in Europe (see figure 3.1.5e).

population	n	min	max	mean	(SE)	SD
Falais	20	-1.510	2.520	0.750	(0.266)	1.188
Guernanville	33	-1.530	3.050	1.591	(0.199)	1.145
Le Quesney	20	0.000	2.080	1.050	(0.162)	0.723
Beauvais	30	-3.100	2.020	0.780	(0.267)	1.464
Feucherolles	20	0.010	2.100	1.150	(0.142)	0.633
Chalons s/M	20	-1.500	2.210	0.260	(0.243)	1.085
Chaumont en B	20	-1.530	2.220	0.260	(0.243)	1.085
Besancon	30	-1.540	2.080	0.200	(0.258)	1.414
Verbier	20	-2.550	-0.500	-1.850	(0.159)	0.709
Brixen	15	-5.200	-1.490	-3.421	(0.312)	1.210
Vienna	30	-5.400	-1.875	-3.360	(0.203)	1.110
Loipersbach	20	-6.800	-1.750	-3.752	(0.293)	1.310
Sopron Umbeg	16	-6.900	-1.520	-4.010	(0.250)	1.001
Kiev	24	-4.000	-1.510	-2.667	(0.210)	1.027

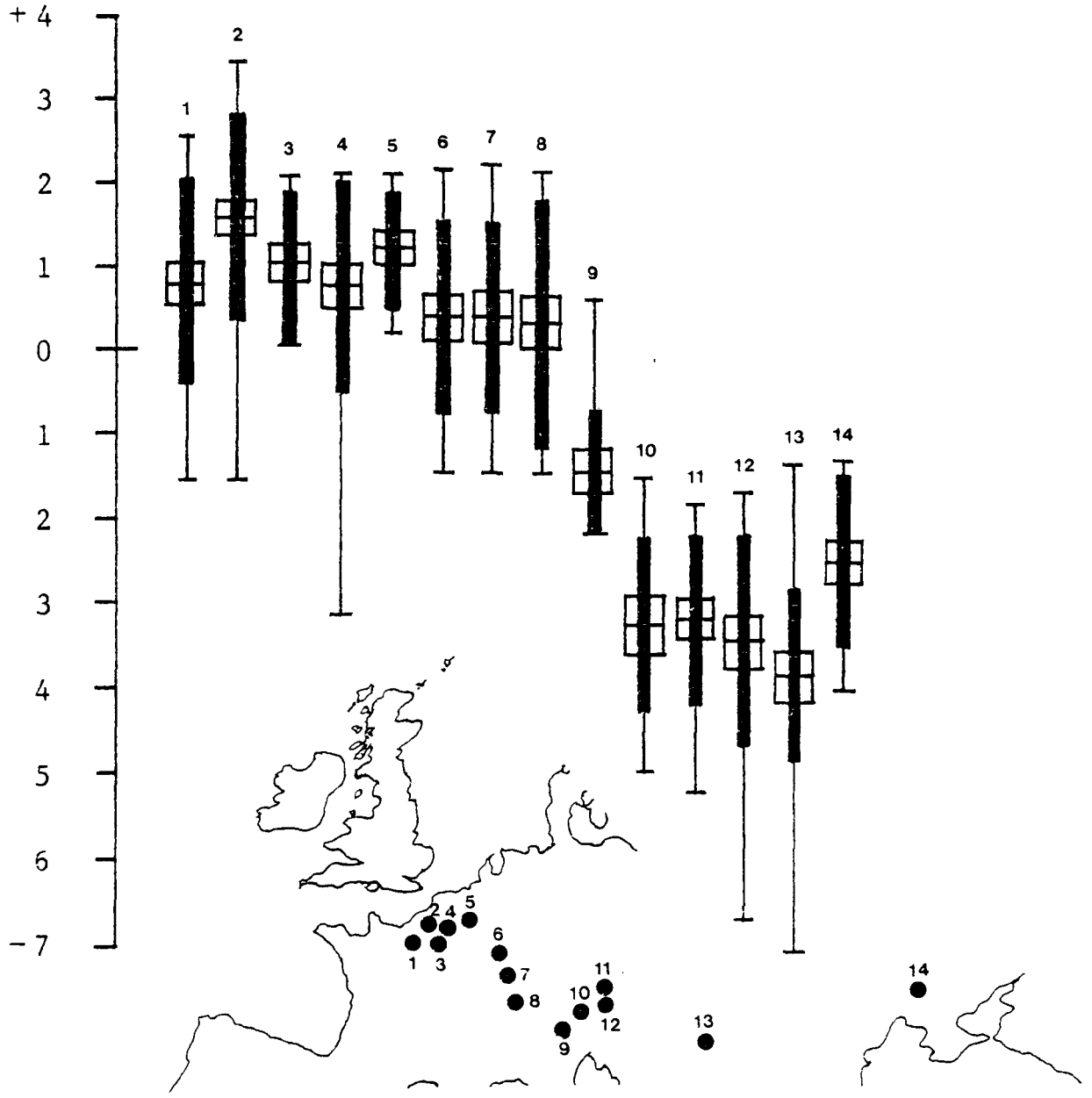


figure 3.1.5e

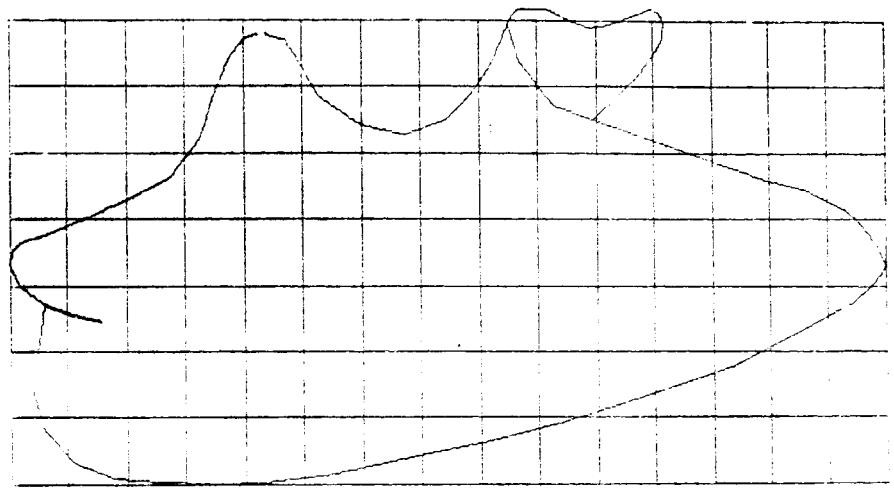
Population range diagram: Maniola jurtina - male genital valve: dorsal process height minus length in populations forming a northern transect in Europe (see table 3.1.5b). The thin line represents the range, the thick line one standard deviation from the mean, the open box the standard error and the horizontal line the mean.

intergradation was recent, the process of stabilising selection is incomplete. It is certainly of note that the area in which the 'transitional' zone is narrowest (the western Maritime Alps) is also the area in which the isolating effects of altitude, in the form of the Alpine chain, must be most effective. Further north, barriers to gene flow must be greatly reduced on a broad geographical front. These considerations support the secondary intergradation hypothesis. There is also a suggestion of a cline represented by decreasing values from west to east in both transects.

If factors other than phylogeny and geographical isolating barriers are determining the variation in the form of the male valve in jurtina, then these have so far escaped identification. It is unlikely that selection would result in these patterns of variation which are present over a wide range of environments. Similarly, it is unlikely that environmental variables would influence the form of these structures: those already examined, temperature and photoperiod (section 3.1.1) do not appear to affect genitalia form.

It seems probable that the 'western' and 'eastern' male genital valves evolved from an ancestral prototype which was not unlike one of these forms, which of the two being dependent upon evolutionary direction (section 4.2). Certainly, dramatic changes have taken place in the structure. As a means of examining these modifications in morphology (displacements), a computer algorithm was written (GENTRANS) which revealed graphically the changes which have taken place. Two techniques were used 1. cartesian transformation using biorthogonal grids (Thompson 1917) and 2. a mean co-ordinate system.

D'Arcy Thompson devised a graphical technique in which the shape of a supposed ancestral (or in the case of growth, immature) form was overlain by a regular grid. Changes derived from homologous parts of the original form incurred relative distortion of the grid. Of necessity, Thompson drew his diagrams manually and, to a degree, subjectively. Computers can now perform the task relatively simply and objectively, although statistical methods of analysing the consequent modifications of the original grid are unsatisfactory (Bookstein 1978). Displacements in the 'western' and 'eastern' genital valve of jurtina are represented in the computer generated plots (figure



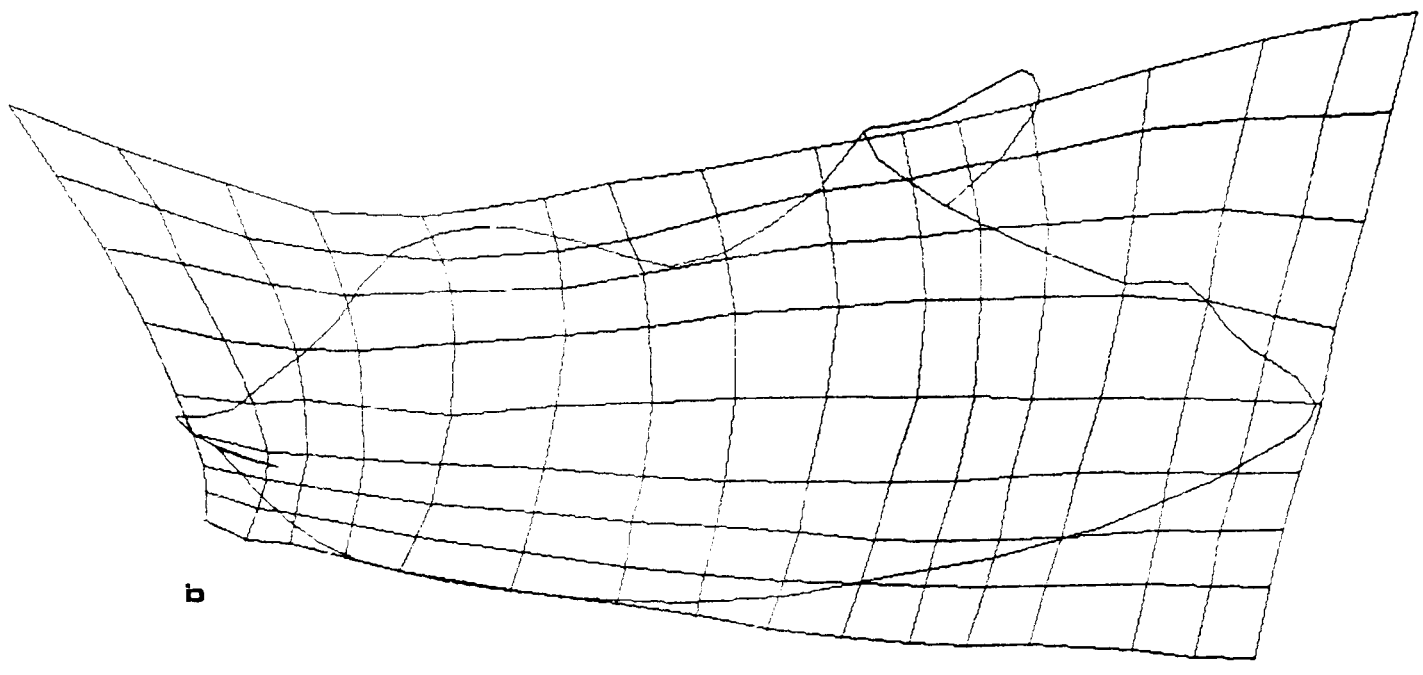


figure 3.1.5f

Transformation of Maniola jurtina male genital valve from the 'Western' form (a) to the 'Eastern' form (b) using biorthogonal grids (see text for explanation).

3.1.5f). These show clearly the lengthening of the valve, the compression of the distal part of the ventral margin, the reduction in the height of the dorsal process and consequent flattening of the upper part of the distal border.

A major problem with Thompson's method is the fact that across large areas of the structure there are no 'landmarks' (see below) so that much of the representation of shape change is based on purely arbitrary choices. The mean co-ordinate system does not attempt to represent the global configuration of a structure: its uses are limited to displacements or transformations of the outline shape of a structure in which any modifications of 'internal' form are either unimportant or irrelevant to the study. Displacement in the jurtina valve form can be seen, perhaps more clearly, in the plots generated by this method. This approach requires the selection of points which are, as far as possible, homologous. These are the 'landmarks' of Walker and Kowalski (1972) and the 'data link points' of Thomson (1971). The displacement sequences in discrete steps from one landmark to the other in relation to two fixed co-ordinates in the original plot are computed. The resulting plot reveals the changes which have taken place between the two forms. In these, form displacement is revealed by non-parallel lines: parallel lines suggest change in size with relatively little change in form (figure 3.1.5f). It should be noted, however, that these illustrations do not represent an evolutionary hypothesis. Each part of the structure could well have changed at different rates or the change may have been sudden rather than gradual. They simply represent a clarification of the differences between two structures.

Studies in the differences between the valves of Maniola species were limited to an examination of the form of the jurtina 'western', 'eastern' and telmessia structures. Mean co-ordinate plots show that there is, overall, less difference between telmessia and the 'western' valves than between jurtina and the 'eastern' valve. This being so it could well have important evolutionary implications (section 4.2).

The reasons why the genitalia form is so variable, and almost always species specific, in Lepidoptera is not known but obviously must be

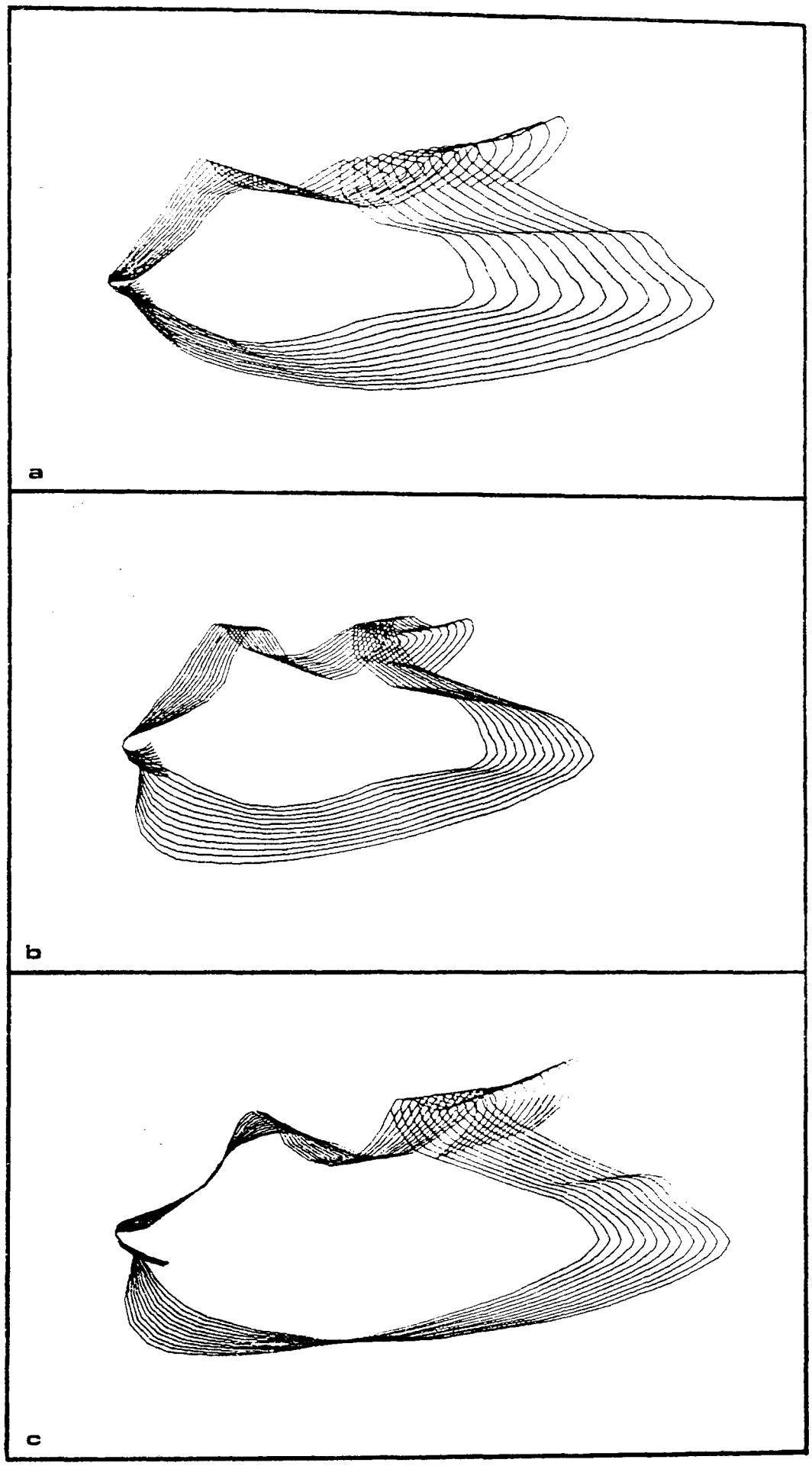


figure 3.1.5g

Transformations of the Maniola male genital valve using the mean co-ordinate system - a M. telmessia to M. jurtina 'Eastern' form, b M. telmessia to M. jurtina 'Western' form and c M. jurtina 'Eastern' form to M. jurtina 'Western' form (see text for explanation).

related to primary sexual function and associated with species affinity. At family and subfamily levels, the male genital armature often shows good characters conforming existing classifications based on other data. It is even more useful at genus level, as wing venation may be constant throughout large groups, and often they are of greater value than any other character (Mayr 1969a). Most studies have utilised the male organ, partly because, in many groups, critical features are clearer and partly because distortion can occur in the microscope preparations of female genitalia. The main reason for the under utilisation of the female structure, however, is that it is more difficult to make good slide mounts in that sex. As pointed out by Ford (1945), the female genitalia in many species reveal more useful characters than the males. In Maniolini, parallel variation to that found in the males occurs in all species (De Lesse 1952a). Thomson (1976) has demonstrated the systematic value of female genitalia variation in Maniola, but this has not been included in the present study.

3.1.6 The Jullien organ

In the course of the survey of morphological characters in Maniolini, the 'Jullien organ' of Maniola was examined. This structure, which is present in males only, comprises a series of hard, black chitinous rods fused together in bundles attached to the last abdominal tergite. They are not found in any other Manioline genus. Similar structures, in some instances homologous, are found in several other Satyrids and their morphology has been used in the identification of certain Hipparchia species (Kudrna 1977, Coutsis 1983(84)). Similar, if not homologous structures occur outwith the family (Reverdin 1894), including Hamadryas (Ageronia s.l.). The seventh, eighth and ninth intersegmental regions of the male abdomen are known frequently to be connected with display, scent-producing and scent-distributing structures consisting of pouches and supports for eversible and erectile masses of modified hairs or scales (Tuxen 1970).

There are some differences in the gross morphology of these structures between Maniola species. The Jullien organ of telmessia, cypricola and nurag is formed of fewer rods than that of jurtina.

Examination of chia and megala indicated that their organs had an affinity with jurtina, being strong and formed of many rods, although in chia these features were a little less pronounced. Using scanning electron microscopy, the ultrastructure of the Jullien organ was examined in detail. It was found to be a set of hollow tubes, with no external punctures or openings (plate 3.1.6a:12). From these initial observations it was concluded that earlier suggestions that the organ was for scent production were highly improbable, although they did not eliminate the possibility, which was also Jullien's own belief, that they were of a 'tactile order' (see Reverdin 1894).

The surface topography of the rods unexpectedly proved to be very much more interesting. It was found to be irregularly fluted, varying from species to species, and, most interestingly of all, they were furnished with an extremely complex pattern of teeth, reminiscent of some stridulatory organs in Orthoptera.

All six Maniola species were examined closely by scanning electron microscopy at high magnification. Some general observations can be summarised.

1. Fluting varied to some extent from rod to rod, but the height of the ridges (or depth of valleys) were similar within species and varied greatly between species.

2. The extent and location of teeth was characteristic of each species. In some Maniola, they were found to be similar on the inner and outer external surfaces of the Jullien organs, while in others they were restricted to specific areas of the inner surfaces only.

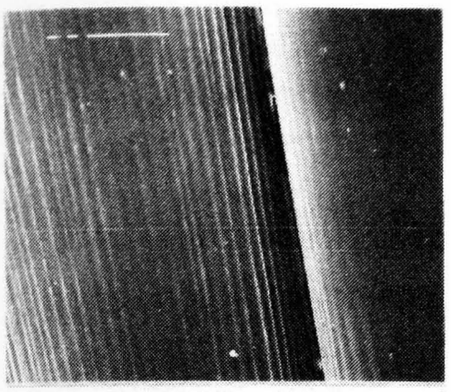
3. Interspecific differences were noted in the frequency of teeth within the toothed area and their length. No teeth were found in megala.

4. The distance between teeth was fairly constant in some parts of the organ and irregular in others. There were considerable differences in the degree of teeth regularity between species. The characteristics of the Jullien organs in Maniola are detailed in table 3.1.6a and illustrated in plate 3.1.6a.

plate 3.1.6a

Scanning electron micrographs of Manioline Jullien organs.

1. Maniola jurtina, Taganana, Canary Islands, X1860, SEM0440/1
2. Maniola jurtina, Great Cumbrae, Scotland, X9700, SEM0471/9
3. Maniola jurtina, Fort, Angus, X9700, SEM0468/1
4. Maniola megala, Marmaris, Turkey, X1400, SEM0589/1
5. Maniola chia, Nea Moni, Chios, X4600, SEM0618/6
6. Maniola chia, Nea Moni, Chios, X9200, SEM0620/8
7. Maniola cypricola, Episkopi, Cyprus, X1930, SEM0465/9
8. Maniola telmessia, Beirut, Lebanon, X4700, SEM 0463/6
9. Maniola telmessia, Filerimos, Rhodes, X9500, SEM0460/1
10. Maniola nurag, Nuoro, Sardinia, X2800, SEM0445/9
11. Maniola nurag, Nuoro, Sardinia, X9200, SEM0444/7
12. Maniola nurag, Nuoro, Sardinia, X450, SEM0443/6 - showing hollow rod structure



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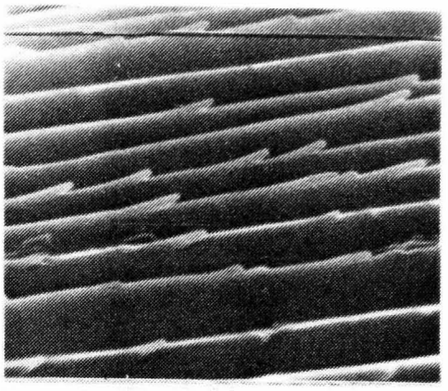

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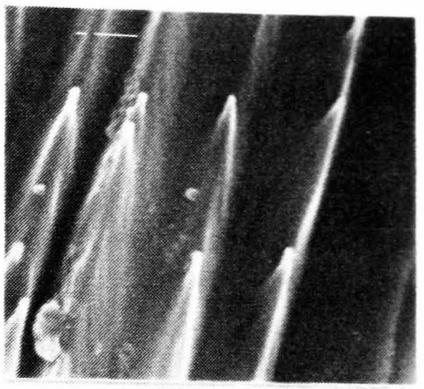

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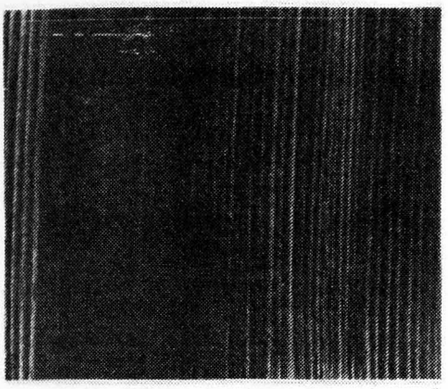

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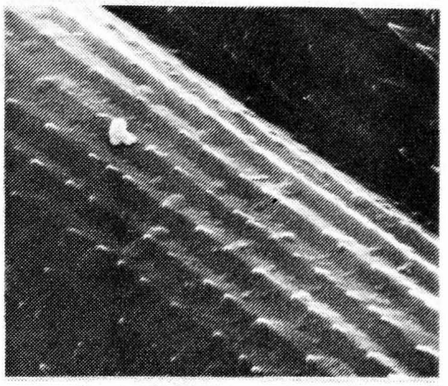

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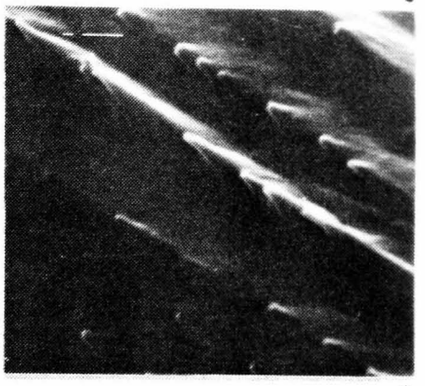

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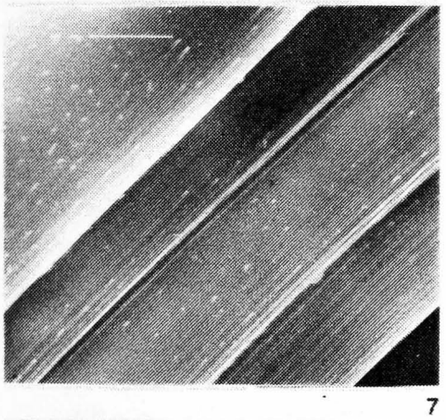

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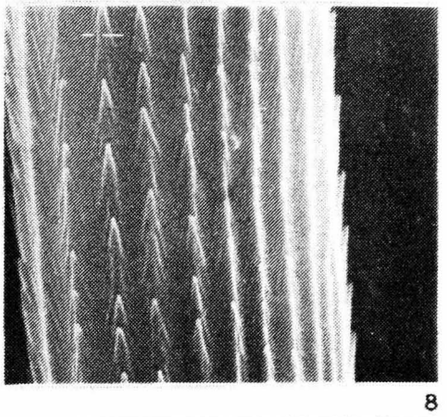

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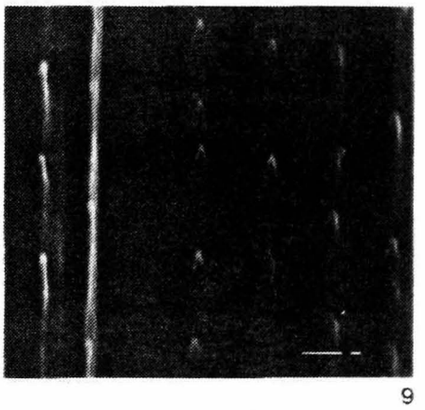

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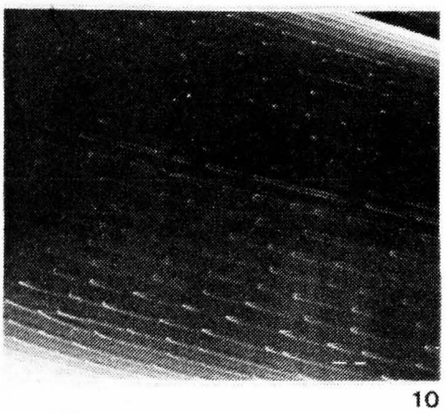

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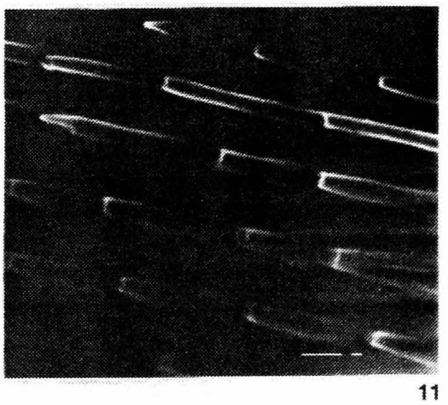

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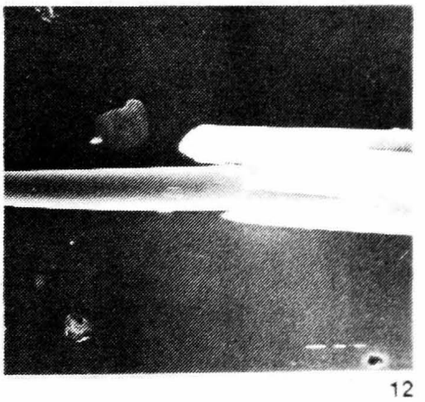

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table 3.1.6a

Ultrastructural characteristics of the Jullien organ of Maniola species.

species	fluting/ ridge heights	teeth length	teeth frequency	teeth regularity
jurtina	fine/high	short	variable	irregular
megala	v.fine/high	? absent	-	-
chia	course/f.high	short	frequent	irregular
telmessia	coarse/low	long	frequent	regular
cypricola	coarse/low	short	frequent	irregular
nurag	coarse/low	v.long	frequent	regular

These observations strengthened the hypothesis that the Jullien organ in Maniola had a sound producing function. If this was so, the most likely structure which would complete the mechanism would be the genital valves. It is significant, perhaps, that those of Maniola are unique in Maniolini in being broad in form. Indeed, broad shaped valves are rare in the Satyridae (Higgins 1975). It is interesting also that the shape of the dorsal process of the valve is characteristic of Maniola species (Thomson 1973b, 1976 - see section 3.1.5). Examination of living jurtina revealed that the juxtaposition of the Jullien organ and the dorsal process would facilitate stridulation (figure 3.1.6a): both structures can be manipulated independently by the insects. The hollow form of the rods would amplify any sound produced by the valve rubbing against the Jullien organ. Unfortunately, the evidence, at present, is still circumstantial.

The suggestion that these structures were sound generators was made by Reverdin (1894) who discussed similar structures (the 'Godman and Salvin organ') in the south American Nymphalid genus Hamadryas, known popularly as 'crackers' because of the sounds they make. However, in this group the organ is inserted in the upper edge of the ventral

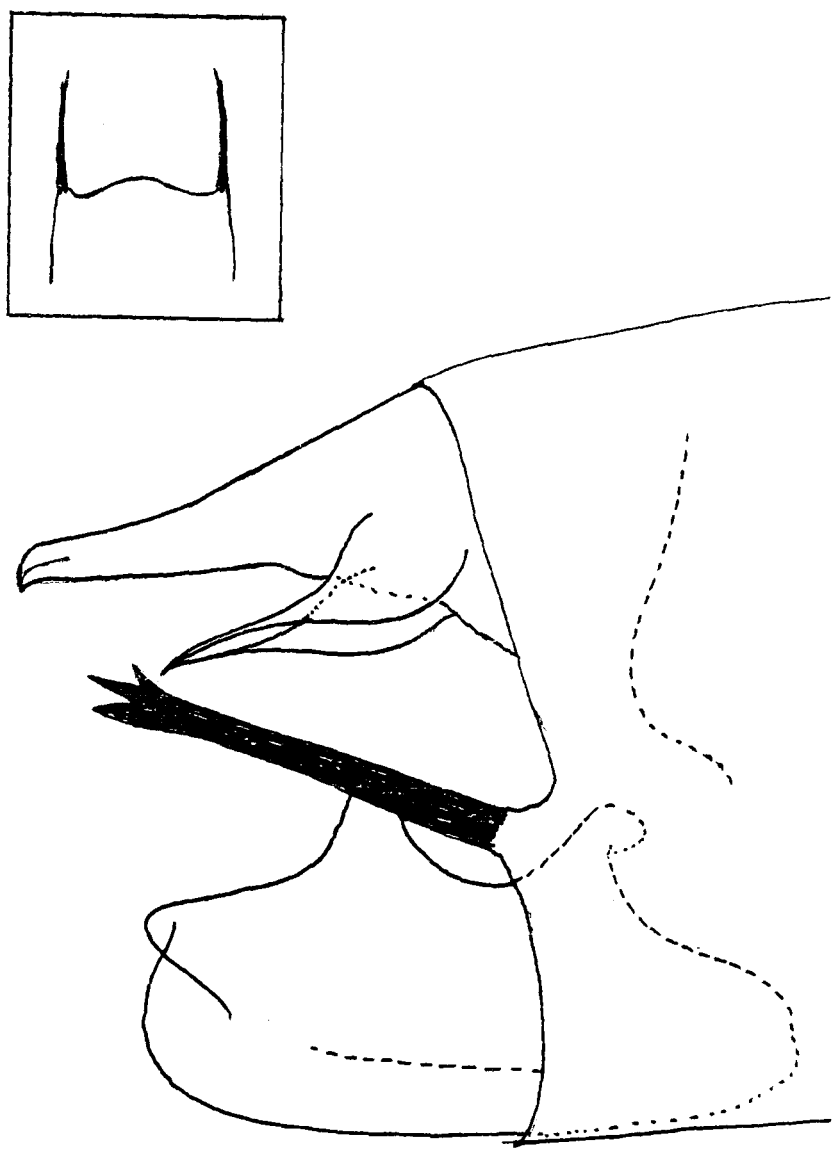


figure 3.1.6a

Relative position of the Jullien organ and dorsal process of the valve in living Maniola butterflies. Inset shows dorsal view of paired organs at each side of vinculum.

portion of the terminal segment (sternite) of the abdomen, not in the eighth segmental tergite as in Maniola. These organs in Hamadryas possess 'hairs' or spiculae varying in number and length, yet Reverdin is the only author to associate these structures with the butterflies' auditory generation (see Fruhstorfer 1924, Ehrlich and Ehrlich 1961, Ross 1963, Swihart 1967). Acoustic signaling is known in only a few other butterflies, Neptis hylas (Scott 1968) and a Neotropical Satyrid species tentatively identified as 'Pharneuptychia nr. pharnabazos' (Kane 1982). No Maniola has been heard to make a sound, although the sounds thus generated might well be beyond our hearing capabilities (about 8kH maximum) and ultra-sonic detecting apparatus (a sonagraph with wide band filter settings) would be required. It is known that the range of auditory perception in insects, especially Lepidoptera, extends into frequencies far beyond those which the human ear can detect (Wigglesworth 1974). The sound spectrogram from pharnabazos illustrates that most of the sounds which this species generates were ultrasonic.

If the Jullien organ in Maniola is a stridulation organ, as these ultrastructural studies would suggest, it could have major implications for evolutionary studies of the genus as a reproductive isolating mechanism. The different tooth pattern observed in the various species would make sounds which would be identified by the species. This, in turn, could be vitally important in mate location and/or stimulation. More importantly, it would act as a mode of species consolidation and accelerate the speciation process through very acute selection pressures.

The possible importance of these scanning electron microscope observations are such that Reverdin must be credited with some foresight and the relevant part of his little known paper should be quoted here.

> '... I have read a remark by Fritz Muller incorporated in Dr G. B. Longstaff's work. "Just as the Ageronia ... only make the remarkable crackling sound on the wing and during courtship, so also, in all probability, butterflies equipped with brands, tufts, etc., only distribute their scent under the same circumstances."

May we not deduce that this crackling sound is caused by the friction of the spiculae against another part of the genitalia, and that the Jullien organ has the same function, the action being modified by their situation; it would seem as though the motionless organ of Ageronia were rubbed by the mobile valves, whereas the mobile spiculae of the Jullien organ in Satyrus [and jurtina] rub against the valves or uncus...'

3.2 Embryonic and pre-imaginal morphology

Evolutionary affinities between major groups of animals are frequently manifest in embryonic stages of the organisms (De Beer 1958). This is often not so in Lepidoptera, in which convergence in the immature insects is commonplace (Miller 1968). Behavioral differences, which might or might not have phylogenetic significance, can contribute towards the evolution of differential shape and form. In Manioline ova, for example, some species (Pyronia bathseba and Aphantopus hyperantus) distribute their eggs freely with little or no adhesion to the larval hostplant (Forster and Wohlfahrt 1955), while the congeneric species, Pyronia cecilia, fixes its eggs firmly (Villa pers. comm.). The general chorionic architecture, therefore, is not necessarily a consequence of the major evolutionary processes. The modifying processes appear to be effective at species level only (see below), being greatly modified by oviposition strategy.

To what extent the same argument applies to larvae cannot be certain on the basis of present knowledge, but recent larval chaetotaxy work, including this study, suggests that the configuration of spines and setae are of considerable importance as indicators of evolutionary relationships. Fleming (1960) believed that the first and, consequently, least adapted larval instar represents an ancestral condition which can be of considerable importance in constructing 'a natural classification'.

3.2.1 Chorionic sculpturing of ova

Not all Manioline ova have been described. The ova of three of these species, Maniola megala, telmessia and cypricola, are described here for the first time. The ovum of Maniola chia was not available for scanning electron microscopy and a brief description from optical observations is given. A description of the ovum of Hyponephele lupina is yet to be published. For outline descriptions of the ova of species of Maniolini see Howarth (1973) for jurtina, tithonus and hyperantus and Simmonds (1930) for nurag. Villa (1966) gives a detailed description of the cecilia ovum. Poor and grossly inaccurate

descriptions of ova are given by Howe (1975) for Cercyonis pegala and Forster and Wohlfahrt (1955) for lycaon.

Ova were obtained from several adults, to preclude the possibility that the females were aberrant individuals. When possible, eggs from five or more females of each species were utilised. The egg laying strategy (ie whether ova were fixed to a substrate or not) was noted in each species. These observations must be treated only as tentative, as oviposition in the wild state may differ from that in captivity (Chew and Robbins 1984). The eggs were examined optically, by stereo microscope and by scanning electron microscopy.

Little importance was placed on size differences which were noted between closely related species. Brakefield (1979a) suggests that the size of jurtina ova decreases northwards through Europe. However, many factors can contribute to ovum size differences (Karlsson and Wiklund 1985), including adult age (Telfer and Rutberg 1960) and imaginal nutrition, although the interactions of nutritional factors and endrocrinology in butterflies are not well understood (Tojo et al. 1981). Variation in egg size in Lepidoptera probably is the norm (Arbogast et al. 1980). In the present study it was found that ovum size varied in most species, with a marked tendency for size to increase as oviposition progressed, with a slight decrease in size at the end of the oviposition period. This is in contrast with the observations of Jones et al. (1982) who noted that eggs produced by individuals became smaller as females aged. Brakefield's data were based on weight, but Karlsson and Wiklund (1985) have demonstrated that this parameter is an invalid measure of egg size. In the present work egg sizes were assessed subjectively and precise measurements of intraspecific variation were not made.

Variation in longitudinal rib (keel) number was recorded in Maniola species in ova laid be the same female. Dennis and Richman (1985) noted that ova with eight and nine keels were found in single batches laid by Aglias urticae, but they were uncertain whether they were the offspring from one or more females. It is presumed that the gross architecture of the ova is determined in the progressively enlarging and maturing follicles within the ovarioles, and certainly before the eggs reach the oviduct (Stern and Smith 1960, Telfer 1965, Ehrlich

and Ehrlich 1978, Dunlap-Pianka 1979, Hinton 1981, Herman and Dallman 1981). No explanation can be suggested at present for such variation in longitudinal rib number. However, as this phenomenon might be widespread throughout Lepidoptera, care must be taken in its interpretation.

Differences in ova coloration was found to be of little evolutionary significance in the present context. Maniola and Pyronia ova are irregularly speckled light and dark brown, with extensive individual and geographical variation. Hyponephele and Cercyonis ova are uniformly pale yellow/grey.

Chorionic sculpturing revealed by scanning electron microscopy was found to a valuable indicator of evolutionary relationships in Maniolini as the illustrations show (plate 3.2.1a, b, c). Ultra-structural studies of chorionic sculpturing of the ova in the Maniolini group have never before been undertaken (section 1.3.3). A whole new taxonomy has been devised since the first electron microscope studies were published. In the following descriptions of the ova, the terminology used by Arbogast et al. (1984) has been followed.

Maniola jurtina

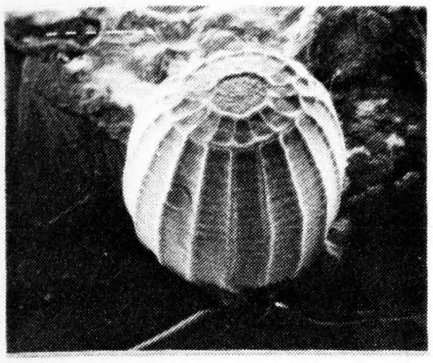
Material examined: 10 ova from 5 females, Parma, Italy; 10 ova from 2 females, Vienna, Austria; 10 ova from 2 females, Easdale Island, Argyll; 10 ova from 2 females, Kinvara, Co. Clare; 10 ova from 2 females, Head of Holland, Orkney.

Ova subspherical to truncated conical, tapering slightly to anterior pole. Longitudinal ribs 18 to 21, distinct. Transverse ribs fine, sinuous, usually incomplete.
Aeropyles numerous, located on the longitudinal rib apices, collars low or absent.
Anterior zone comprised of two distinct regions of primary and secondary cells, separated and bounded outwardly by distinct concentric ridges, formed of subparallel intersecting subridges. A third concentric tertiary cell region lies outwith the secondary cell ring,

plate 3.2.1a

Scanning electron micrographs of Manioline ova.

1. Maniola jurtina, Dunblane, Perthshire, X85, SEM0540/3
2. Maniola jurtina, Parma, Italy, X110, SEM0400/6
3. Maniola megala, Marmaris, Turkey, X110, SEM0580/2
4. Maniola telmessia, Hakkari, Turkey, X91, SEM0578/12
5. Maniola cypricola, Episkopi, Cyprus, X91, SEM0500/1
6. Maniola nurag, Nuoro, Sardinia, X85, SEM0532/9
7. Pyronia tithonus, St Bees, Cumbria, X87, SEM0558/12
8. Maniola bathseba, Madrid, Spain, X63, SEM0593/7
9. Pyronia cecilia, Sardinia, X90, SEM0511/2
10. Aphantopus hyperantus, Drumloist, Perthshire, X83, SEM0496/8
11. Hyponephele lycaon, Planet, Italy, X78, SEM0420/7
12. Cercyonis pegala, Macomb, USA, X73, SEM0457/5



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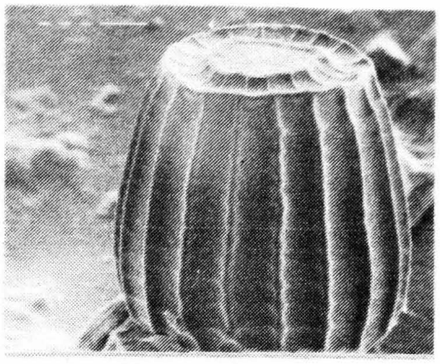

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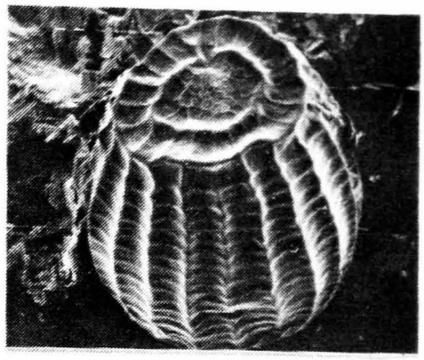

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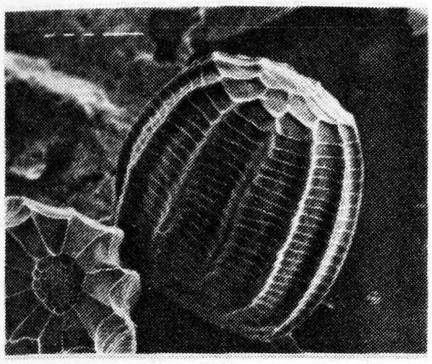

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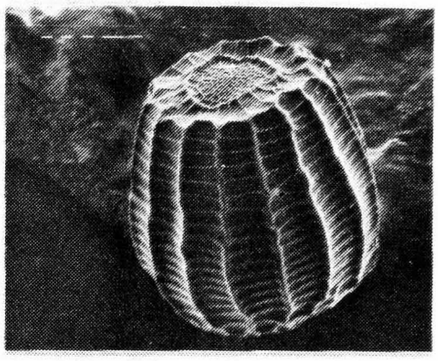

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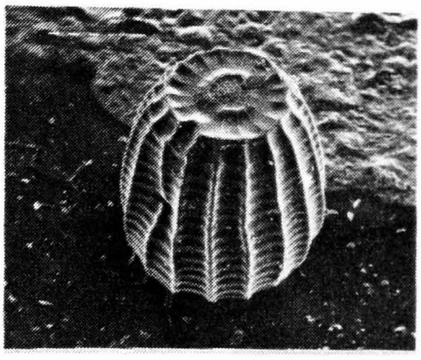

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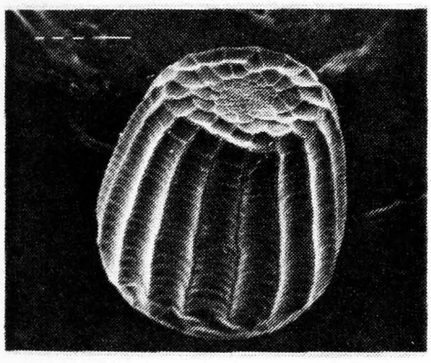

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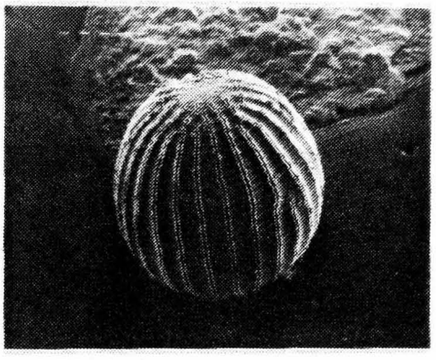

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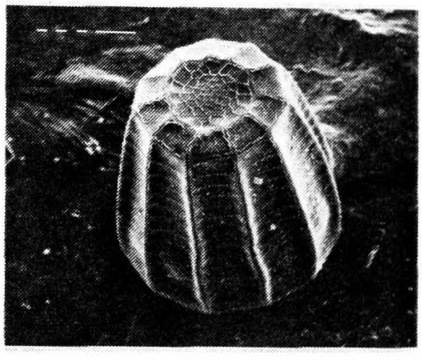

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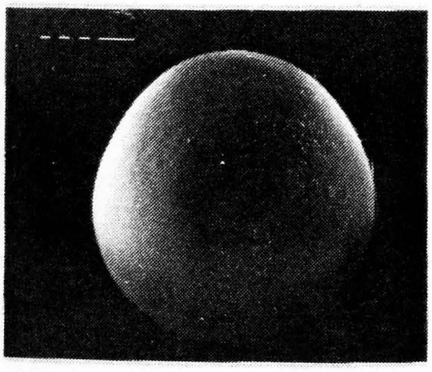

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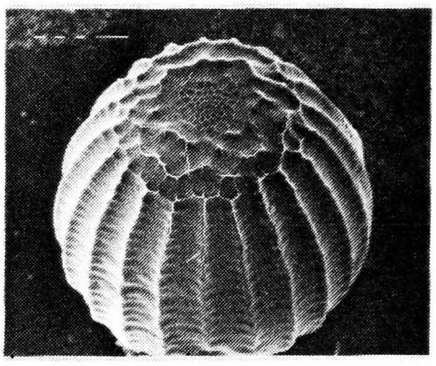

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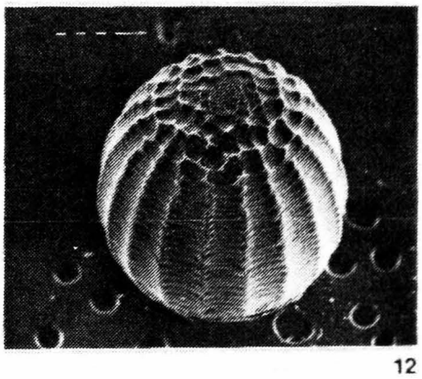

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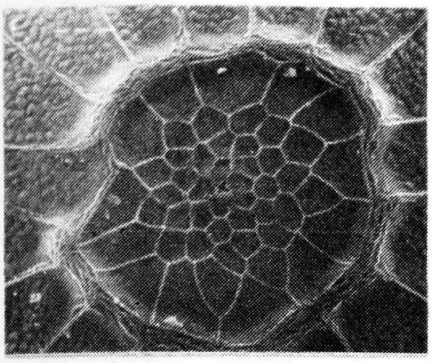
plate 3.2.1b

Scanning electron micrographs of Manioline ova - micropyle area

1. Maniola jurtina, Dunblane, Perthshire, X430, SEM0543/6
2. Maniola jurtina, Dunblane, Perthshire, X1790, SEM0540/5
3. Maniola megala, Marmaris, Turkey, X1410, SEM0582/4
4. Maniola telmessia, Hakkari, Turkey, X1970, SEM0573/6
5. Maniola cypricola, Episkopi, Cyprus, X1910, SEM0505/7
6. Maniola nurag, Nuoro, Sardinia, X1790, SEM0534/7
7. Pyronia tithonus, St Bees, Cumbria, X2600, SEM0551/4
8. Maniola bathseba, Fountain de Vaucluse, France, X730, SEM0406/1
9. Pyronia cecilia, Sardinia, X1910, SEM0515/7
10. Aphantopus hyperantus, Drumloist, Perthshire, X4100, SEM0498/11
11. Hyponephele lycaon, Planet, Italy, X5000, SEM0418/4
12. Cercyonis pegala, Macomb, USA, X1500, SEM0454/3



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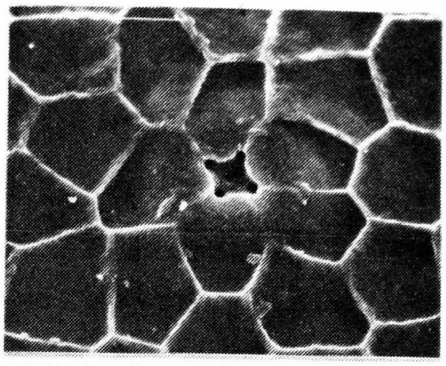

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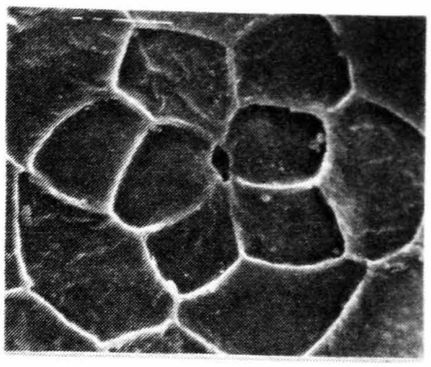

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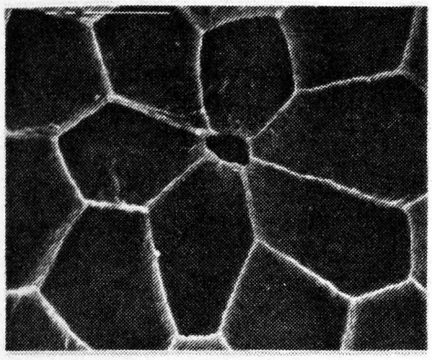

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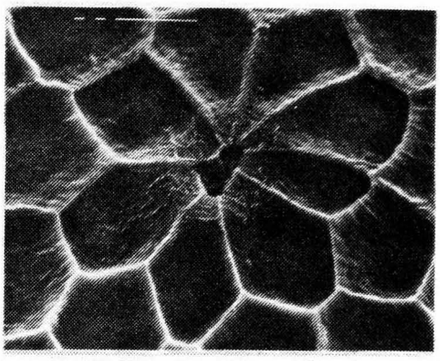

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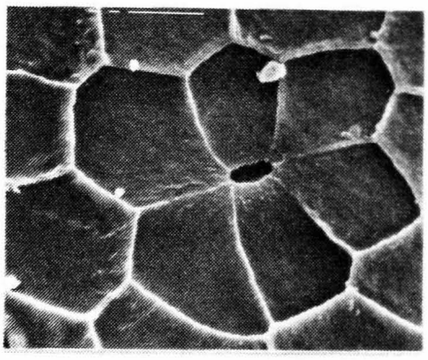

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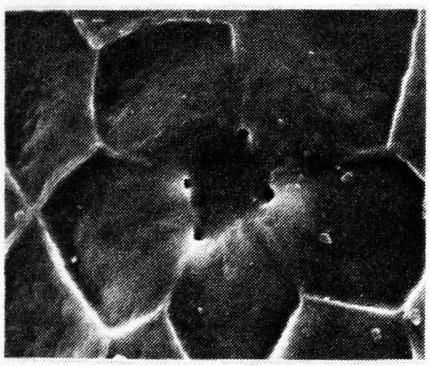

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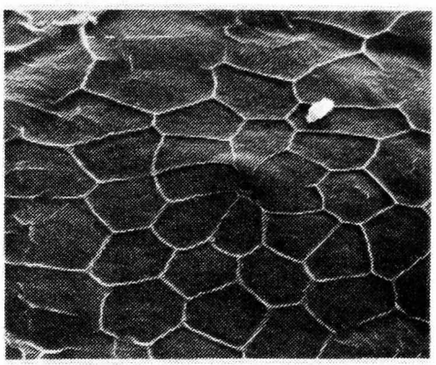

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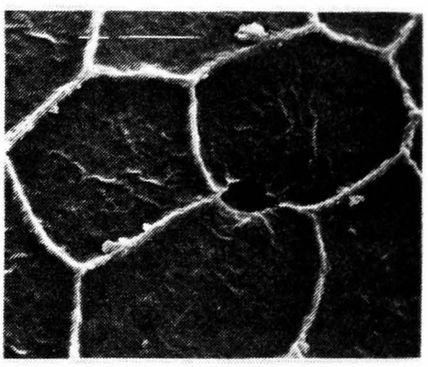

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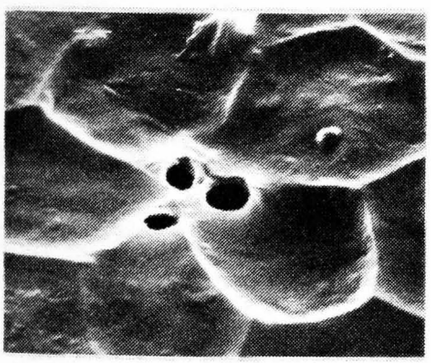

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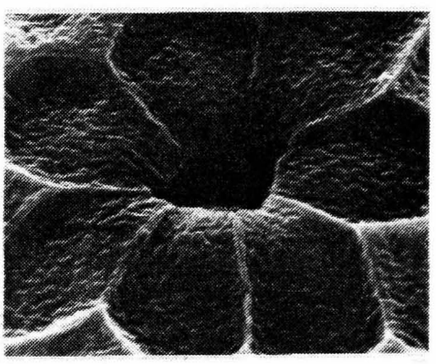

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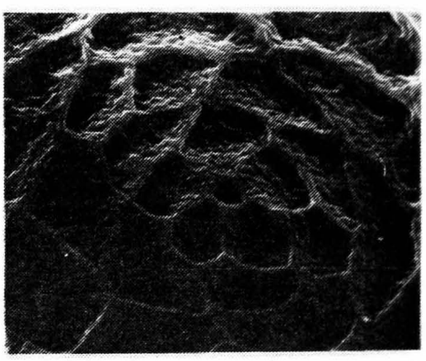

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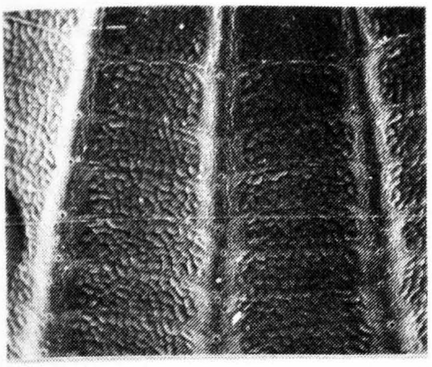
plate 3.2.1c

Scanning electron micrographs of Manioline ova - rib structure.

1. Maniola jurtina, Dunblane, Perthshire, X430, SEM0539/2
2. Maniola telmessia, Hakkari, Turkey, X390, SEM0570/2
3. Maniola cypricola, Episkopi, Cyprus, X450, SEM0501/2
4. Maniola nurag, Nuoro, Sardinia, X610, SEM0530/2
5. Pyronia tithonus, St Bees, Cumbria, X620, SEM0556/10
6. Maniola bathseba, Madrid, Spain, X250, SEM0595/10
7. Aphantopus hyperantus, Drumloist, Perthshire, X830, SEM0491/2
8. Pyronia cecilia, Sardinia, X440, SEM0512/4
9. Cercyonis pegala, Macomb, USA, X2120, SEM0455/2



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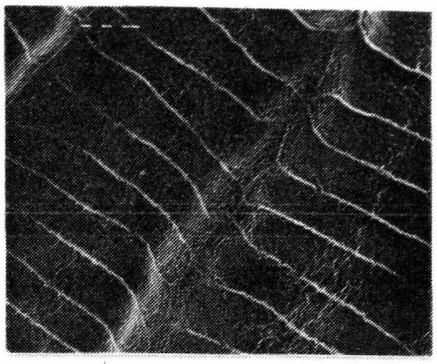

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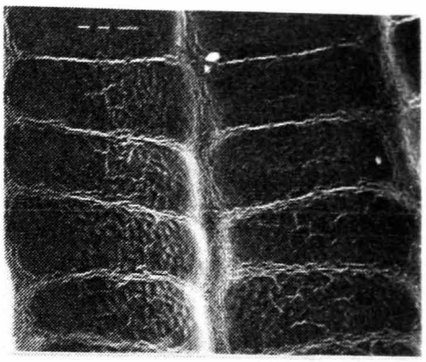

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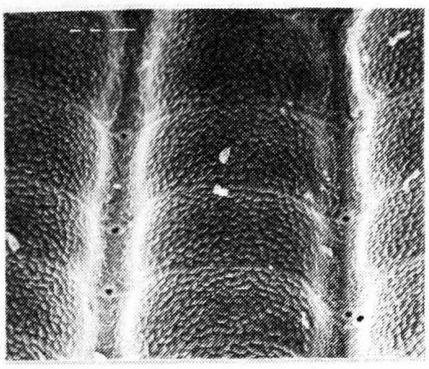

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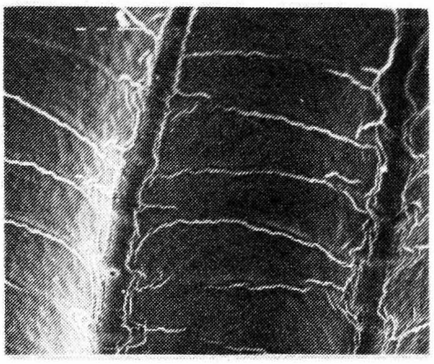

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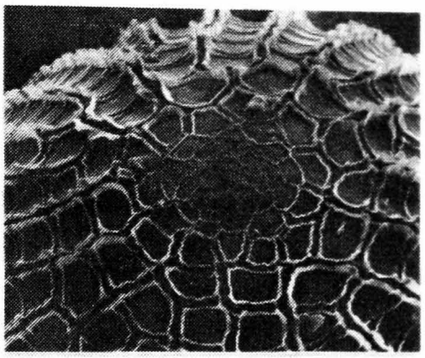

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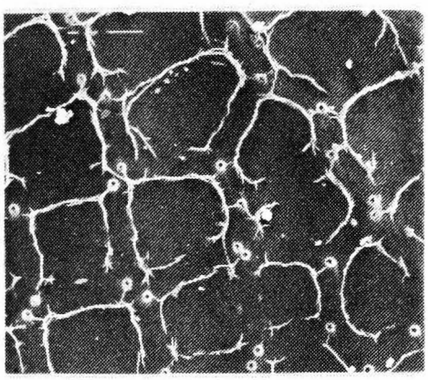

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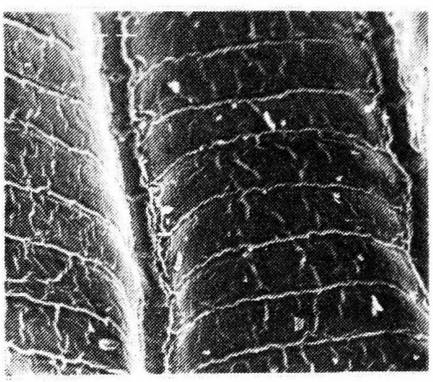

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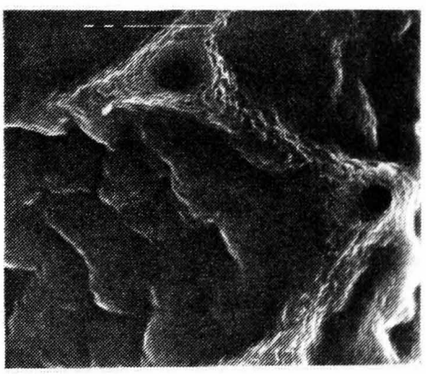

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indistinctly separated from and forming part of the longitudinal/transverse rib zone.
Micropylar canals two to four in number, set in a deep micropylar pit, surrounded by a rosette of three to six petal shaped primary cells, defined by distinct ridges. Other primary cells polygonal, complex, forming a web-like pattern. Ribs dividing secondary cells approximately similar to longitudinal ribs in number.
Chorion surface reticulate, covered with adjacent, irregularly shaped polygonal prominences.

Maniola megala

Material examined: 5 Ova from 1 female, Marmaris, Turkey.

Ova subspherical to truncated conical, tapering to anterior pole: taller than jurtina ova. Longitudinal ribs 19 to 21, distinct. Transverse ribs more distinct than in jurtina, usually complete.
Aeropyles numerous, located on the longitudinal rib apices, collars low or absent.
Anterior zone similar to jurtina, concentric ridges rather higher.
Micropylar canals two to four in number, set in a deep micropylar pit, surrounded by a rosette of five petal shaped primary cells, defined by distinct ridges. Other primary, secondary and tertiary cells similar to jurtina.
Chorion surface like jurtina.

Maniola chia

Material examined: 11 ova from 1 female, Nea Moni, Chios.

Ova shape as jurtina and megala, but shorter than either species. Longitudinal ribs 13 to 14, distinct.

Ova were not available for electron microscopy.

Maniola telmessia

Material examined: 5 ova from 1 female, Hakkari, Turkey; 15 ova from 1 female, Antalya, Turkey.

Ova barrel shaped to truncated conical. Longitudinal ribs 14 to 16, prominent. Transverse ribs distinct, complete.
Aeropyles numerous, located on the longitudinal rib apices, collars distinct.
Anterior zone comprised of primary, secondary and tertiary cell regions as jurtina. Primary and secondary regions separated by a prominent concentric ridge. Ridge separating secondary and tertiary regions usually less prominent.
Micropylar canals four in number, set in a deep micropylar pit, surrounded by a rosette of four to six petal shaped primary cells, defined by distinct ridges. Other primary and secondary cells fewer in number than jurtina.
Chorion surface sparsely covered with low prominences and fine, sinuous, irregular ridges: ridges most frequent in secondary cell region and in the interlongitudinal rib depressions.

Maniola cypricola

Material examined: 15 ova from 5 females, Paphos, Cyprus.

Ova barrel shaped to truncated conical. Longitudinal ribs 13 to 14, prominent (as telmessia). Transverse ribs distinct, sinuous and complete.
Aeropyles numerous, located on the longitudinal rib apices, collars distinct.
Anterior zone comprised of primary and secondary cell regions. Tertiary cell region indistinguishable.
Micropylar canals to three in number, set in a deep micropylar pit, surrounded by a rosette of eight petal shaped primary cells, defined by very distinct ridges. Other primary cells more numerous than in other Maniola species.
Chorion surface similar to telmessia.

Maniola nurag

Material examined: 6 ova from 1 female, Correboi, Sardinia.

Ova barrel shaped. Longitudinal ribs 16 to 17, prominent. Transverse ribs low, complete, not sinuous as in other Maniola.
Aeropyles located only at the junctures of the longitudinal and transverse ribs, collars distinct.
Anterior zone comprised of primary, secondary and tertiary cell regions.
Micropylar canals two in number, set in a deep micropylar pit, surrounded by a rosette of six petal shaped primary cells, defined by very distinct ridges. Other primary and secondary cells fewer in number than other Maniola species.
With the exception of primary, secondary and tertiary cell regions, chorion surface reticulate, covered with low polygonal prominences. Primary cell area irregularly sinuous. Secondary and tertiary cell regions with numerous sinuous ridges.

Pyronia tithonus

Material examined: 30 ova from 6 females, St Bees, Cumbria.

Ova barrel shaped, tapering along whole length to anterior pole. Longitudinal ribs 16, prominent. Transverse ribs fine, sinuous, usually complete.
Aeropyles numerous, located on the longitudinal rib apices, collars low or indistinct.
Anterior zone indistinctly divided into primary and secondary cell regions, separated and bounded by distinct single or double ridges. Concentric region of tertiary cells indistinctly separated from the longitudinal/transverse rib zone.
Micropylar canals four in number, set in a shallow, indistinct micropylar pit, surrounded by a rosette of five petal shaped primary cells, outwardly defined by distinct ridges. Other primary cells numerous, complex, forming a web-like pattern. Ribs dividing secondary cells approximately similar to longitudinal ribs in number.

Chorion surface indistinctly reticulate, covered with irregularly shaped prominences.

Pyronia bathseba

Material examined: 15 ova from 5 females, Fountain de Vaucluse, France; 10 ova from 1 females, Madrid, Spain; 10 ova from 1 female, Guadalajara, Spain.

Ova spherical to subspherical. Longitudinal ribs 22, distinct, and formed of paired, parallel, membranous ridges. Transverse ribs less distinct than longitudinal ribs, similarly formed.
Aeropyles absent or undetected.
Anterior zone comprised of a single area of primary and (possibly) secondary cells, defined by single distinct ridges.
Micropylar canals two in number, set in a shallow, distinct micropylar pit, surrounded by a rosette of five to six petal shaped primary cells, defined by distinct ridges. Secondary cells merge into longitudinal/transverse rib zone.
Chorion surface smooth.

Pyronia cecilia

Material examined: 10 ova from 2 females, Nuoro, Sardinia.

Ova truncated conical, sides straight or concave. Longitudinal ribs 10 to 12, very prominent. Transverse ribs fine, sinuous and complete. Aeropyles present only at the junctures between longitudinal and transverse ribs, collars distinct.
Anterior zone slightly concave, flat or slightly convex (possibly dependent upon age and/or desiccation), divided very distinctly into primary and secondary cell regions by prominent concentric ridges.
Micropylar canals two (possibly up to five) in number, set in a deep micropylar pit, surrounded by a rosette of four or five petal shaped primary cells. Other primary cells numerous, complex forming a web-like pattern. Ribs defining secondary cells of the same number as longitudinal ribs.

Chorion surface covered with fine sinuous, irregular and incomplete ridges.

Aphantopus hyperantus

Material examined: 20 ova from 5 females, Gignod, Italy; 30 ova from 5 females, Doune, Perthshire.

Ova spherical to subspherical, tapering slightly to anterior pole. Longitudinal and transverse ribs detectable only as subregular patterns of sinuous, paired, parallel ridges on barely distinguishable, very slightly raised ribs.
Aeropyles present over whole chorion surface, collars distinct.
Anterior zone detectable only as a very slight depression, not clearly divided into primary and secondary cell regions.
Micropylar canals three in number, not set in a micropylar pit, surrounded by a very indistinct rosette of petal shaped primary cells. Ridges low or absent. Secondary cells, when detectable, with low, single ridges.
Chorion surface smooth.

Hyponephele lycaon

Material examined: 20 ova from 5 females, Monti Sibillini, Italy.

Ova spherical. Longitudinal ribs 21, prominent. Transverse ribs numerous, low and detectable only at their junctures with the longitudinal ribs.
Aeropyles numerous, collars indistinct.
Anterior zone comprised of two regions of primary and secondary cells.
Micropylar canals three in number, set in a deep micropylar pit, surrounded by a rosette of five to seven petal shaped primary cells, defined by prominent ridges. Other primary cells polygonal, complex, increasing in size radially. Secondary cells incomplete, partially defined by distinct ridges.

table 3.2.1a

Principal character states of chorionic sculpturing in ova of Maniolini.

species	shape	longitudinal ribs	transverse ribs	anterior zone areas	micropyle canal number	primary cell petals	surface
jurtina	subcylindrical to truncated conical	18-21/ distinct	fine incomplete sinuous	3	2-4	3-6	polygonal promins**
megala	subcylindrical to truncated conical	19-21/ distinct	fine complete sinuous	3	2-4	5	polygonal promins**
telmessia	barrel shaped to truncated conical	14-16/ prominent	distinct complete sinuous	3	4	4-6	polygonal promins** + s/ribs*
cypricola	barrel shaped to truncated conical	13-14/ prominent	distinct complete sinuous	2	3	8	polygonal promins** + s/ribs*
nurag	barrel shaped	16-17/ prominent	low complete not sinuous	2	2	6	polygonal promins** + s/ribs*
tithonus	barrel shaped	16/ prominent	fine complete sinuous	3	4	5	polygonal promins**

* sinuous ribs ** prominences

table 3.2.1a (continued)

species	shape	longitudinal ribs	transverse ribs	anterior zone areas	micropyle canal number	primary cell petals	surface
bathseba	spherical to subspherical	20/ distinct	distinct paired sinuous	2	2	5-6	smooth
cecilia	truncated conical	10-12/very prominent	fine complete sinuous	2	2 (-5)	5	sinuous
hyperantus	spherical	very indistinct (? absent)	very indistinct	1	3	0	smooth
lycaon	spherical	21/ prominent	low	2	3	5-7	pitted
pegala	spherical	18/ prominent	low	1-2	2	4	deeply wrinkled

* sinuous ribs ** prominences

table 3.2.1b

Oviposition strategy of Maniolini in captivity: degree of ova adhesion to foodplant or other substrate.

	very firm	firm	light	very light	not fixed
Maniola jurtina			*	*	*
Maniola megala			*	*	*
Maniola chia			*	*	*
Maniola telmessia		*	*		
Maniola cypricola		*	*		
Maniola nurag		*	*		
Pyronia tithonus			*	*	
Pyronia bathseba				*	*
Pyronia cecilia	*				
Aphantopus hyperantus					*
Hyponephele lycaon	*				
Cercyonis pegala	*				

Chorion surface deeply pitted over whole surface, except primary cell area. Primary cell area irregularly and deeply wrinkled.

Cercyonis pegala

Material examined: 10 ova from 2 females, Macombe, Illinois, USA.

Ova spherical. Longitudinal ribs 18, prominent. Transverse ribs numerous, low and detectable only at their junctures with the longitudinal ribs.
Aeropyles numerous, collars indistinct.

Anterior zone comprised of two illdefined regions of primary and secondary cells.
Micropylar canals two in number, set in a very deep micropylar pit, surrounded by a rosette of four petal shaped primary cells, defined by very prominent ridges. Secondary cells incomplete, outwardly defined by prominent ridges.
Chorion surface, including primary cell area, entirely covered with a complex series of deep, convoluted rib-like structures.

The principal character state differences described above are summarised in table 3.2.1a. Oviposition strategy is listed in table 3.2.1b.

The complex morphology of these ova suggests evolutionary relationships which are in agreement with accepted systematic arrangements, although the ova of Pyronia tithonus is remarkably similar to that of Maniola species, and much closer to them than to its congeners. Ova of Maniola are very similar to each other, with only minor, although important, differences in ribbing and ultrastructure. Hyponephele and Cercyonis ova have similarities which suggest close affinity. The simple, spherical ova of Aphantopus are unique in the group, although the structure of the ribs, when present, is similar to that of bathseba ova. Interspecific differences between Pyronia ova are greater than between species in other genera.

It is suggested that these large intrageneric differences in Pyronia and occasional transgeneric similarities in ova morphology (between Maniola species and Pyronia tithonus, and between Pyronia bathseba and Aphantopus hyperantus) are a consequence of oviposition strategy. In some instances, selection associated behavioural adaptations of this sort are so great (as in Pyronia) that they appear to contribute to changes in the expected genetically determined archetype. Although not a direct corollary of this hypothesis, it is worth noting that the mechanism for similar evolutionary events, in the form of large 'jumps' to alternative foodplants, was proposed by Chew and Robbins (1984). However, some features of the ova must be determined genetically. The relationships between jurtina and megala and between telmessia, cypricola and nurag can be seen in characteristics of the chorionic ultrastructure, while evolutionary affinities must be

inferred from the very similar ova of Hyponephele and Cercyonis, genera found in two continents separated by several thousand kilometers.

3.2.2 Larval chaetotaxy

The larvae of Manioline butterflies, including those of the first instar, are identical in basic morphology to larvae of all Lepidoptera, being formed of the head (or cranium), three thoracic segments, each with a pair of 'true' legs, and ten abdominal segments. On the third, fourth, fifth and sixth abdominal segments are pairs of ventral prolegs and on the tenth segment a pair of anal prolegs (figure 3.2.2a). On the distal end of each proleg is a planta to which the crochets are attached. Several names have been advocated for the areas of the cranium (figure 3.2.2b). Those of Fleming (1960) have been adopted here. The head, thorax and abdomen are furnished with spines or setae of which Hinton (1946) distinguished two types, macrosetae, mainly tactile receptors, and microsetae, the proprioceptors. Some setae, the subprimaries and usually the secondaries, are not present in the first instar. This study is concerned with the setae of the first instar larvae only.

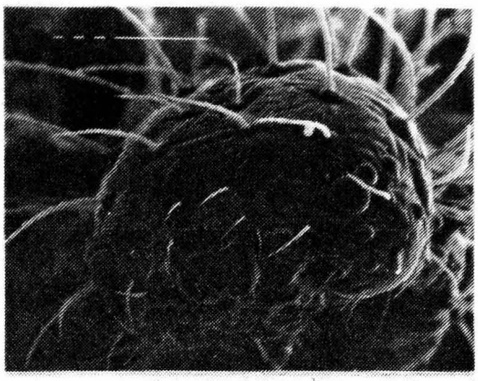
Although not strictly chaetotaxy as such, in the course of chaetotaxy work differences were noted in larval cranial ultrastructure. The cranium surface of Maniola species was smooth. That of Hyponephele and Cercyonis was heavily textured by a series of convolutions covering the whole surface and similar to that already described in the ova of these genera. Pyronia and Aphantopus were intermediate in this respect having a sparse series of 'wrinkles' over several parts of the head (plate 3.2.2a). In optical magnification the cranial texture gave the appearance of speckling.

The principal aim of the chaetotaxy analysis was to examine interspecific relationships. The first instar larval setae of 12 Manioline species were examined. Unfortunately, larvae of Maniola megala were not available for study. Larvae were obtained from the same localities as those for SEM studies of ova (section 3.2.1).

plate 3.2.2a

Scanning electron micrographs of Manioline first instar larval crania.

1. Maniola jurtina, County Clare, Ireland, X216, SEM0477/5
2. Maniola telmessia, Hakkari, Turkey, X1170, SEM0613/12 - 'punctures' F_a
3. Pyronia tithonus, St Bees, Cumbria, X264, SEM0568/12
4. Pyronia tithonus, St Bees, Cumbria, X630, SEM0566/10
5. Aphantopus hyperantus, Drumloist, Perthshire, X153, SEM0525/7
6. Aphantopus hyperantus, Drumloist, Perthshire, X540, SEM0528/12
7. Hyponephele lycaon, M. Sibillini, Italy, X174, SEM0478/6
8. Hyponephele lycaon, M. Sibillini, Italy, X760, SEM0487/10



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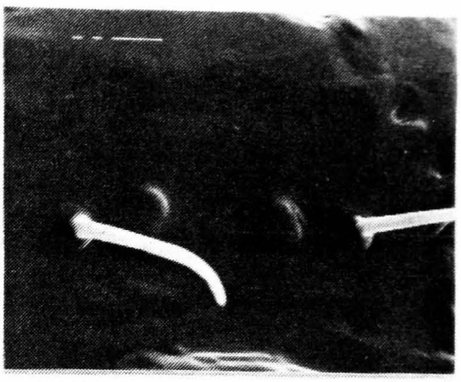

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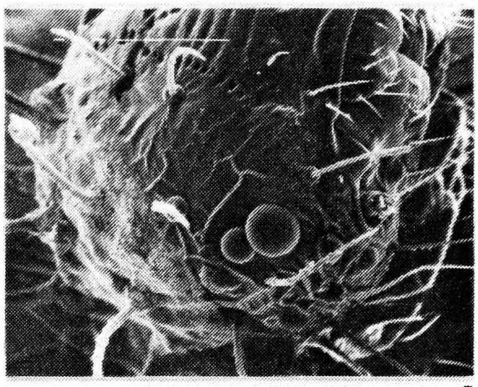

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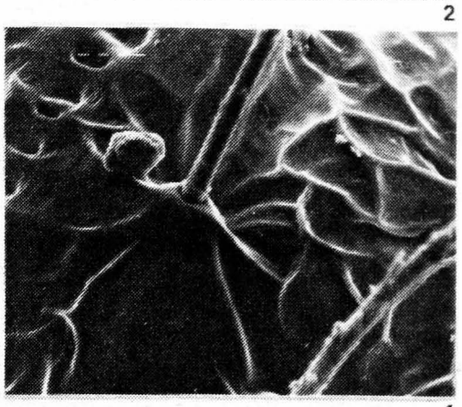

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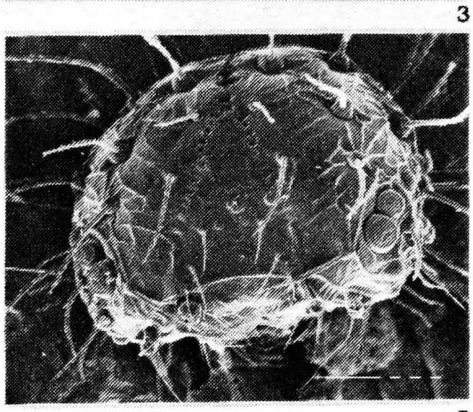

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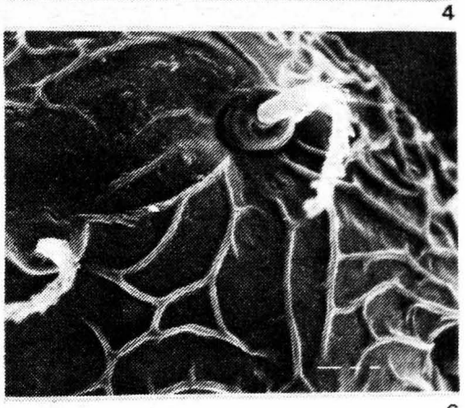

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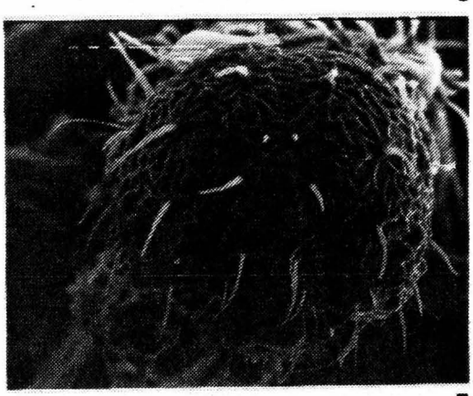

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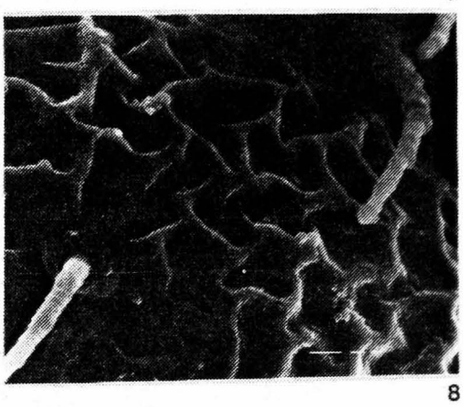

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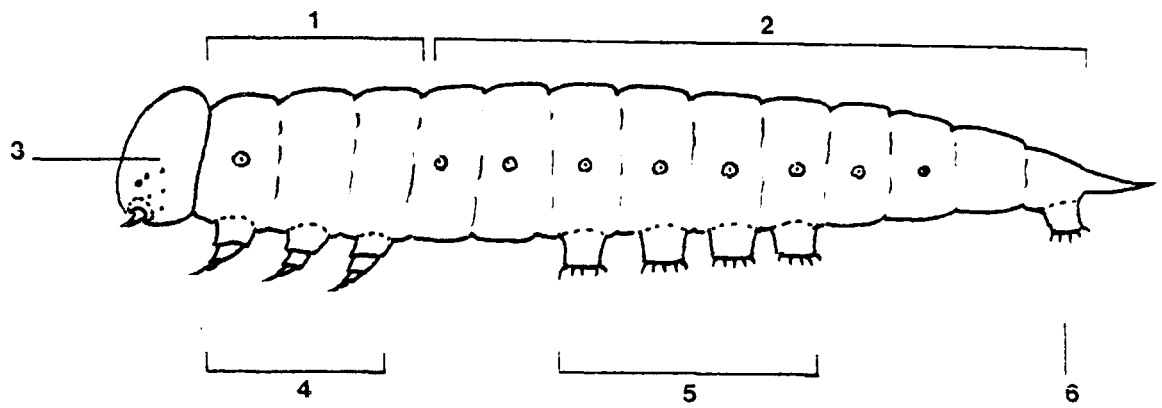


figure 3.2.2a

External anatomy of a Manioline larva 1. thoracic segments 2. abdominal segments 3. cranium 4. 'true' legs 5. ventral prolegs 6. anal prolegs

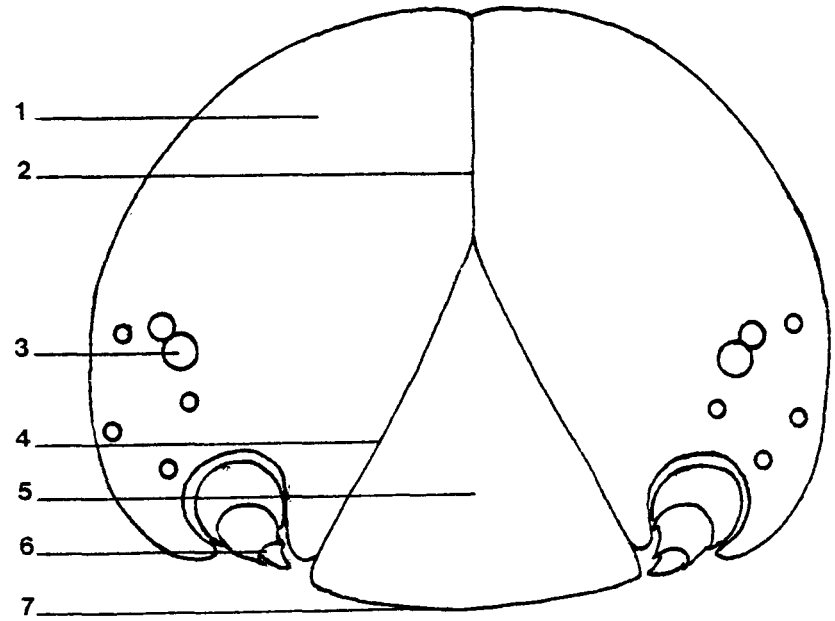


figure 3.2.2b

Nomenclature of larval cranium (Fleming 1960) 1. epicranium 2. epicranial stem 3. ocellus 4. frontal suture 5. front 6. antenna 7. clypeus

Homologies are not easy to ascertain and that of some setae must remain tentative. Most authors follow the accounts of Hinton (1946, 1947) and his illustrations of the chaetotaxy of Pieris brassicae. No exception has been made here, except that the nomenclature proposed for the tenth abdominal segment by Kitching (1984) is adopted in preference to the older systems. Homologies were also established by comparison with the illustration of Fleming (1960) and Kitching (1984).

Almost all published butterfly larval chaetotaxy studies have been concerned with the presence or absence, relative positions and relative lengths of setae. Many, if not most, of these have relied on subjective assessments of relative position and length, a notable exception being Fleming (1960) who tabulates the length of all cranial, thoracic and abdominal setae in Heliconius.

The juxtaposition of setae was noted, but not measured as it was obvious at an early stage that there appeared to be observable differences only between Maniola/Pyronia/Aphantopus and Hyponephele/Cercyonis (figure 3.2.2c). If other interspecific differences in setal topography exist, these were not detected.

Lengths of 74 cranial, thoracic and abdominal setae were measured by projection from prepared tissues (sections 1.3.4 and 1.3.6). The lengths of the cranial setae were checked against measurements from scanning electron micrographs of preparations from different specimens of the same species. If discrepancies were found between the optical and SEM dimensions, the mean was calculated. From most samples, 12 specimens each per species were measured. The setae of 12 specimens from three populations of Maniola jurtina were measured, Rabat (Morocco), Vienna (Austria) and Dunblane (Perthshire). In addition, the width of the cranium of each species at its widest point was recorded. The measurement error was estimated (by repeat measurement tests) at less than 4% for the longest spines and less than 6% for the shortest. The setae of Maniola, Pyronia and Aphantopus were found to be much straighter than those of Hyponephele and Cercyonis. This gave them the superficial appearance of being much longer than they actually were.

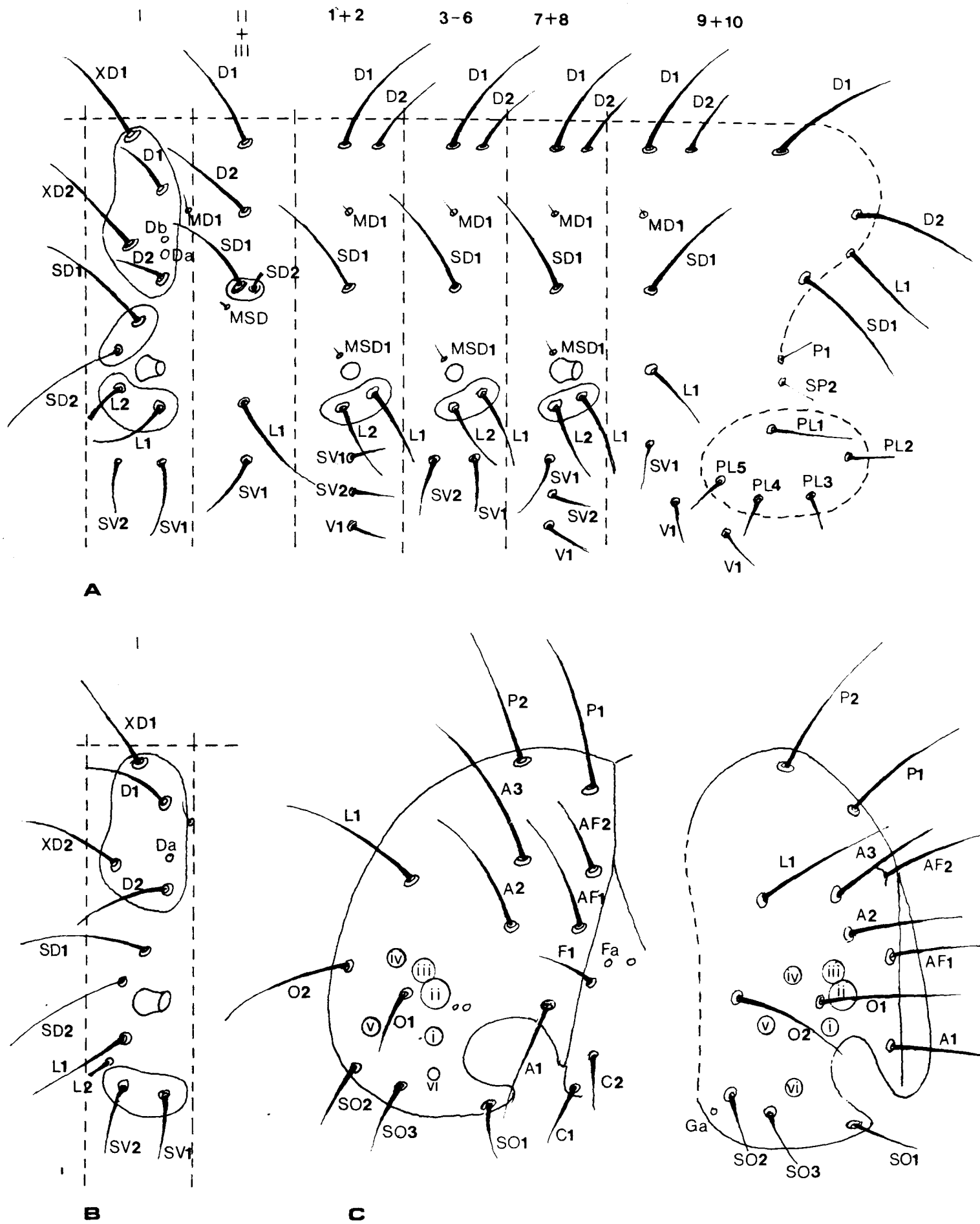


figure 3.2.2c

Chaetotaxy of Maniolini (first instar larva) A Hyponephele B Maniola, Pyronia and Aphantopus - segment I (all other segments identical to A) C cranium - all genera

Examination of the raw data confirmed that the measurements from each species within single samples was distributed normally about the mean.

Spine lengths and their percentage of cranial widths are given in table 3.2.2a. The standard error for spine lengths within samples was remarkably small and it was felt that data from 12 specimens could be utilised with some confidence. Geographical variation was observed in the three jurtina populations, the mean spine lengths decreasing northwards in the samples, but by very little. The presumption made in earlier chaetotaxy studies that setae on thoracic segments II and III, abdominal segments 3 to 6 and abdominal segments 7 and 8 are identical was checked and found to be true also in Maniolini. Thoracic segment II and abdominal segment 3 were measured to represent setae on the other segments.

It should be noted that the precise homologies of the lineal and subventral groups on the thoracic segment I have not been determined, especially those of L2 and SV2. This also applies to the cranial setae O2 and O3 and the designation of these setae must be treated as provisional.

Scanning electron microscopy revealed that the clypeal feature described in papers as 'puncture F_a' was not a puncture or hole as believed, but a raised nipple (plate 3.2.2a:2). Whether all so-called punctures are of this nature was not discovered and, until a full survey has been completed, the term 'puncture' has been retained.

Description of the larval chaetotaxy of Maniola jurtina

HEAD

Frontals and clypeals

Puncture C_a absent. C1 in angle formed by frontal and clypeal suture. C2 about one third the distance between C1 and F1 and slightly mesad of a line between the setae and less than half the length of C2. F1

table 3.2.2a

Larval chaetotaxy of Maniola, Pyronia, Aphantopus, Hyponephele and Cercyonis - setal lengths in microns and their percentage of cranial width.

Thoracic segment I

		XD1	XD2	D1	D2	SD1	SD2	L1	L2	SV1	SV2	V1
M. jurtina		347	271	184	47	217	173	103	23	103	27	-
	SE	0.58	0.64	0.53	0.57	0.60	0.43	0.69	0.47	0.84	0.60	
	%	93.1	73.1	49.1	12.3	58.2	46.1	27.3	6.0	27.0	7.1	-
M. chia		238	161	119	24	179	119	161	18	77	89	-
	SE	0.70	0.67	0.60	0.83	0.64	0.45	0.73	0.75	0.57	0.83	-
	%	80.0	54.0	40.0	8.0	60.0	40.0	54.0	6.0	26.0	30.0	-
M. telmessia		192	154	139	31	208	162	89	0	92	58	-
	SE	0.80	0.76	0.72	1.05	0.73	0.49	0.90	0	0.70	1.01	-
	%	54.9	44.0	39.7	8.9	59.4	46.3	25.4	0	26.3	16.6	-
M. cypricola		225	217	150	67	217	167	67	11	67	54	-
	SE	0.80	0.76	0.72	1.05	0.73	0.49	0.90	0.91	0.70	1.01	-
	%	59.2	57.1	39.5	17.6	57.1	44.0	17.6	3.0	17.6	14.2	-
M. nurag		208	167	200	71	200	158	54	5	75	46	-
	SE	0.72	0.70	0.66	0.92	0.65	0.46	0.79	0.81	0.61	0.90	-
	%	59.4	47.7	57.1	20.3	57.1	45.1	15.4	1.5	21.4	13.1	-
P. tithonus		281	180	313	47	281	125	109	0	117	51	-
	SE	0.86	0.78	0.72	1.09	0.82	0.52	0.95	0	0.75	1.05	-
	%	63.9	40.9	71.1	10.7	64.9	28.4	24.8	0	26.6	11.6	-
P. bathseba		199	192	167	90	186	173	128	0	77	55	-
	SE	0.72	0.70	0.66	0.92	0.65	0.46	0.79	0	0.61	0.90	-
	%	44.3	42.9	37.1	20.0	41.4	38.6	28.6	0	17.1	12.1	-
P. cecilia		250	152	258	46	110	133	83	0	83	68	-
	SE	0.70	0.67	0.60	0.83	0.64	0.45	0.73	0	0.57	0.83	-
	%	69.4	42.2	71.7	12.8	30.6	37.0	23.1	0	23.1	18.9	-
A. hyperantus		333	283	283	292	267	150	133	75	58	58	-
	SE	0.65	0.64	0.63	0.86	0.56	0.41	0.74	0.86	0.56	0.76	-
	%	67.0	57.0	57.0	58.0	53.0	30.0	27.0	15.0	12.0	12.0	-
H. lycaon		141	125	67	66	135	130	86	21	66	66	-
	SE	0.57	0.51	0.47	0.72	0.57	0.34	0.63	0.69	0.50	0.63	-
	%	34.0	30.1	16.0	16.0	32.0	31.2	20.0	5.0	16.0	16.0	-
H. lupina		163	192	146	38	142	92	58	21	42	52	-
	SE	0.50	0.50	0.48	0.64	0.44	0.32	0.55	0.65	0.41	0.56	-
	%	42.6	50.1	38.1	9.9	37.1	24.0	15.1	5.5	11.0	13.6	-
C. pegala		152	146	65	63	94	157	73	27	36	35	-
	SE	0.53	0.55	0.51	0.66	0.46	0.34	0.57	0.65	0.42	0.57	-
	%	30.1	29.2	13.1	12.3	18.0	31.4	14.2	5.0	7.0	7.0	-

table 3.2.2a (continued)

Thoracic segments II and III

		XD1	XD2	D1	D2	SD1	SD2	L1	L2	SV1	SV2	V1
M. jurtina		-	-	201	195	141	23	163	-	81	-	-
	SE	-	-	0.66	0.54	0.63	0.42	0.58	-	0.47	-	-
	%	-	-	54.0	52.4	38.3	6.0	44.0	-	21.1	-	-
M. chia		-	-	202	125	143	39	107	-	60	-	-
	SE	-	-	0.66	0.72	0.53	0.32	0.79	-	0.58	-	-
	%	-	-	68.0	42.0	48.0	13.0	36.0	-	20.0	-	-
M. telmessia		-	-	165	127	142	19	81	-	69	-	-
	SE	-	-	0.91	0.75	0.86	0.56	0.36	-	0.97	-	-
	%	-	-	47.1	36.3	40.6	5.4	23.1	-	19.7	-	-
M. cypricola		-	-	208	188	217	25	175	-	100	-	-
	SE	-	-	0.75	0.86	0.56	0.36	0.97	-	0.65	-	-
	%	-	-	54.7	49.5	57.1	6.6	46.1	-	26.3	-	-
M. murag		-	-	179	158	192	33	158	-	92	-	-
	SE	-	-	0.70	0.76	0.53	0.33	0.86	-	0.61	-	-
	%	-	-	51.1	45.1	54.9	9.4	45.1	-	26.3	-	-
P. tithonus		-	-	277	133	242	59	188	-	125	-	-
	SE	-	-	0.96	0.76	0.92	0.60	0.39	-	0.99	-	-
	%	-	-	63.0	30.2	55.0	13.4	42.7	-	28.4	-	-
P. bathseba		-	-	180	183	151	61	67	-	87	-	-
	SE	-	-	0.81	0.70	0.76	0.53	0.33	-	0.86	-	-
	%	-	-	40.0	40.7	33.6	13.6	15.0	-	19.3	-	-
P. cecilia		-	-	212	133	242	49	159	-	114	-	-
	Se	-	-	0.75	0.66	0.72	0.53	0.32	-	0.79	-	-
	%	-	-	58.9	36.9	67.2	13.6	44.2	-	31.7	-	-
A. hyperantus		-	-	308	208	325	83	166	-	83	-	-
	SE	-	-	0.64	0.70	0.50	0.30	0.83	-	0.58	-	-
	%	-	-	62.0	42.0	65.0	17.0	33.0	-	17.0	-	-
H. lycaon		-	-	168	92	119	27	119	-	65	-	-
	SE	-	-	0.48	0.61	0.41	0.27	0.65	-	0.39	-	-
	%	-	-	40.2	22.0	29.2	6.1	29.0	-	15.3	-	-
H. lupina		-	-	133	58	104	17	67	-	67	-	-
	SE	-	-	0.50	0.53	0.40	0.24	0.62	-	0.45	-	-
	%	-	-	34.7	15.1	27.2	4.4	17.5	-	17.5	-	-
C. pegala		-	-	173	146	173	32	108	-	54	-	-
	SE	-	-	0.55	0.54	0.41	0.25	0.64	-	0.49	-	-
	%	-	-	33.8	29.1	33.8	6.0	20.9	-	10.0	-	-

table 3.2.2a (continued)

Abdominal segments 1 and 2

		XD1	XD2	D1	D2	SD1	SD2	L1	L2	SV1	SV2	V1
M. jurtina		-	-	227	108	184	-	168	97	70	32	32
	SE	-	-	0.61	0.44	0.63	-	0.59	0.45	0.76	0.57	0.62
	%	-	-	74.5	29.0	49.1	-	45.4	26.0	18.0	8.1	8.1
M. chia		-	-	196	66	125	-	86	83	48	39	36
	SE	-	-	0.75	0.67	0.54	-	0.56	0.58	0.72	0.57	0.48
	%	-	-	66.0	22.0	42.0	-	29.0	28.0	16.0	13.0	12.0
M. telmessia		-	-	192	65	162	-	146	108	42	23	15
	SE	-	-	0.65	0.90	0.79	-	0.63	0.61	0.69	0.83	0.65
	%	-	-	54.9	18.6	46.3	-	41.7	30.9	12.0	6.6	4.6
M. cypricola		-	-	225	75	158	-	250	117	75	42	42
	SE	-	-	0.90	0.79	0.63	-	0.61	0.69	0.83	0.65	0.58
	%	-	-	59.2	19.7	41.6	-	65.8	30.8	19.7	11.1	11.1
M. murag		-	-	238	71	179	-	175	92	33	33	33
	SE	-	-	0.80	0.73	0.58	-	0.58	0.63	0.74	0.60	0.52
	%	-	-	68.0	20.3	51.1	-	50.0	26.3	9.4	9.4	9.4
P. tithonus		-	-	258	125	137	-	223	125	102	55	35
	SE	-	-	0.65	0.95	0.80	-	0.65	0.61	0.71	0.91	0.68
	%	-	-	58.6	28.4	31.1	-	50.7	28.4	23.2	12.5	8.0
P. bathseba		-	-	205	87	167	-	160	186	48	16	14
	SE	-	-	0.61	0.80	0.73	-	0.58	0.58	0.63	0.74	0.60
	%	-	-	45.7	19.3	37.1	-	35.7	41.4	10.7	3.6	3.1
P. cecilia		-	-	239	106	155	-	174	133	61	30	15
	SE	-	-	0.58	0.75	0.67	-	0.54	0.56	0.58	0.72	0.57
	%	-	-	66.4	29.4	43.0	-	48.3	36.9	16.9	8.3	4.2
A. hyperantus		-	-	283	100	242	-	242	75	50	42	42
	SE	-	-	0.74	0.70	0.52	-	0.56	0.60	0.67	0.56	0.49
	%	-	-	57.0	20.0	48.0	-	48.0	15.0	10.0	8.0	8.0
H. lycaon		-	-	163	97	103	-	119	108	45	43	38
	SE	-	-	0.64	0.52	0.44	-	0.39	0.47	0.61	0.45	0.41
	%	-	-	39.0	23.3	25.1	-	28.8	26.0	10.0	10.4	9.1
H. lupina		-	-	217	88	113	-	92	83	77	33	27
	SE	-	-	0.56	0.54	0.41	-	0.44	0.46	0.51	0.44	0.38
	%	-	-	56.7	23.0	29.5	-	24.0	21.7	20.1	8.6	7.1
C. pegala		-	-	195	152	157	-	163	141	38	27	21
	SE	-	-	0.58	0.57	0.46	-	0.47	0.48	0.53	0.47	0.39
	%	-	-	39.0	30.0	31.2	-	32.0	27.5	7.0	5.0	4.0

table 3.2.2a (continued)

Abdominal segments 3 to 6

		XD1	XD2	D1	D2	SD1	SD2	L1	L2	SV1	SV2	V1
M. jurtina		-	-	277	108	184	-	168	97	32	32	-
	SE	-	-	0.53	0.66	0.70	-	0.56	0.62	0.62	0.54	-
	%	-	-	74.3	29.3	49.0	-	45.1	26.0	8.0	8.0	-
M. chia		-	-	173	83	113	-	95	60	30	36	-
	SE	-	-	0.81	0.72	0.60	-	0.68	0.66	0.80	0.65	-
	%	-	-	58.0	28.0	38.0	-	32.0	20.0	10.0	12.0	-
M. telmessia		-	-	192	54	131	-	146	42	35	35	-
	SE	-	-	0.58	0.97	0.86	-	0.76	0.83	0.78	0.99	-
	%	-	-	54.9	15.4	37.4	-	41.7	12.0	10.0	10.0	-
M. cypricola		-	-	233	100	225	-	192	92	42	63	-
	SE	-	-	0.97	0.86	0.76	-	0.83	0.78	0.99	0.80	-
	%	-	-	61.3	26.3	59.2	-	50.5	24.2	11.1	16.6	-
M. murag		-	-	217	92	183	-	167	92	17	17	-
	SE	-	-	0.87	0.77	0.68	-	0.74	0.68	0.88	0.70	-
	%	-	-	62.0	26.3	52.3	-	47.7	26.3	4.9	4.9	-
P. tithonus		-	-	246	125	156	-	215	133	74	117	-
	SE	-	-	0.60	1.01	0.90	-	0.76	0.88	0.86	1.03	-
	%	-	-	55.9	28.4	35.5	-	48.9	30.2	16.8	26.6	-
P. bathseba		-	-	192	135	122	-	96	103	48	31	-
	SE	-	-	0.52	0.87	0.77	-	0.68	0.74	0.68	0.88	-
	%	-	-	42.9	30.0	27.1	-	21.4	22.9	10.7	6.9	-
P. cecilia		-	-	227	106	182	-	144	114	34	30	-
	SE	-	-	0.48	0.81	0.72	-	0.60	0.68	0.66	0.80	-
	%	-	-	63.1	29.4	50.6	-	40.0	31.7	9.4	8.3	-
A. hyperantus		-	-	308	100	242	-	242	75	58	42	-
	SE	-	-	0.81	0.71	0.66	-	0.69	0.60	0.83	0.62	-
	%	-	-	62.0	20.0	48.0	-	48.0	15.0	12.0	8.0	-
H. lycaon		-	-	163	97	103	-	119	108	38	38	-
	SE	-	-	0.66	0.60	0.50	-	0.59	0.60	0.68	0.59	-
	%	-	-	39.0	23.1	25.3	-	29.4	26.1	9.0	9.0	-
H. lupina		-	-	158	79	105	-	154	54	42	42	-
	SE	-	-	0.62	0.54	0.49	-	0.52	0.47	0.62	0.47	-
	%	-	-	41.3	20.6	27.4	-	40.2	14.1	11.0	11.0	-
C. pegala		-	-	271	173	157	-	125	114	36	36	-
	SE	-	-	0.64	0.57	0.51	-	0.54	0.49	0.63	0.50	-
	%	-	-	54.0	34.4	30.9	-	25.0	22.4	7.0	7.0	-

table 3.2.2a (continued)

Abdominal segment 7 and 8

		XD1	XD2	D1	D2	SD1	SD2	L1	L2	SV1	SV2	V1
M. jurtina		-	-	239	103	184	-	152	108	38	32	32
	SE	-	-	0.43	0.55	0.53	-	0.62	0.49	0.64	0.68	0.64
	%	-	-	64.1	27.0	48.8	-	41.0	29.1	10.0	8.1	8.1
M. chia		-	-	131	45	149	-	173	66	61	30	21
	SE	-	-	0.47	0.61	0.88	-	0.44	0.44	0.60	0.56	0.57
	%	-	-	44.0	15.0	50.0	-	58.0	22.0	21.0	10.0	7.0
M. telmessia		-	-	200	54	81	-	123	54	35	42	27
	SE	-	-	0.80	0.54	0.74	-	1.08	0.51	0.49	0.76	0.64
	%	-	-	57.1	15.4	23.1	-	35.1	15.4	10.0	12.0	7.7
M. cypricola		-	-	233	71	179	-	167	63	67	54	33
	SE	-	-	0.54	0.74	1.08	-	0.51	0.49	0.76	0.64	0.69
	%	-	-	61.3	18.7	47.1	-	44.0	16.6	17.6	14.2	8.7
M. murag		-	-	238	50	217	-	158	83	38	25	25
	SE	-	-	0.48	0.66	0.95	-	0.46	0.44	0.68	0.59	0.61
	%	-	-	68.0	14.3	62.0	-	45.1	23.7	10.9	7.1	7.1
P. tithonus		-	-	199	94	106	-	172	117	78	66	70
	SE	-	-	0.86	0.59	0.76	-	1.13	0.56	0.55	0.77	0.65
	%	-	-	45.2	21.4	24.1	-	39.1	26.6	17.7	15.0	15.9
P. bathseba		-	-	256	122	135	-	93	77	80	22	26
	SE	-	-	0.70	0.48	0.66	-	0.95	0.46	0.44	0.68	0.59
	%	-	-	57.1	27.1	30.0	-	20.7	17.1	17.9	5.0	5.7
P. cecilia		-	-	220	53	98	-	129	83	45	38	30
	SE	-	-	0.65	0.47	0.61	-	0.88	0.44	0.44	0.60	0.56
	%	-	-	61.1	14.7	27.2	-	35.8	23.1	12.5	10.6	8.3
A. hyperantus		-	-	308	100	167	-	242	125	67	50	42
	SE	-	-	0.43	0.64	0.89	-	0.39	0.39	0.67	0.57	0.56
	%	-	-	62.0	20.0	33.0	-	48.0	25.0	13.0	10.0	8.0
H. lycaon		-	-	168	98	102	-	119	108	39	38	36
	SE	-	-	0.43	0.52	0.74	-	0.39	0.39	0.51	0.40	0.49
	%	-	-	40.3	23.0	24.1	-	28.7	26.0	9.1	9.1	8.0
H. lupina		-	-	150	67	79	-	71	75	29	29	27
	SE	-	-	0.35	0.49	0.66	-	0.31	0.31	0.50	0.44	0.43
	%	-	-	39.2	17.5	20.6	-	18.5	19.6	7.6	7.6	7.1
C. pegala		-	-	260	163	141	-	152	119	36	36	21
	SE	-	-	0.35	0.50	0.68	-	0.33	0.32	0.50	0.46	0.46
	%	-	-	50.0	32.5	28.0	-	30.2	23.0	7.0	7.0	4.0

table 3.2.2a (continued)

Abdominal segment 9

		XD1	XD2	D1	D2	SD1	SD2	L1	L2	SV1	SV2	V1
M. jurtina		-	-	239	104	271	-	150	-	38	-	27
	SE	-	-	0.53	0.64	0.55	-	0.57	-	0.48	-	0.63
	%	-	-	63.7	28.1	73.1	-	40.4	-	10.0	-	7.1
M. chia		-	-	200	48	220	-	45	-	15	-	15
	SE	-	-	0.77	0.52	0.44	-	0.57	-	0.55	-	0.58
	%	-	-	74.0	16.0	74.0	-	32.0	-	5.0	-	5.0
M. telmessia		-	-	223	35	92	-	54	-	39	-	19
	SE	-	-	0.69	0.91	0.58	-	0.51	-	0.63	-	0.62
	%	-	-	63.7	10.0	26.3	-	15.4	-	11.1	-	5.5
M. cypricola		-	-	242	71	113	-	96	-	21	-	21
	SE	-	-	0.91	0.58	0.51	-	0.63	-	0.62	-	0.69
	%	-	-	63.7	18.7	29.7	-	25.3	-	5.5	-	5.5
M. murag		-	-	221	58	71	-	96	-	25	-	25
	SE	-	-	0.83	0.53	0.46	-	0.59	-	0.58	-	0.62
	%	-	-	63.1	16.6	20.3	-	27.2	-	7.1	-	7.1
P. tithonus		-	-	258	94	160	-	90	-	47	-	23
	SE	-	-	0.72	0.93	0.63	-	0.54	-	0.66	-	0.64
	%	-	-	58.6	21.4	36.4	-	20.5	-	10.7	-	5.2
P. bathseba		-	-	206	157	106	-	96	-	42	-	29
	SE	-	-	0.61	0.83	0.53	-	0.46	-	0.59	-	0.58
	%	-	-	46.0	35.0	23.6	-	21.4	-	9.4	-	6.4
P. cecilia		-	-	258	61	98	-	87	-	38	-	19
	SE	-	-	0.57	0.77	0.52	-	0.44	-	0.57	-	0.55
	%	-	-	71.7	16.9	27.2	-	24.2	-	10.6	-	5.3
A. hyperantus		-	-	308	100	167	-	108	-	58	-	58
	SE	-	-	0.80	0.44	0.40	-	0.56	-	0.56	-	0.54
	%	-	-	62.0	20.0	33.0	-	22.0	-	12.0	-	12.0
H. lycaon		-	-	169	103	121	-	163	-	32	-	32
	SE	-	-	0.59	0.45	0.37	-	0.44	-	0.41	-	0.49
	%	-	-	41.3	25.0	29.1	-	39.0	-	7.1	-	7.1
H. lupina		-	-	188	79	79	-	38	-	34	-	21
	SE	-	-	0.61	0.37	0.32	-	0.44	-	0.43	-	0.42
	%	-	-	49.1	20.6	20.6	-	9.9	-	8.9	-	7.6
C. pegala		-	-	293	195	163	-	173	-	36	-	34
	SE	-	-	0.63	0.41	0.35	-	0.46	-	0.44	-	0.47
	%	-	-	58.0	39.1	32.3	-	34.0	-	7.0	-	6.0

table 3.2.2a (continued)

Abdominal segment 10

		D1	D2	SD1	L1	V1	SV1	P1	P2	PL1	PL2	PL3	PL4	PL5
M. jurtina		271	250	271	41	27	38	25	21	108	59	59	59	59
	SE	0.33	0.50	0.72	0.58	0.59	0.48	0.59	0.67	0.67	0.62	0.66	0.78	0.66
	%	73.0	67.0	73.2	11.0	7.0	10.0	6.0	5.0	29.4	15.0	15.0	15.0	15.0
M. chia		214	173	250	137	15	15	24	15	33	48	45	54	48
	SE	0.77	0.51	0.56	0.56	0.67	0.55	0.49	0.54	0.59	0.74	0.39	0.64	0.84
	%	72.0	58.0	84.0	46.0	5.0	5.0	8.0	5.0	11.0	16.0	15.0	18.0	16.0
M. telmessia		231	154	208	62	19	39	14	14	89	50	50	50	42
	SE	0.69	0.92	0.59	0.64	0.67	0.63	0.84	0.52	0.62	0.69	0.84	0.42	0.75
	%	66.0	44.0	59.4	17.7	5.5	11.1	3.9	3.9	25.4	14.3	14.3	14.3	12.0
M. cypricola		275	150	233	83	21	21	13	13	117	58	58	54	42
	SE	0.92	0.59	0.64	0.67	0.84	0.62	0.52	0.62	0.69	0.84	0.42	0.75	1.04
	%	72.4	39.5	61.3	21.8	5.5	5.5	3.4	3.4	30.8	15.3	15.3	14.2	11.1
M. murag		275	175	200	75	25	25	13	13	133	58	58	58	50
	SE	0.81	0.55	0.59	0.59	0.75	0.58	0.49	0.58	0.62	0.76	0.40	0.68	0.91
	%	78.6	50.0	57.1	21.4	7.1	7.1	3.7	3.7	38.0	16.6	16.6	16.6	14.3
P. tithonus		242	191	176	90	20	47	16	16	148	78	78	39	35
	SE	0.73	0.98	0.59	0.65	0.70	0.66	0.86	0.55	0.63	0.70	0.90	0.44	0.77
	%	55.0	43.4	40.0	20.5	4.6	10.7	3.6	3.6	33.6	17.7	17.7	8.9	8.0
P. bathseba		250	205	224	87	26	42	19	19	157	90	64	77	67
	SE	0.62	0.81	0.55	0.59	0.59	0.59	0.75	0.49	0.58	0.63	0.76	0.40	0.68
	%	55.7	45.7	50.0	19.3	5.7	9.4	4.3	4.3	35.0	20.0	14.3	17.1	15.0
P. cecilia		292	212	182	106	19	38	23	23	106	45	83	68	45
	SE	0.58	0.77	0.51	0.56	0.56	0.57	0.67	0.49	0.54	0.59	0.74	0.39	0.64
	%	81.1	58.9	50.6	29.4	5.3	10.6	6.4	6.4	29.4	12.5	23.1	18.9	12.5
A. hyperantus		342	275	275	250	50	58	42	42	117	83	83	83	83
	SE	0.75	0.53	0.56	0.55	0.74	0.56	0.44	0.57	0.62	0.68	0.36	0.67	0.84
	%	68.0	55.0	55.0	50.0	10.0	12.0	8.0	8.0	23.0	17.0	17.0	17.0	17.0
H. lycaon		217	168	171	84	32	32	27	21	108	38	38	38	38
	SE	0.65	0.38	0.44	0.47	0.54	0.41	0.35	0.38	0.46	0.61	0.32	0.48	0.74
	%	52.2	40.2	41.0	20.0	7.1	7.1	6.0	5.0	26.4	9.2	9.2	9.2	9.2
H. lupina		171	146	138	138	21	34	38	33	88	67	63	58	63
	SE	0.56	0.41	0.45	0.42	0.54	0.43	0.35	0.42	0.47	0.54	0.30	0.50	0.62
	%	44.6	38.1	36.0	36.0	5.5	8.9	9.9	8.6	23.0	17.5	16.5	15.1	16.5
C. pegala		300	271	282	250	34	36	38	36	152	48	48	48	48
	SE	0.58	0.43	0.48	0.44	0.54	0.44	0.38	0.42	0.48	0.57	0.32	0.50	0.63
	%	62.0	54.2	56.0	50.0	6.0	7.0	7.1	7.0	3.0	9.0	9.0	9.0	9.0

table 3.2.2a (continued)

		Cranial setae							
	cranial width	P1	P2	A1	A2	A3	AF1	AF2	L1
M. jurtina	370	241	271	81	173	255	108	65	239
	SE 1.05	0.71	0.76	0.64	0.61	0.67	0.49	0.64	0.61
		% 65.1	73.0	22.0	46.8	69.1	29.0	17.0	64.3
M. chia	297	214	167	66	137	173	128	83	167
	SE 0.86	0.45	0.54	0.80	0.72	0.56	0.41	0.80	0.72
		% 72.0	56.0	22.0	46.0	58.0	43.0	28.0	56.0
M. telmessia	350	208	208	35	165	192	181	73	200
	SE 1.55	1.04	0.52	0.63	0.95	0.84	0.63	0.46	0.94
		% 59.4	59.4	10.0	47.1	54.9	51.7	20.9	57.1
M. cypricola	380	250	230	90	183	220	138	131	240
	SE 1.25	0.52	0.63	0.95	0.84	0.63	0.46	0.94	0.88
		% 65.8	60.5	23.7	48.2	57.9	36.3	34.5	63.2
M. murag	350	230	230	83	175	225	146	113	183
	SE 0.95	0.48	0.58	0.85	0.76	0.58	0.43	0.85	0.77
		% 65.7	65.7	23.7	50.0	64.3	41.7	32.3	52.3
P. tithonus	440	281	324	130	145	273	195	168	270
	SE 2.10	1.11	0.53	0.65	1.00	0.89	0.66	0.48	1.00
		% 63.9	73.6	29.6	33.0	62.1	44.3	38.2	61.4
P. bathseba	449	285	333	110	214	357	143	191	214
	SE 1.80	0.91	0.40	0.58	0.85	0.76	0.58	0.43	0.85
		% 63.5	74.2	24.5	47.7	79.6	31.9	42.6	47.7
P. cecilia	360	318	292	83	155	242	72	139	250
	SE 0.98	0.85	0.45	0.54	0.80	0.72	0.56	0.41	0.80
		% 88.3	81.1	23.1	43.1	67.2	20.0	38.6	69.4
A. hyperantus	497	333	333	60	83	283	75	75	333
	SE 0.88	0.46	0.53	0.79	0.68	0.55	0.37	0.78	0.69
		% 67.0	67.0	12.0	17.0	57.0	15.0	15.0	67.0
H. lycaon	406	110	119	54	76	78	65	23	66
	SE 1.30	0.34	0.41	0.66	0.59	0.43	0.32	0.67	0.64
		% 27.1	29.0	13.0	18.0	19.0	15.1	5.0	16.2
H. lupina	384	107	96	67	71	85	38	50	83
	SE 1.02	0.35	0.41	0.60	0.52	0.42	0.30	0.59	0.52
		% 27.9	25.1	17.5	18.5	22.2	9.9	13.1	21.7
C. pegala	515	103	97	70	108	103	97	70	108
	SE 1.30	0.36	0.46	0.61	0.57	0.44	0.34	0.62	0.55
		% 20.0	19.1	14.3	21.2	20.0	19.0	14.0	21.2

table 3.2.2a (continued)

Cranial setae (continued)

		F1	C1	C2	O1	O2	SO1	SO2	SO3
M. jurtina		59	63	27	86	119	76	65	48
	SE	0.59	0.68	0.68	0.50	0.67	0.68	0.57	0.46
	%	16.1	17.0	7.0	23.3	32.4	20.0	17.2	13.1
M. chia		30	24	18	83	89	27	27	24
	SE	0.60	0.52	0.62	0.74	0.51	0.66	0.73	0.76
	%	10.0	8.0	6.0	28.0	30.0	9.0	9.0	8.0
M. telmessia		46	28	28	65	135	85	62	46
	SE	0.88	0.72	0.64	0.74	0.91	0.54	0.82	0.82
	%	13.1	8.0	8.0	18.6	38.6	24.3	17.7	13.1
M. cypricola		45	50	50	100	115	30	30	30
	SE	0.72	0.64	0.74	0.91	0.54	0.82	0.82	0.92
	%	11.8	13.2	13.2	26.3	30.3	7.9	7.9	7.9
M. murag		33	21	21	108	158	33	33	33
	SE	0.65	0.57	0.66	0.80	0.52	0.72	0.75	0.81
	%	9.4	6.0	6.0	30.9	45.1	9.4	9.4	9.4
P. tithonus		30	62	23	145	156	95	137	90
	SE	0.94	0.76	0.66	0.79	0.95	0.55	0.85	0.87
	%	6.8	14.1	5.2	33.0	35.5	21.6	31.1	20.5
P. bathseba		191	38	30	191	179	36	119	35
	SE	0.77	0.69	0.57	0.66	0.80	0.52	0.72	0.75
	%	42.6	8.4	6.7	33.6	32.1	8.0	26.6	7.9
P. cecilia		100	62	39	121	167	77	85	51
	SE	0.72	0.60	0.52	0.62	0.74	0.51	0.66	0.73
	%	27.8	17.2	10.8	33.6	46.4	21.4	23.6	14.2
A. hyperantus		33	35	35	150	300	15	15	15
	SE	0.62	0.54	0.62	0.75	0.47	0.67	0.68	0.75
	%	7.0	7.0	7.0	30.0	60.0	3.0	3.0	3.0
H. lycaon		54	54	38	54	65	59	65	65
	SE	0.52	0.46	0.55	0.63	0.35	0.56	0.56	0.67
	%	13.0	13.0	9.0	13.1	15.0	14.2	15.0	15.0
H. lupina		113	58	71	83	67	63	58	54
	SE	0.47	0.42	0.48	0.56	0.38	0.51	0.52	0.56
	%	29.5	15.1	18.5	21.7	17.5	16.5	15.1	14.1
C. pegala		60	51	51	81	114	65	54	54
	SE	0.47	0.43	0.48	0.58	0.43	0.54	0.56	0.56
	%	12.1	10.0	10.0	16.2	22.4	13.1	10.0	10.0

dorsal to C2, close and ventral to puncture F_a, about midway between the anteclypeal suture and the junction of frontal sutures.

Adfrontals

AF_a and AF3 absent. AF2 in line with junction of frontal sutures and a short distance from it. AF1 ventral to AF2 and about one third the distance between AF2 and the anteclypeal suture and very close to the frontal suture. AF1 about 3X the length of AF2.

Anteriors

A_a absent. A1 dorsal and very close to the antennal cavity, slightly mesad of central. A2 and A3 in line with A2, somewhat caudal of a line between A1 and A3 and closer to A3 than A2. A3 slightly mesad of midway between AF2 and L1. A2 about 2X and A3 about 3X A1.

Ocellars

O3 absent. O2 longer than O1. O1 ventral and caudal of ocelli ii and iii and very close to them. O2 caudal to midway between ocellus iv and occiput and in line with O1. Puncture O_b absent. O_a very close to ocellus ii and 45^{o} ventral and mesad to it.

Laterals

L1, the only lineal present, caudal to A3 and on a line running from AF2 through A3. L_a absent.

Genals

Setae absent. Puncture G_a close to caudo-ventral angle.

Posteriors

P2 close to cranial apex. P1 midway between AF2 and P2, close to coronal suture. P_a immediately above ocellus iii and slightly caudal to a line between ocellus iii and A2, closer to it than to A2. P_b absent.

Verticals

Absent or undetected.

BODY

Thoracic segment I

Prothoracic shield on midline, comprising a single, fairly heavily sclerotised plate encompassing setae XD1, XD2, D1 and D2. XD1 and XD2 subparallel to D1 and D2. XD1 dorsal to D1. XD2 dorsal to D2. D1 about midway between XD1 and XD2, but caudal to them. XD1 in median-anterior angle, D2 diagonally opposite XD1 and about 1.25X XD2. XD2 about 1.5X D1. D2 very short, about one third the length of D1.
Puncture D_a midway between D_b and D2. D_b in line with, ventral and caudal to XD2, smaller than D_a. SD1 and SD2 usually on a subdorsal shield. SD1 dorsal to SD2. SD2 very thin, shorter than and about 45^o ventral and anterior to SD1. L1, L2, SV1 and SV2on a single lineal-subventral shield. L1 caudal to L2. L2 absent or rudimentary, sometimes thick and irregular, but always short when present. SV1 and SV2 ventral to and closer together than the lineals. SV2 short.
Microseta MD_1 central and caudal to the prothoracic shield. Other microsetae absent or undetected.

Thoracic segments II and III

All setae except SD2 in a straight vertical line. D1 and D2 subequal. D1 ventral to XD1 on the thoracic segment I. D2 slightly ventral to

midway between D1 and SD1. SD1 and SD2 on a common pinaculum. SD1 about 0.75X L2. SD2 very short and rudimentary, blunt ended and sometimes malformed, but always present. L1 in line with L1 on thoracic segment I and slightly longer than SD1. SV1 in line with subventral on this segment and about 0.5X L1. L2, V1 and V2 absent.
Microseta MSD_1 only present anterior, ventral and close to SD1.

Abdominal segments 1 and 2

D1 and D2 in line with D1 on thoracic segments II and III and all other abdominal dorsals. D1 more than 2X D2. SD1 vertically below D1, about midway between it and L2. SD1 slightly shorter than D1. SD2 absent. L1 and L2 on a lineal shield. L2 in line with D1 and SD1. L1 caudal and slightly dorsal to L2 and a little less than 2X L2. SV1, SV2 and V1 in line, equidistant. SV2 and V1 subequal. SV1 more than 2X SV2.
Microseta MD_1 midway between D1 and SD1 and slightly anterior to them. MSD_1 absent. MSD_2 dorsal, anterior and close to spiracle.

Abdominal segments 3 to 6

D1, D2, SD1, L1, L2, MD_1 and MSD_2 as in segments 1 and 2, except D1 longer. SV1 and Sv2 subequal in line with each other and with SV1 in segments 1 and 2. SV1 caudal to SV2, about one third the length of L2.

Abdominal segments 7 and 8

Identical to segments 1 and 2, except SV1 is subequal to SV2 and V1.

Abdominal segment 9

Similar to segments 7 and 8, but SD1 longer. L2, SV1 and SV2 absent. L1 more dorsal than L1 in segments 7 and 8. V1 more caudal than in these segments.

Abdominal segment 10

Epiproctal setae D1, D2 and SD1 subequal. L1 short, ventral to a line between D2 and SD1. Paraproctal setae P1 and SP2 subequal and short, almost microsetae. SV1 anterior to and V1 ventral to the proleg shield. P1, P2, P3 P4 and P5 present on proleg shield, P2, P3, P4 and P5 forming a semicircle with P1 slightly dorsal to its centre. P1 nearly 2X the length of the other setae.
Punctures absent or undetected.

Differences in setal lengths compared with those of jurtina can be found in table 3.2.2a in which data from measurement is presented. The following differences in other chaetotaxy characters were recorded.

The puncture D_b on thoracic segment I was absent in Maniola telmessia, cypricola and nurag, in Pyronia tithonus, bathseba and cecilia and in Aphantopus hyperantus.

In hyperantus the ocelli were found to be set in a very dark shield-like area. The prothoracic and lineal-subventral shields of thoracic segment I were weak or missing and puncture D_b was absent.

The species of the genus Hyponephele had the cranial seta AF2 below the line of the junction between the frontal sutures and close to them. The prothoracic shield was covered with sclerotised nipples and punctures D_a and D_b were absent. L1 and L2 only were set in a lineal shield. L2 was dorsal to L1 and only just ventral of the spiracle. The subventral shield was missing. The seta L2 was fully developed and subequal to SV1.

In Cercyonis pegala the seta AF2 was dorsal to line of junction between the frontal sutures and more distant from them than in Hyponephele. All thoracic and abdominal microsetae absent or undetected in pegala. The prothoracic shield was cover with nipples as in Hyponephele. The lineal shield, lineals and subventrals were similar to those of Hyponephele. The punctures D_a and D_b were present in the same location as in jurtina. L2 was fully developed, subequal

table 3.2.2b

Factors, eigenvalues and variance estimates for the first 10 factors from 74 setal length variables in three populations of *Maniola jurtina*, all Manioline species except *jurtina* and all Maniolini.

	factor	eigenvalue	percentage of variation
Maniola jurtina 3 populations accumulated	1	57.630	80.0
	2	5.956	8.3
	3	1.673	2.3
	4	1.031	1.4
	5	0.928	1.3
	6	0.846	1.2
	7	0.658	0.9
	8	0.600	0.8
	9	0.558	0.8
	10	0.441	0.6
Maniolini excluding *Maniola jurtina*	1	24.522	34.1
	2	12.071	16.8
	3	9.805	13.6
	4	8.029	11.3
	5	5.415	7.5
	6	4.027	5.6
	7	2.707	3.8
	8	2.267	3.1
	9	1.773	2.5
	10	1.060	1.5
Maniolini all species	1	22.968	31.9
	2	10.915	15.2
	3	8.891	12.3
	4	7.397	10.3
	5	7.186	10.0
	6	3.930	5.5
	7	3.310	4.6
	8	2.365	3.3
	9	2.020	2.8
	10	1.540	2.1

table 3.2.2c

Maniolini first instar larval setae lengths: Euclidean distances (d) from normalised data between species, figures given as similarity values $s=e^{-d}/n \times 10^4$

	2	3	4	5	6	7	8	9	10	11	12
1 jurtina	9964	9964	9966	9964	9958	9952	9959	9954	9955	9949	9942
2 chia		9960	9958	9956	9952	9945	9952	9952	9946	9944	9939
3 telmessia			9970	9970	9963	9956	9963	9954	9946	9947	9937
4 cypricola				9978	9964	9954	9965	9960	9951	9948	9941
5 nurag					9962	9956	9967	9961	9949	9947	9940
6 tithonus						9953	9967	9953	9945	9947	9930
7 bathseba							9959	9944	9939	9940	9933
8 cecilia								9959	9944	9948	9936
9 hyperantus									9944	9946	9938
10 lycaon										9955	9964
11 lupina											9948
12 pegala											

---

to SV1.

The possibility was recognised that there would be some association between setal lengths and that they would not vary independently. Factor analysis performed on the data (section 1.3.6) revealed that the variation in setal lengths was highly correlated. A single factor accounted for 80% of variation in jurtina. The situation in the case of data from all species together was rather different, although this analysis also suggested that only a few factors were involved. Six factors alone accounted for over 85% of the variation in this instance. When all species except jurtina were considered, five factors account for over 83% of the variation, the first two accounting for over 50% (table 3.2.2b). It has been suggested that the data possibly indicate that, although setal lengths are highly correlated, within each species a different factor, or factors might be involved in each case (Dennis pers. comm.). Further analyses will help to associate the factors with actual setal groups and reveal more clearly the nature of these interesting morphological characters.

However, although data redundancy might bias the results in favour of spines exhibiting character dependence, it was felt that initial analysis should utilise data from all measured setae. The actual setal lengths, rather than lengths relative to the cranium width, were utilised which meant that a significant contribution from a larval size component might be present in the data. However, the indications from factor analysis would not support this. Very little difference was observed between lengths of the same setae within each sample.

Interspecific Euclidean distances (section 1.3.6) between each species pair were calculated (table 3.2.2c).

Clustering procedures, single, complete and average linkage, similar to those performed on electrophoretic data (section 2), were undertaken. Dendrograms from the analysis are illustrated in figures 3.2.2d - f. While there are major discrepancies between the dendrograms produced by the three clustering methods, there are also several features in common. Although interspecific differences are fairly large, Hyponephele lycaon and lupina together with Cercyonis pegala associate as a distinct group, as do Pyronia tithonus and cecilia. Maniola telmessia, cypricola and nurag comprise a cluster of closely related species, suggesting also that cypricola and nurag have a very close affinity. Aphantopus hyperantus lies within the Maniola/Pyronia complex by single, complete and average clustering.

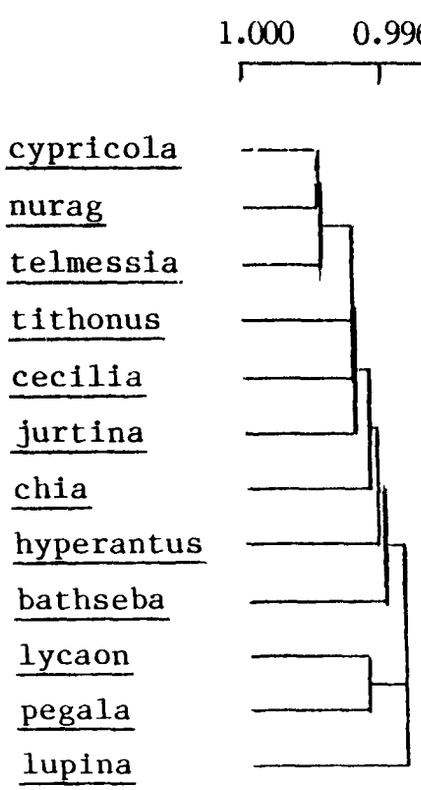
The independence of the Pyronia species from each other, which is suggested by these analyses, is surprising. By single and complete linkage clustering, tithonus and cecilia lie between jurtina/chia and telmessia/nurag/cypricola. However, in the UPGMA plot the only anomalies are the isolation of bathseba and the location of Aphantopus hyperantus between bathseba and other Pyronias.

Perhaps more confidence in major grouping can be placed in the inferred relationships from nonmetric multidimensional scaling (figure 3.2.2g). In this ordination the genera Maniola, Pyronia and Hyponephele form loosely discrete clusters, with Aphantopus hyperantus located in an unexpected isolated position. The stress value for the NMMS ordination was very low (0.078 r^2=0.976), and the product-

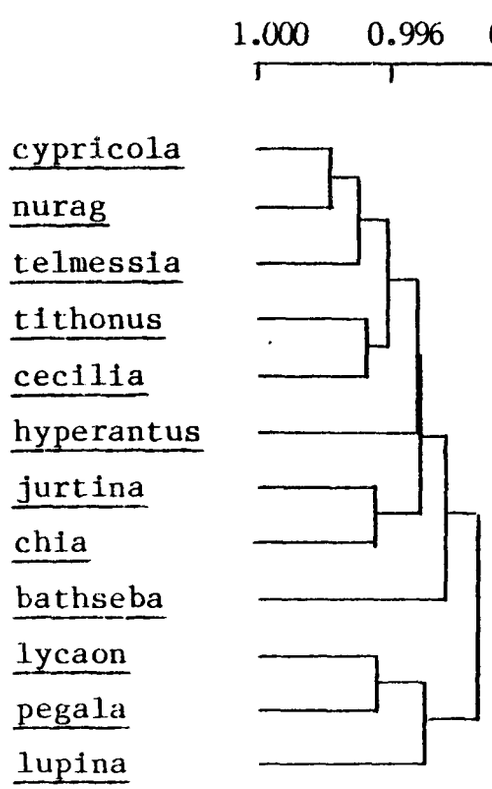
figures 3.2.2d - f

Single, complete and average linkage clustering (UPGMA) from setae lengths - Maniolini

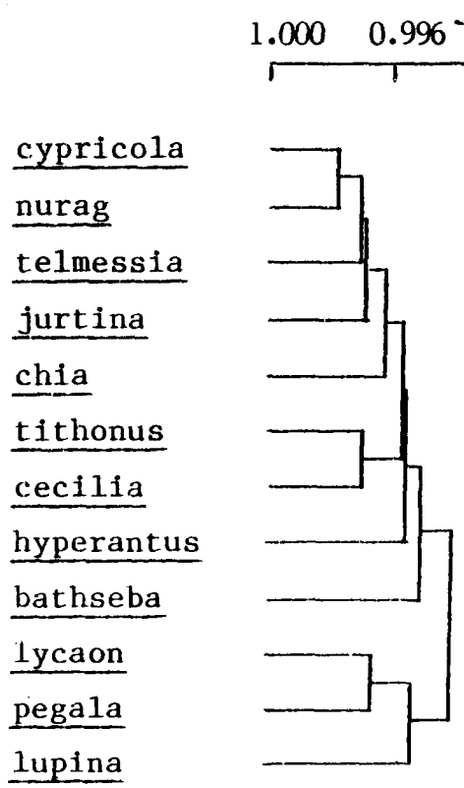
d) single



e) complete



f) average



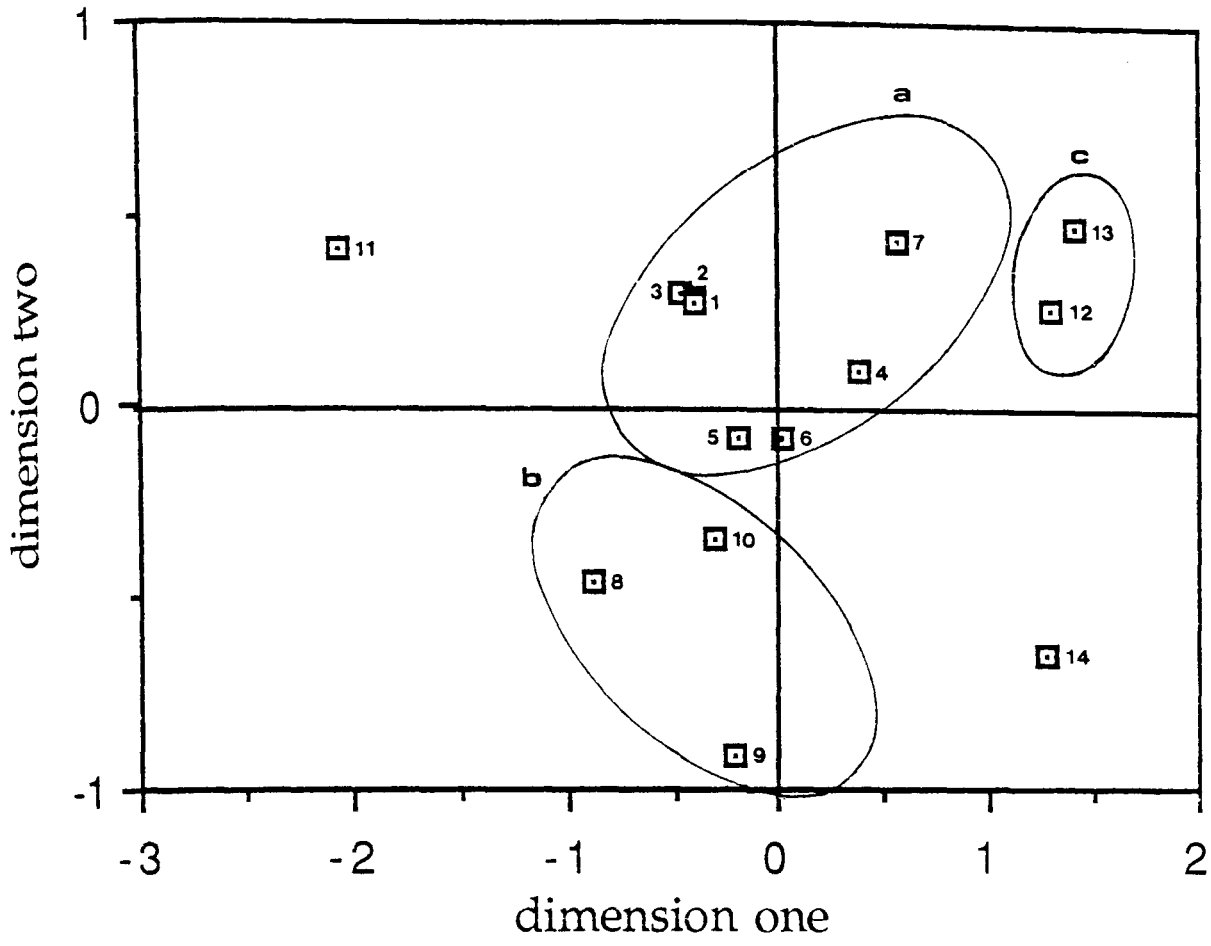


figure 3.2.2g

Nonmetric multidimensional scaling plot of Euclidean distance coefficients from species of Maniolini: setal lengths in first instar larvae.

Envelopes

a Maniola

b Pyronia

c Hyponephele

Populations

1 Maniola jurtina (Britain)
2 Maniola jurtina (Austria)
3 Maniola jurtina (Morocco)
4 Maniola telmessia
5 Maniola cypricola
6 Maniola nurag
7 Maniola chia
8 Pyronia tithonus
9 Pyronia bathseba
10 Pyronia cecilia
11 Aphantopus hyperantus
12 Hyponephele lycaon
13 Hyponephele lupina
14 Cercyonis pegala

moment co-efficient was high. The calculated r_p values from the distance (similarity) matrix and corresponding cophenetic values suggest that the best fit of the clustering methods was for complete linkage, which, taking account of relationships inferred by other methods, was also the most anomalous.

single linkage	r_p=0.875	df 65
complete linkage	r_p=0.880	df 65
UPGMA	r_p=0.873	df 65
NMMS two-dimensional	r_p=0.974	df 65

The number of crochets on the ventral prolegs usually differ from those of the anal prolegs and between species, frequently differing in number, position and length. It was not practical to measure crochet lengths in Maniolini, but the number and position were recorded. In Maniola chia and Pyronia bathseba the number of crochets in the samples varied, but in other species the number was constant (table 3.2.2d). Excluding supernumery crochets (in aberrant individuals) from the counts, the numbers in M. jurtina and chia were identical. An important difference was identified between M. telmessia/cypricola/nurag and jurtina/chia, these species having one fewer crochet on the ventral prolegs. Pyronia tithonus and cecilia had an identical configuration, as had most bathseba and pegala. Other Maniolini species were unique in their crochet numbers.

The crochets of Maniola species were closely adjacent, forming only partial ellipses, while those of Hyponephele and Cercyonis were virtually contiguous. Pyronia, on the other hand, had more widely spaced crochets.

The value of the proleg crochets in species differentiation is not often considered and few interspecific studies have utilised the interesting variation in these structures (Fleming 1960). Kitching (1984) found such considerable variation in the crochet number in Danaids, both between individuals and between species, that he did not find the character of value. The Maniolini transgeneric similarities are interesting, but of doubtful phylogenetic significance. Intrageneric differences are, probably, more important.

table 3.2.2d

Crochet numbers on ventral and anal prolegs of first instar larvae in Maniola, Pyronia, Aphantopus, Hyponephele and Cercyonis. Figures in brackets represent aberrant crochet numbers occasionally found in individuals.

	M. jurtina	M. chia	M. telmessia	M. cypricola	M. nurag
ventral	4	4 (5)	4	4	4
anal	6	6 (7)	5	5	5

	P. tithonus	P. bathseba	P. cecilia	A. hyperantus
ventral	4	5 (6)	4	5
anal	6	7 (8)	6	6

	H. lycaon	H. lupina	C. pegala
ventral	3	4	5
anal	5	5	7

Although chaetotaxy is a young science and its potential in evolutionary studies is only now being realised, the systematic importance of the pre-imaginal instars of Lepidoptera was recognised by Muller in his classification of south American Nymphalid larvae (Muller 1886). The chaetotaxy of Satyrid species has not been tackled before. The closest relatives to the group which have been studied are the Heliconius (Fleming 1960) and Danaidae (Kitching 1984) and these provide the only material for comparison with the present work. However, although these studies were useful in identifying homologies, few comparisons of value could be made between them and Maniolini.

An objective evaluation of the Maniolini chaetotaxy results is not easy. It is difficult to divorce the relationships inferred by traditional methods and the relatively unambiguous relationships suggested by electrophoresis from those suggested by chaetotaxy. The best fit to these data could be chosen. If so, it must also be noted that NMMS on chaetotaxy infers that Aphantopus is a much more distant and isolated genus than suggested by electrophoresis. It has been proposed here (section 3.2.1) that egg morphology in some species owes much to the oviposition behaviour of the adult butterflies. The anomalies observed in larval chaetotaxy could accrue from behavioural attributes of the first instar larvae significantly affecting the setal lengths to a degree not reflected in genetic and other morphological analyses.

Clearly, the fact that utilisation of the lengths of 74 setae in Maniolini first instar larvae can result in indications of meaningful relationships, by some statistical methods, shows that these characters are of some importance at interspecific level. However, the implication is that care must be exercised in the interpretation of setal lengths in chaetotaxy.

It can been seen from the Maniolini that the relative positions of setae are characteristic of major genus groups. In the present study, the location of setae on thoracic segment I distinguishes the Maniola/Pyronia/Aphantopus group from Hyponephele/Cercyonis, although it is doubtful if an analysis of their relative positions could detect intrageneric differences, in Maniolini at least. Perhaps measurement of a reduced number of setae, selected via factor analy-

sis, and measurements of intersetal distances (possibly cranial and thoracic), together with more sensitive statistical techniques, would be more positive in revealing relationships between and within very close groups.

There appears to be little phylogenetic significance in the presence or absence of punctures in Maniolini.

4 EVOLUTIONARY AND OTHER IMPLICATIONS

4.1 Comparison of morphological and electrophoretic analyses

Very few studies using objective methods have been made of the covariance of electrophoretic and morphological characters. As Ferguson (1980) suggests, the reason for this is due, in part, to the difficulties in quantifying morphological differences. As a relatively large number of enzyme loci had been utilised in this study, electrophoretic data was extensive. Similarly, as an evaluation of a number of morphological characters (genitalia, egg morphology, larval chaetotaxy and scales) had been made in the present work, and there was substantial agreement between them, it was felt that some confidence could be placed in the selection of these characters as being representative of interspecific relationships in Maniolini. This presented the opportunity to compare biochemical and morphological data in several ways. Even so, the analyses presented here must be considered only as a preliminary examination of the available data and there is great potential for further study in this area.

4.1.1 The Cramond Island polymorphisms in Maniola jurtina

The unusual isozyme and phenotypic polymorphisms in jurtina on Cramond Island were used to test the possibility that there was some link between enzyme genotypes and a single morphological character, in this instance, an aberration of the apical eyespot, f.erymanthoides. It has already been shown (section 2.4.2) that this population is unusual in both the very high frequency of rare genotypes detected by electrophoresis and morphology. Clearly, a direct association between the alleles at functional loci and the erymanthoides phenotype would not be expected, although Johnson (1976) suggests that there is clear evidence that particular alleles at the different loci preferentially associate together at specific locations. If this is so, some undetected factor could relate the expression of the genetic and morphological characters to each other.

Allozyme frequencies at polymorphic loci for erymanthoides and normal

table 4.1.1a

Allele frequencies at variable loci in normal Maniola jurtina and form erymanthoides from Cramond Island - standard errors in parenthesis below.

Locus	allele	normal	erymanthoides
GK	100	0.703	0.774
		(0.042)	(0.046)
	109	0.297	0.226
		(0.042)	(0.046)
GOT-1	84	0.237	0.274
		(0.039)	(0.049)
	100	0.669	0.571
		(0.043)	(0.054)
	114	0.093	0.155
		(0.027)	(0.039)
IDH-1	44	0.008	0.012
		(0.008)	(0.012)
	100	0.992	0.998
		(0.008)	(0.012)
IDH-2	22	0.008	-
		(0.008)	-
	57	0.068	0.048
		(0.024)	(0.023)
	100	0.924	0.952
		(0.023)	(0.023)
MPI	84	0.008	0.024
		(0.008)	(0.017)
	96	0.305	0.274
		(0.042)	(0.049)
	100	0.627	0.667
		(0.045)	(0.051)
	105	0.059	0.036
		(0.022)	(0.020)
PGI-2	22	0.008	-
		(0.008)	-
	100	0.975	0.988
		(0.014)	(0.012)
	165	0.017	0.012
		(0.012)	(0.012)
PGM	78	0.325	0.183
		(0.043)	(0.043)
	88	0.008	-
		(0.008)	-
	100	0.608	0.695
		(0.045)	(0.051)
	113	0.058	0.122
		(0.021)	(0.036)

table 4.1.1b

Allozyme differentiation between normal and erymanthoides samples Maniola jurtina from Cramond Island.

locus	F_{ST}	χ^2	df	p
GK	0.006	0.540	1	>0.30
GOT-1	0.010	0.864	1	>0.30
IDH-1	0.000	0.035	1	>0.90
IDH-2	0.003	0.273	1	>0.50
MPI	0.002	0.146	1	>0.70
PGI-2	0.002	0.185	1	>0.50
PGM	0.010	0.864	1	>0.30
	mean	total		
	0.005	2.907	7	>0.80

morphs were calculated (table 4.1.1a). The F_{ST} and contingency χ^2 values were found for each locus. No significant difference was found between the electrophoretic data from normal and erymanthoides samples. The samples were least similar at the GOT-1 and PGM loci ($p>0.30$), but overall they were highly homogeneous ($p>0.80$ - table 4.1.1b).

4.1.2 Comparison of morphological and electrophoretic analyses - species, subspecies and populations

As the two dimensional single, complete and average linkage clustering analyses produced results which were, to a degree, inconsistent with each other, the nonmetric multidimensional scaling ordination was considered to be the best representation resulting from multivariate analyses of the input morphological data from larval chaetotaxy. It is important that, without exception, linkage clustering analysis and ordinations from electrophoretic data corresponded very closely with existing accepted taxonomies (table 1.2.a), although it could be argued that this is is no reflection on the appropriateness of the techniques, but more an indication of the fact that the input data was extremely robust and withstood distortions well. The

table 4.1.2a

Comparison of electrophoretic and morphological (larval chaetotaxy) data. Residuals (converted to positive values) from regression based on genetic identities (I) and Euclidean distances. Values greater than 0.100 bold.

	2	3	4	5	6	7	8	9	10	11	12
1 jurtina	**.114**	**.120**	**.146**	**.172**	.081	.048	.053	.061	.072	.037	.070
2 telmessia	–	**.122**	**.120**	**.185**	.055	.088	**.120**	.011	.015	.018	.071
3 cypricola		–	.041	**.195**	.062	.068	**.123**	.066	.047	.020	.020
4 nurag			–	**.224**	.047	.094	**.162**	.081	.007	.012	.040
5 chia				–	.008	.014	.008	.046	.028	.014	.074
6 tithonus					–	**.101**	.092	.054	.043	.044	**.114**
7 bathseba						–	.009	.096	.087	.088	.024
8 cecilia							–	.009	.095	**.160**	.067
9 hyperantus								–	.062	.059	.031
10 lycaon									–	.051	.026
11 lupina										–	.021
12 pegala											–

position of Aphantopus hyperantus was confirmed within Maniolini, between Pyronia and Hyponephele.

Electrophoretic data for Manioline species were compared with data from larval chaetotaxy. Regression analysis performed on the electrophoretic genetic identity (I) values and Euclidean distances from larval chaetotaxy (figure 4.1.2a) suggests a very close correlation between the analytical methods (r_p=0.746 df65). Examination of the residuals reveals that the greatest difference between electrophoretic and morphological data appears to be between Maniola species, bathseba and cecilia, suggesting that in these species electrophoretically detectable variation does not correspond so well with that of larval chaetotaxy (table 4.1.2a), although taking Maniola species alone the correlation is still highly significant (r_p=0.749 df9 $p<.001$).

Although there is a clear overall agreement in the configuration of plots from electrophoretic and morphological data, a detailed comparison between them reveals some discrepancies. The most obvious dif-

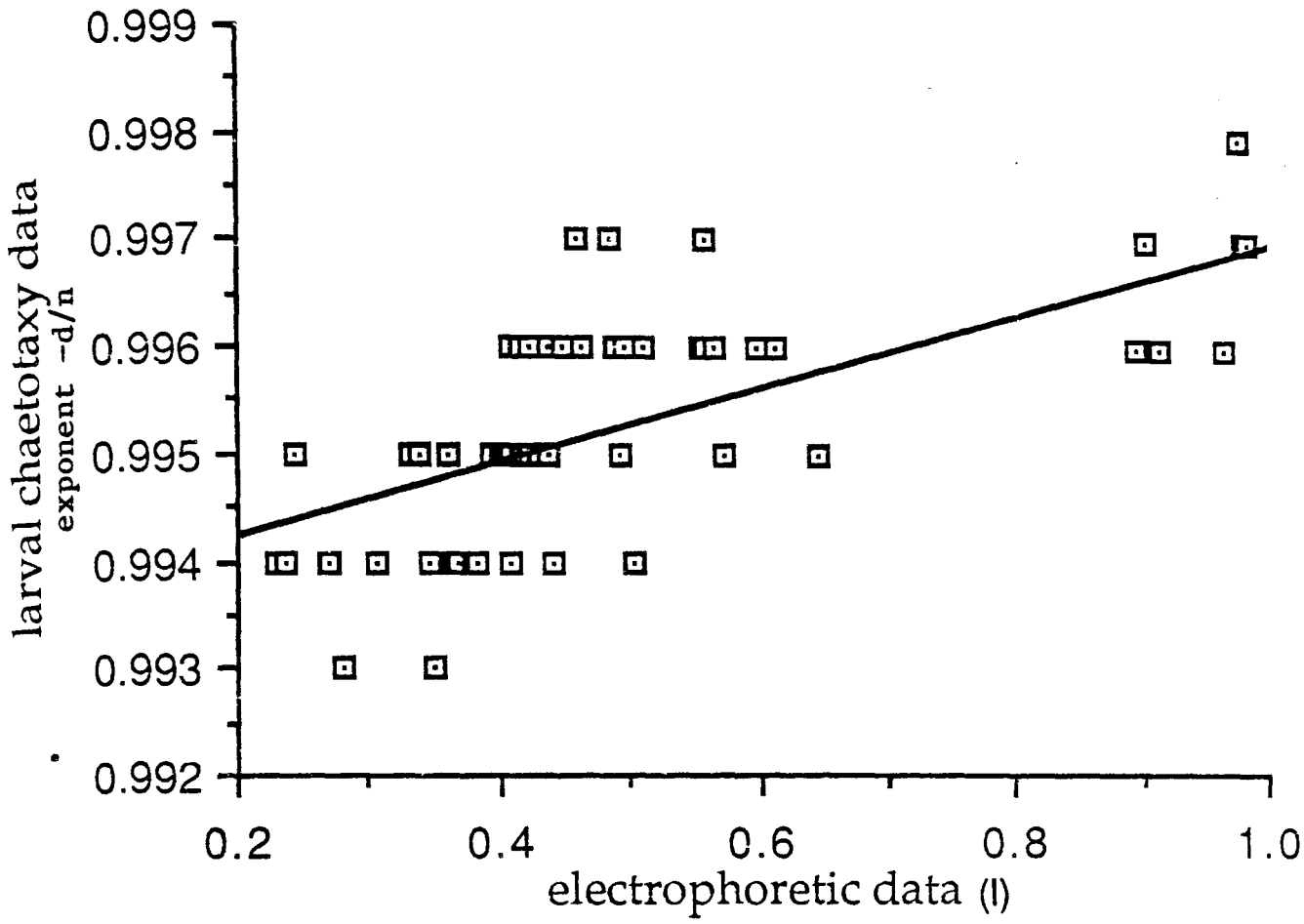


figure 4.1.2a

The relationship between electrophoretic data (genetic identity I) and larval chaetotaxy data (Euclidean distance converted to a similarity coefficient). The fitted regression line is $y = 0.9935 + 0.0035x$.

ferences are the distant location of Aphantopus from other genera, the proximity of Hyponephele and Cercyonis to Maniola and the position of Maniola between Hyponephele and Aphantopus suggested by morphological characters, but not confirmed by electrophoresis. Maniola telmessia and chia appear to be much closer in morphology than is suggested by electrophoresis. The principal differences between the results of the analysis can be summarised.

1. By single and complete linkage clustering of morphological data the Pyronia species locate between Maniola jurtina / chia and the telmessia group (telmessia, cypricola and nurag), whereas by electrophoresis Maniola forms a major cluster of closely related species.

2. By single and complete linkage clustering of morphological data Cercyonis pegala lies between Hyponephele lycaon and lupina, although average clustering correlates with clustering from electrophoretic data by separating pegala.

3. Aphantopus hyperantus is represented as a very isolated taxon by NMMS of morphological data, but it relates closely to Pyronia by electrophoresis.

Other NMMS ordinations of interspecific and population data show a remarkably close correspondence between those produced from electrophoretic and morphological data.

The almost unanimous treatment of the species relationships within each genus by clustering and ordination procedures from electrophoretic and morphological data is confirmed by the results from other morphological methods in this study. The forms of the ova chorion and scale ultrastructure are characteristic of each of these groups. They also confirm the indications from electrophoretic analysis that Hyponephele and Cercyonis are closely related genera which, in turn, are very much more distinct from Pyronia and Maniola than these are from each other. Egg and scale morphology also confirm the relationships of telmessia, cypricola and nurag to each other, as a distinct subgroup within Maniola.

At the intraspecific level in Maniola jurtina, the correspondence between electrophoretic data and genitalia form is very close indeed. All clustering procedures and ordinations show clearly the 'eastern' and 'western' groups: their representation in UPGMA and NMMS is remarkable (section 2.5.2). The analysis of jurtina subspecies by electrophoresis is not so clear and is, in part, ambiguous. This is, perhaps, not surprising when most of the infra-specific taxa have been recognised on the basis of wing markings, which are affected greatly by environmental factors.

Mickevich and Johnson (1976) were first to demonstrate the congrence between molecular (electrophoretic) and morphological (cladistic) data. The close agreement between classifications based on electrophoretic data and accepted taxonomies in butterflies is usual (Geiger and Rezbanyai 1982, Geiger 1978, 1980, 1982, Courtney 1980, 1982). This is often also the case with other invertebrates, eg birds (Smith and Zimmerman 1976, Avise et al. 1980) and fish (McAndrew and Majumdar 1984). In an extremely interesting study, Carson et al. demonstrate the relationship between chromosomal characters and allozyme differentiation. Some workers have shown that there is little electrophoretically detectable genetic change in otherwise distinct forms of Drosophila (Prakash 1972), minnows (Avise and Ayala 1976, Avise et al. 1980) and ribbon snakes (Gartside et al. 1977), while others have demonstrated a significant difference between results from biochemical and morphological analysis (Schnell et al. 1978, Turner et al. 1979). Where such differences are detected, they can be due to independent evolution of protein and morphological characters, convergent evolution or incomplete or incorrect data.

The work of Handford (1972a, 1973) on esterase loci in Maniola jurtina is not convincing. He claims to have detected an area of 'genetic disturbance' in the boundary region of south-western England corresponding to the spotting morphs, but the recent work of Brakefield serves only to invalidate further the suggested hypothesis (section 3.1.2). The more sensitive multi-locus study which formed a major part of the present research did not detect anything which would suggest that the electrophoretically detectable genetics of the populations in that region differed from each other in any way

different from other jurtina populations throughout the species' range (section 2.5.1).

Mitton (1978), working on the killifish (Fundulus heteroclitis), and Eanes (1978), working on the monarch butterfly (Danaus plexippus), claim to have demonstrated that genetic heterozygozity as indicated by proteins in individuals is more likely to be morphologically less variable than homozygous individuals, but McAndrew et al. (1982) have shown that this is not the case in plaice (Pleuronectes platessa). Although this aspect of genetic/morphological covariance was not examined in detail in the present study, a cursory examination of the data from Cramond Island did not support Mitton's hypothesis.

4.2 Evolution of Maniolini

4.2.1 Maniolini species and genera

Detailed interpopulation analyses of the species belonging to the genera Pyronia, Aphantopus, Hyponephele and Cercyonis, as performed in this study of Maniola, would be required before a serious attempt could be made at reconstructing the evolutionary history of these butterflies. However, some observations can be made on the available data.

Miller (1968) suggests that Maniolini evolved from an Ypthimine stock in the late Tertiary and that Cercyonis, which he included in Coenonymphini, split from that same common ancestor about the same time to enter northern America via the Bering Straight. The overwhelming evidence from both electrophoretic and morphological studies points to the probability that the Manioline species have evolved from a common ancestor which diverged into three main groups, 1. Hyponephele and Cercyonis, 2. Pyronia and Aphantopus and 3. Maniola. It has already been shown that Maniola species are unique in possessing the Jullien organ and in having a broad valve in the male genitalia. It is equally evident that the mean genetic and morphological differences between the genera groups are approximately the same, suggesting a tripartide branching sometime in the past. It can also be seen from both electrophoretic and morphological data that the computed

differences between species in each of the genera are very different. Pyronia and Hyponephele species differentiate at about the same level in the electrophoretic analysis, ie mean difference I=0.590 and I=0.627 respectively. However, the data for levels of genetic differentiation for Maniola species suggest that they are much more closely related to each other, with a mean value of I=0.936, and a maximum similarity of I=0.994 between jurtina and megala. It could be argued that the differences in species differentiation is a consequence of widely varying evolutionary rates detected by electrophoresis. However, similar differences are suggested by larval chaetotaxy, although smaller differences between Maniola species than those between Pyronia and Hyponephele species are evident in the ova and, to a certain extent, in scale morphology.

It should be noted that the interpopulation genetic differences in lycaon, lupina (section 2.5.5) and Cercyonis (unpublished) were far greater than in any two species of Maniola. Indeed, the interpopulation, interspecific and intergeneric differences between Pyronia and Hyponephele taxa (table 2.5.5e) are much greater than that so far found in butterflies and most invertebrates and very similar to that associated with vertebrates (Ayala 1975, Avise and Smith 1977, Britton and Thaler 1978, see Ferguson 1980).

4.2.2 Evolution of Maniola species

From the accumulated data, including those from androconia, there seems to be little doubt that Maniola is the most recent group within the tribe Maniolini, although there is no certainty of the actual overall differences between species in the genus (section 4.2). The close affinities of the Maniola species suggest that they evolved from a common ancestor in relatively recent times. The two groups of species, most clearly distinguished by electrophoresis, but corroborated by morphological studies, comprise 1. jurtina, megala and chia (the jurtina group) and 2. telmessia, cypricola and nurag (the telmessia group). For the first time, it has been shown here that nurag relates closely to telmessia and cypricola. Bernardi (1961) argued that nurag was a jurtina-form species, in spite of sympatry with jurtina.

The fact that a telmessia group species is now found in Sardinia suggests that a telmessia-like ancestor once penetrated the western Mediterranean, at least as far west as Sardinia. The telmessia group species generally require, or at least are tolerant of higher thermal conditions than jurtina, although nurag and megala are anomalous in this respect. It is dangerous to speculate on the past ecological requirements of an animal on the basis of observations of existing species: these requirements and tolerances can evolve and change as much as other aspects of behaviour, morphology or genetics. However, it is likely that an early Maniola form was, at one time, widespread throughout much, if not all of Europe and western Asia, at least as far north as the present northern limits of the range of jurtina. During a cold climatic interlude in the Pleistocene, the northern limit of this Maniola would have been pushed far to the south at which time the 'species' would have fragmented into relict populations. From a group of these relict populations, two telmessia forms probably evolved, one in Sardinia and perhaps elsewhere (nurag), and another in southern Anatolia and other parts of western Asia (telmessia). An ancestral jurtina form must have survived and evolved in different directions from relict populations in north Africa and possibly Iberia (jurtina) and in southern Anatolia (megala). When warmer conditions returned, jurtina was able to recolonise the areas vacated by the telmessia ancestor. By this time, nurag was sufficiently distinct to be able to live sympatrically with jurtina. Similarly, both megala and telmessia had become sufficiently distinct and were able to retain their species identity in the presence of colonising jurtina.

At the time of retreat when the telmessia ancestor evolved, one of two situations could have prevailed 1. the telmessia ancestor could have become restricted to a relatively small part of southern Anatolia and/or western Asia and extinct in Cyprus, or 2. the ancestral telmessia could have been rather more widespread and survived in Cyprus, possibly in small numbers. This being so, cypricola could have evolved in one of two ways. If the first hypothesis is correct, then cypricola could have evolved from a few founders at the time of ameliorating climatic conditions. If, on the other hand, a telmessia form survived as postulated, then cypricola evolved as a geographical isolate, almost certainly the evolutionary rate being accelerated by

bottlenecking during periods of population stress. A similar situation could have prevailed in the Aegean and Dodecanes islands, especially those adjacent to the Turkish mainland. In another instance, chia could have evolved from a surviving jurtina-form relict, or as a result of founder effect at a time of rapid population expansion.

Important clues relevant to the evolution of Maniola species can be gleaned from the electrophoretic interpopulation studies detailed in section 2.2. It has been suggested here that, in jurtina, there is a level of gene flow between populations which is so low that a small increase in genetic isolation, be it geographic or some other form, can cause a sudden and substantial increase in genetic differentiation. Conversely, periodic behavioural changes result in dispersal activity not normally associated with the species.

It is now believed that the colonisation of the Scottish Western Isles was post-glacial (Dennis 1977, Thomson 1980). The distinct genetic configuration of populations on Barra, Tiree, Orkney and Cramond Island must reflect something of the genetics of these post-glacial founders. It is clear from the analysis of Scottish and Irish populations, and their genotypic affinities with other jurtina populations, that the Western Isles of Scotland were colonised from mainland Scotland. This has considerable bearing on the old argument that the fauna of these islands was Hibernian in origin (Harrison 1943, 1948). As far as jurtina is concerned, the evidence is quite contrary to that suggestion.

The more subtle differences between British groups of populations which have been detected electrophoretically (section 2.5.1) might owe something of their genetic configuration to climatic and vegetational changes during the Holocene (Perring 1974) or to selection, but further studies on interpopulation differences are needed before a weight of evidence can be presented.

The Irish jurtina undoubtedly have had a very different origin from those in the rest of Britain, one which relates more to the post-glacial recolonisation of Europe than to more local and later evolutionary events in Britain. During the last (Weichselian) glaciation, it is unlikely that jurtina would have survived in all but the most

southern parts of Europe (Dennis 1977). It certainly could not have survived in the periglacial environment of Britain during the Loch Lomond Advance of 10,800-10,300BP (Dennis 1977, Thomson 1980). It has already been suggested that the relict colonies of the species were probably centred in Iberia and north Africa, but there probably also were relict populations in south Italy and Greece. Faunal depauperation on islands would have been inevitable. It would have been from these populations that the recolonisation of Europe and western Asia by the species would have originated. The evolution of the 'western' and 'eastern' genitalia types probably derive from this disjunction. The direction of colonisation by the western form would have been in an eastern, western and northern direction. As it moved eastwards it would have met the eastern form expanding its range. Some islands would have been colonised from the west (the Balearic Islands, Sardinia, Capri) while on others both eastern and western forms were able to integrate (Corsica, Elba, Giglio). Somehow, the western form which populated Capri and the 'transitional' populations in Corsica and the Elba group have not been greatly affected by latter immigration, nor have the populations on either side of the Straits of Messina between Calabria and Sicily lost their eastern and western morphological characteristics. Selective forces, no doubt, have helped to maintain the characteristics of these populations: to what extent selection has contributed to the forms on these islands is much more difficult to ascertain. Further work on jurtina on Capri might detect evidence of character displacement.

The rapidly colonising western jurtina would have met the barrier of the Alps. This would have effectively isolated the two groups in southern Europe. The effect of the Atlas Mountains and Pyrenees in restricting gene flow has already been shown (section 2.4.1). However, as the effectiveness of the altitude barrier broke down in the Jura, Vosges and further to the north, the two forms could mix freely, creating the zone of secondary intergradation which has been clearly identified by an analysis of the genitalia form. It should be noted that the intergradation zone broadens northwards, corresponding to the decreasing effectiveness of altitude as an isolation mechanism. Although this zone is clearly seen in the analysis of the genital armature, and the eastern and western forms are clearly identifiable by both morphological and electrophoretic methods, the genetic

data do not suggest a similar area in which intermediate or mixed populations are found. Populations which would be classified as 'transitional' on morphological grounds grouped with the eastern forms in the analysis of electrophoretic data. If, as is suggested here, this zone of intergradation is relatively recent, either it is being maintained by stabilising selection or else the boundaries of the zone will be changing slowly over a period of time. It is possible that this change could be detected by monitoring the situation over a number of years.

The close correspondence of allozyme data with genitalia in the 'eastern' and 'western' forms and their geographic distribution, together with the effects of the Atlas, Pyrenees and Alps on these organisms, strongly suggest that the major population groups derive their allozyme configuration and genital morphology from their evolutionary ancestors, far more than from selection.

Electrophoretic analysis related the south-west European hispulla with jurtina from Alderney, the Isles of Scilly and Ireland. It is likely that post-glacial dispersal and colonisation would have been very rapid, especially in the western coastal part of Europe (Dennis 1977). It is probable that some of these early founders reached and colonised Alderney, the Isles of Scilly and Ireland - true Lusitanian elements of the fauna (Scharff 1899). The fact that the sex-associated heterozygote deficient GOT-1 populations are found in Ireland and not in mainland Britain (section 2.1.3) would support this hypothesis.

Almost all attempts at reconstructing the evolutionary zoogeography of animals have sought evidence of landbridges at appropriate time to establish the geomorphological conditions which would have facilitated colonisation of present day islands (Ford 1945, Harrison 1943, 1948, Beirne 1943, 1947, 1952, Dennis 1977 and many other works on non-Lepidopterous faunas). While such landbridges undoubtedly would have been important to the most sedentary terrestrial creatures, the importance of involuntary colonisation by such mechanisms as introduction by man is probably greatly underestimated (Berry 1979). It could be thought that the absence of some species in Ireland which are present in mainland Britain (Heath, Pollard and Thomas 1984)

would suggest that insularity has effectively isolated post-glacial colonisers from some islands. However, we have no evidence whatsoever that these species did not occur there in historical times and have subsequently been eliminated by adverse climatic or other environmental conditions.

The periodic dispersal behaviour of relatively sedentary species could easily account for the presence of the organisms on remote islands, without the need for land connections. Maniola jurtina has been reliably recorded on two occasions on the Fair Isle, between Orkney and Shetland (Biological Records Centre), nearly 100km north of its most northerly, precariously established colony on Orkney. Only one or two vagile females in several thousand years would be required to establish a colony, in these circumstances, provided climatic and other environmental conditions fitted the species ecological needs.

Using a mathematical model, Maruyama and Fuerst (1984) suggested that allelic polymorphism can be achieved from zero variability in less than 20 generations, although this rate did not take account of the possible effects of selection. However, it is likely that selection pressures would be less in a species, colonising virgin territory and occupying a vacant niche. These conditions probably would have been met for many hundreds, if not thousands of years after the last glaciation. A rapid increase in population variation could then take place following colonisation by a few founders. Nei et al. (1975) proposed that it would take from 100,000 to 10,000,000 generations for a population to recover fully from the effects of bottlenecking, although much appears to depend upon the size of the bottleneck and the rate of population growth. They suggested that, even if the size of the bottleneck was very small, if population size increased rapidly - populations of Maniola species can, and do, increase very rapidly (Pollard 1981) - the reduction in average heterozygozity would be very small. They also suggest that a substantial fraction of the variability is retained by the population, even in extreme cases, although the average number of alleles can be profoundly affected. This is exactly the situation described in this work for islands which are suspected of being colonised by a few founders - Orkney, Isles of Scilly, Barra, Gottland - and established after a short

period of extremely rapid population growth (section 2.4.2 and 2.5.1), or populations which have been bottlenecked - Feldthunes (section 2.5.2). This being so, the potential exists for successful colonisation and establishment of Maniola on islands from adventitious founders, without land connections.

Recent work on geomorphological conditions in western Scotland and the Western Isles (Gray and Lowe 1977, Price 1980, 1983) suggests that it is unlikely that the Outer Hebrides would have been connected to the Inner Hebrides and mainland Scotland in the post-glacial period. However, an explanation of the colonisation and establishment by jurtina (of the Western Isles of Scotland from mainland Scotland, Ireland and the Isles of Scilly from continental Europe, the Balearic Islands, Corsica, Sardinia, Capri, Sicily and Malta from Iberia and/or north Africa and the western Aegean islands from mainland Greece) in post-glacial times need not be inhibited by the fact that land connections did not exist at that time. Similarly, telmessia could easily have reached the Dodecanes and other eastern Aegean islands over water.

As the geomorphological and palaeobotanical evidence is slight, little is known of the past environmental conditions in Anatolia until after the middle Pleistocene (Butzer 1978). It has been suggested that the environment of Greece could be compared with that of northern Scotland today during the last glacial period (Higgs 1978). Eastern Mediterranean conditions in the upper full glacial were of steppe vegetation (Bottema 1978). It is likely that jurtina could have survived in southern Anatolia at that time, but certainly not telmessia, except in warm relict environments, possibly at sea level and perhaps further to the east.

Although landbridges need not be a prerequisite for colonisation, an increased degree of isolation, which insularity provides, could contribute to the establishment of conditions which would accelerate allopatric speciation. In the eastern Mediterranean, the late glacial mean sea level would have been about 100m lower than today (Erinc 1978), which would be far less than required to connect Cyprus with mainland Turkey, and the conditions for the evolution of cypricola from the ancestral telmessia form would have been appropriate for a

considerable period stretching well back into the previous interglacial.

The situation with Chios is much more ambiguous. The isobath between the island and mainland Turkey is less than 100m. There is some evidence that late- and post-glacial sea level changes in that part of the eastern Aegean were not so great as elsewhere in that part of the Mediterranean region (Eisma 1978), although the situation is not at all clear. If spatial isolation is required for speciation to take place then very recent colonisation and speciation is easier to explain in the light of isostatic[1], eustatic[2] and relative sea levels. If Maniola chia evolved from a jurtina rather than a telmessia ancestor, as is suggested by electrophoretic and morphological data, Chios could have been colonised from the west, across the Aegean, or from the east across the short 15km straight between the island and the Cesme peninsular. In ameliorating climatic conditions, jurtina would have been indigenous in the adjacent part of Turkey before telmessia, and could have colonised the island before telmessia had the opportunity to do so. However, Brice (1978) indicated that the prevailing winds between 18,000BP and 8,500BP in the eastern Mediterranean were westerly, which could have assisted the jurtina ancestor in its colonisation from the west. Certainly, in the expression of wing markings, chia has more in common with some large Greek island form than those of north-western Turkey, although this might be due to environmental effects. Electrophoretic analysis of north-western Turkish jurtina and more extensive samples of Greek island jurtina could provide further information on this interesting problem.

Thus, several factors appear to have been important in the evolution of geographical forms and species in Maniola.

1. Disjunction during glacial times created geographical isolates.

2. Founders reaching islands established populations which were ylittle affected by later immigrants (probably due to rapid population growth and stabilising selection).

3. Colonisation was rapid in the ameliorating climatic conditions.

[1] resulting from adjustments in the balance between parts of the earth's crust
[2] resulting from worldwide changes in sea level

4. Bottlenecking, in some instances, accelerated evolutionary rates.

5. High mountains (especially the Atlas Mountains, Pyrenees and Alps) were very effective in creating isolating barriers to population dispersal and gene flow, whereas stretches of sea did not effectively restrict the colonisation of islands.

Selection would undoubtedly have played an important part in the evolution of jurtina forms, but the degree to which it was effective, although difficult to judge, could have been less than the other factors taken together. However, if the Jullien organ is shown to be a sound producing organ used in the context of behavioral ecology, then the role of selection will have to be reassessed.

4.2.3 Evolutionary rates

Although random amino acid substitution is a consequence of the concept of selective neutrality, the existence of the molecular clock does not depend upon the validity of the neutral hypothesis (Van Valen 1974, Fitch and Langley 1976, Sarich and Cronin 1976, Zuckerkandl 1976, Thorpe 1982). However, there would appear to be little strength left in the argument for an accurate molecular clock as proposed by extreme 'neutralists' (Zuckerkandl and Pauling 1962, Jukes and Holmquist 1972, Fitch 1973, 1976, Radinski 1978, Throckmorton 1978, Lessios 1979, 1981, Korey 1981,). When all aspects of an organism are considered, including molecular, morphological, behavioural and physiological, it is clear that evolutionary rates differ. It has been shown in the present work that biochemistry (electrophoretic data) and morphology (chaetotaxy data) do not correspond in the levels of evolutionary divergence between species, and indicate very different levels between groups of species. The large electrophoretically detectable differences between populations and species in Pyronia and Hyponephele are not reflected in their relatively similar genitalia morphology. Whether this is due to some protein characters being 'selectively neutral' (although subject to selection in time) is not clear, but the conclusion which can be drawn is quite simply that the evolution of proteins in these butterflies is not matched by similar rates in morphology.

Variation in Maniola jurtina populations, however, shows patterns, both biochemical and morphological, which suggest evolutionary developments which run parallel in space and time. It has been shown here that the 'eastern' and 'western' forms of the male genitalia (also, although not included here, the female genitalia) are matched by electrophoretically detectable 'eastern' and 'western' groups. It would be difficult to explain how a situation in which these patterns of genitalia variation in Europe and western Asia could be a consequence of, and be maintained by selection. These patterns of variation are similar over a very wide range of environmental conditions, yet the two forms remain distinct, except in the 'transitional zone', both biochemically and morphologically. This would suggest that these characters in jurtina are not being maintained by selection. Clearly, there will be 'fine adjustments' to the expression of these characters which are subject to such modifications, but the various conditions to which these butterflies are exposed throughout their range have not resulted in substantial electrophoretically detectable changes or morphological modifications. Therefore, in jurtina, it is suggested that these biochemical and morphological characters owe their form and expression more to important evolutionary events in their past than to selection pressures in the present.

One of the principal arguments against the accuracy of the molecular clock is that rates of protein change vary (Dayhoff 1972, Fitch and Margoliash 1970, Dickerson 1971, Tashian et al. 1972, 1976, Fitch 1972, 1973, 1976, Fitch and Langley 1976, Goodman 1976, De Jong et al. 1977). It has been suggested (Ward test Thorpe 1982) that the notion of bimodal 'fast' and 'slow' groups of evolving proteins (Sarich 1977) cannot be supported and that the variation in protein evolutionary rates approximates to a normal distribution. The problems of differential rates can be overcome, to a some extent, by imposing on the calculation of these rates a number of constraints.

1. The same proteins must be utilised, so that there will no differences due to different proteins evolving at different rates. In this study, the same group of isozymes were used throughout the genus.

2. Restrict the analysis to a very closely related group, so that different rates of evolution in different species are minimised. Maniola has been shown to be a very closely related group

3. Establish the overall rate of change from a known standard. Maniola jurtina could not have survived the last glaciation or Loch Lomond Advance in Britain and almost certainly arrived sometime in the last 10,000 years, possibly even more recently than that. The maximum genetic distance must then have accrued in that period of time if the populations were established from founders with a common gene pool. This would have been the case in mainland Britain (Dennis 1977).

4. Accept that their will be a large error in the results.

To determine the evolutionary rate from electrophoretic data the following procedure is suggested.

1. Let T_{max} equal the maximum time for divergence to occur between the populations - 10,000 years in this case.

2. Let D_{max} be the maximum genetic distance between any two populations - mainland Britain in this case.

(As I values were extrapolated from 1-G/2 based on large sample using polymorphic loci (see section 1.3.6), two values can be found, $D_{max95\%}$ and $D_{max99\%}$. Two further values of D_{max} can be taken to represent the maximum genetic distance in British populations 1. the maximum genetic distance overall - Huna/Barra and 2. the genetic distance between the two most geographically isolated mainland populations, eliminated possible 'island effect' - Huna/Tiptree. Therefore, $D_{max95\%}$ for the smaller of the two distance values can be used to calculated the lower limit of T and $D_{max99\%}$ for the greater of the two distance values can be used to calculate the higher limit of T)

3. As $D = 2\alpha T$ (Nei 1971, 1972)

when α is the number of electrophoretically detectable codon substitutions per locus per year, then

$$\alpha = D_{max} / 2T_{max}$$

Note that, as Maniola species are univoltine, T_{years} = number of generations.

Thus, for jurtina

$$\alpha_{min} = 1.6^{-6}$$

$$\alpha_{max} = 6.5^{-7}$$

These rates are somewhat faster than the average given by Nei of 10^{-7}. Thus

$$T_{min} = D_{max95\%} / 2\alpha_{min} = 3.2 \times 10^{5} D_{max95\%}$$

$$T_{max} = D_{max99\%} / 2\alpha_{min} = 7.6 \times 10^{5} D_{max99\%}$$

From these evolutionary rates a number of divergence times for Maniola populations and species were estimated (table 4.3.2a). These estimates could confirm the suggestion that the divergence of species and populations of Maniola was very recent. They would suggest that divergence occurred just before, during or just after the last full glacial period, that is in the last 100,000 years. The interpretation of climatic changes during the last 10 millennia has been complicated by the somewhat discordant evidence from ^{16}O and ^{18}O isotopes (Dansgaard et al. 1969), palaeobotany (see West 1972) and invertebrate faunas, especially Coleoptera (Bell et al. 1972, Coope and Angus 1975, Briggs et al. 1975). Although the period from about 75,000BP to about 14,000BP was predominantly cold, there is some evidence that, for a very short time about 43,000BP (the Upton Warren Interstadial in Britain), summer temperatures were somewhat higher than those of today (Coope and Angus 1975). From the estimated divergence times for Maniola, it seems more likely that the divergence of the jurtina group ancestor from that of the telmessia group took

table 4.2.3a

Estimated divergence times (years) for some populations of *Maniola jurtina* and species of *Maniola*.

jurtina populations	divergence time
Digne / Marzabotto (maximum for species)	20,170 - 59,827
hispulla / Verbier	10,873 - 33,020
hispulla / Gignod	14,535 - 44,214
Gignod / Verbier	10,873 - 33,020
***Maniola* species**	
jurtina / *telmessia*	30,179 - 71,676
jurtina / *megala*	1,926 - 4,574
jurtina / *chia*	11,733 - 27,865
telmessia / *cypricola*	5,813 - 13,805
telmessia / *nurag*	7,119 - 16,907

plate 3.2.1c

Scanning electron micrographs of Manioline ova - rib structure.

1. Maniola jurtina, Dunblane, Perthshire, X430, SEM0539/2
2. Maniola telmessia, Hakkari, Turkey, X390, SEM0570/2
3. Maniola cypricola, Episkopi, Cyprus, X450, SEM0501/2
4. Maniola nurag, Nuoro, Sardinia, X610, SEM0530/2
5. Pyronia tithonus, St Bees, Cumbria, X620, SEM0556/10
6. Maniola bathseba, Madrid, Spain, X250, SEM0595/10
7. Aphantopus hyperantus, Drumloist, Perthshire, X830, SEM0491/2
8. Pyronia cecilia, Sardinia, X440, SEM0512/4
9. Cercyonis pegala, Macomb, USA, X2120, SEM0455/2

place during the warm interlude about 43,000BP, rather than more than 100,000BP during the last interglacial. Indeed, if these figures give even a hint of approximate divergence times, they suggest that all other Maniola species appeared at the end of, or just after the last glaciation. The divergence of the 'eastern' and 'western' forms of jurtina would then have occurred during the last glacial period (about 40,000 to 14,000BP). The very recent divergence time for megala is of considerable interest. Even if one additional and entirely diagnostic locus (ie monomorphic for alternative alleles) was included, it would still suggest a divergence time very similar to that of telmessia/cypricola/nurag and jurtina/chia (approximately 12,500 to 29,500BP). On the other hand, the estimated divergence time of Karpathos Maniola from telmessia (Appolakkia), approximately 17,500 to 54,000 years, lends support to the probability that these butterflies represent a distinct taxon.

Of course, it could be that, since the time of speciation of megala, chia, cypricola and nurag, these insects have been in a state of slow or even suspended evolutionary development. However, if this was so, then these species must be resisting very different selection pressures and other external influences. Convergent evolution as an explanation must be dismissed for similar reasons.

These calculations have been based on the formula proposed by Nei (1971). Using the formula of Gorman et al. (1976), divergence time = Dn where n equals an evolutionary unit of 18×10^5 years, on jurtina gives divergence times of over 300,000 years for the most distantly related populations and over 66,000 years for those which are most closely related. Obviously, bearing in mind the climatic history of Britain, this cannot be correct unless every British population arose from a different and totally isolated group of founders! Clearly, the Gorman molecular clock requires very careful calibration.

These calculations, of course, could be fundamentally flawed if evolutionary rates of individual proteins are not constant within themselves, as is almost certainly the case. In some circumstances, if the appropriate conditions were met, a species might encounter some sort of 'genetic inertia' which would result in an estimated evolutionary rate which was initially slower than that suggested, but which accelerated in time. This would mean that the divergence times

indicated for Maniola were underestimated. However, the converse might be the case, that is that evolving species experience an initial relatively rapid rate of evolution which slows in time. If this was so, then the calculated Maniola divergence times are overestimated. Certainly, if the view that speciation is accelerated during periods of disjunction and as a consequence of founder effect is accepted, the history of Maniola as detailed here would support the hypothesis that very rapid evolution in these butterflies has taken place in recent times.

4.3 Systematic conclusions

It has been shown that electrophoretic and morphological data largely confirmed the accepted systematic arrangement and taxa (sections 2 and 3). It has also been shown that Aphantopus and Cercyonis are Manioline genera and that Aphantopus appears to lie somewhere between Pyronia and Hyponephele.

Results from biochemical and morphological analyses confirm a number of clearly defined groups within the genera Maniola and Hyponephele.

The most problematic situations relate to decisions at species level. It has been shown that electrophoretically detected differences between populations of Hyponephele lupina strongly suggest that H. lupina rhamnusia and H. lupina mauritanica are distinct species. However, these differences were similar to the levels of genetic differentiation which were detected between populations of Hyponephele lycaon and Cercyonis pegala. More extensive studies in this group, including other species, are indicated and, until further information is to hand, it is suggested that the present systematic arrangement for this genus is retained (table 4.3a).

The evidence which has been presented indicates that the Maniola jurtina complex is a single species unit, and that the 'eastern' and 'western' forms represent major subspecies groups.

All electrophoretic analyses show that the relationship of the Karpathos Maniola to telmessia is much less close than was expected.

Indeed, the indications are that the telmessia-like butterfly there is a quite different species. No morphological differences have been detected so far, but these might have been overlooked. Morphological differences between telmessia and cypricola without differentiation identified by electrophoresis have been demonstrated.

table 4.3a

Suggested systematic arrangement for Maniolini

tribe	genus	subgenus or group	species
Maniolini	Maniola	jurtina group	jurtina
			megala
			chia
		telmessia group	telmessia
			cypricola
			nurag
			[Karpathos]
	Pyronia	Pyronia	tithonus
		Pasiphana	bathseba
			[janiroides][1]
		Idata	cecilia
	Aphantopus		hyperantus
	Hyponephele	lycaon[2] group	lycaon
			maroccana
		lupina[2] group	lupina
	Cercyonis[2]		pegala

[1] position uncertain

[2] position of other species not known

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APPENDIX

PAPER

Maniola chia - a new satyrid from the Greek island of Chios (Lepidoptera: Nymphalidae: Satyrinae) - Phegea **15**(1), 13 - 22 (1 January 1987)

Maniola chia - a new Satyrid from the Greek island of Chios (Lepidoptera : Nymphalidae : Satyrinae)

George THOMSON

Samenvatting. *Maniola chia* - een nieuwe Zandoog van het Griekse eiland Híos (Lepidoptera : Nymphalidae : Satyrinae)
De auteur beschrijft een nieuwe soort uit het genus *Maniola* SCHRANK van Híos. De identifikatie van het nieuwe taxon is gebaseerd op kenmerken van de uiterlijke morfologie, genitalia, morfologie van het ei, chaetotaxie van de rups en enzyme-elektroforese. Verondersteld wordt dat deze soort voornamelijk evolueerde als een gevolg van het «founder effect».

Résumé. *Maniola chia*- un nouveau Satyride de l'île grècque de Chios (Lepidoptera : Nymphalidae : Satyrinae)
L'auteur décrit une nouvelle espèce dans le genre *Maniola* SCHRANK provenant de Chios. Pour l'identification du nouveau taxon la morphologie externe, les genitalia, la morphologie de l'oeuf, la chaetotaxie larvaire et l'électrophorèse enzymatique sont utilisés. Il est supposé que l'espèce a évolué principalement par la suite du «founder effect».

Abstract. *Maniola chia* - a new Satyrid from the Greek island of Chios (Lepidoptera : Nymphalidae : Satyrinae)
A new species of the genus *Maniola* SCHRANK from Chios is described. External morphology, genitalia, embryonic morphology, larval chaetotaxy and enzyme electrophoreses were used in an identification of the new taxon. It is suggested that the species evolved principally as a result of founder effect.

Keywords : *chia, Maniola*, Chios, Satyrinae, speciation, morphology, electrophoresis.

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The butterfly fauna of the eastern Greek islands is poorly documented. The early works of REBEL (1916, 1924, 1935, 1936), TURATI (1929), TURATI & FIORI (1930), HARTIG (1940), REISSER (1946) and even the more recent papers by BENDER (1963) and BRETHERTON (1966) have been shown to be superficial in their coverage (THOMSON, 1985; RIEMIS, 1986; GASKIN & LITTLER, 1986; OLIVIER, 1986). The distribution of *Maniola telmessia* (ZELLER, 1847) and *jurtina* (LINNAEUS, 1758) in the eastern Aegean islands was known, until recently, only from some of the abovementioned lists together with the paper of TAUBER & TAUBER (1968). *Maniola telmessia* has been recorded from Karpathos, Rhodes, Symi, Kos, Kirykos, Samos and Lesbos. It was also reported from Chios by REBEL (1935) as '*Epinephele telmessia* Z. : Insel Chios, 10 und 11.VI und Insel Mytilene 13 bis 16.VI (W.W.), ein ♂, fünf ♀.' On the other Greek islands from which there are records *jurtina* is found (COUTSIS, 1969, 1972), but there are many where the situation is not known.

In May 1986 a short series of Satyrid butterflies which had been collected on the Greek island of Chios (GASKIN & LITTLER, 1986) was received from Professor David GASKIN. These had been designated *Maniola telmessia*, but

there was some doubt about their true identity. The males resembled rather large *cypricola* GRAVES (1928) or *megala* (OBERTHÜR, 1909). The females were superficially indistinguishable from the large *jurtina* phenotypes which are found in some of the eastern Mediterranean, Ionian and Aegean islands, including Crete, Corfu (THOMSON, 1969) and Zakynthos (GASKIN, pers. comm.). Dissection of both male and female genitalia revealed that the Chios butterflies were certainly not *telmessia* and, if they were to be assigned to an existing taxon, they would accord more appropriately with *Maniola jurtina*. While the form of the female genitalia suggested this affinity (THOMSON, 1976), the anomalous structure of the male genitalia in relation to known geographical variation in southern and eastern Europe (THOMSON, 1973), left considerable doubt.

Through the generous help of Mr Alain OLIVIER, 20 further individuals (10 males and 10 females) of this butterfly, collected in the Nea Moni Monastery area of the island, were received alive. Two females were subjected to photoperiod regulation in an attempt to induce oviposition. By July 12, 16 ova were laid from which 12 larvae hatched. These did not survive beyond the first instar. In preparation for enzyme electrophoresis 10 males and 9 females, including one of the laying females, were frozen.

On examination of this material it became clear that these butterflies represented an entirely new taxon. Both larval chaetotaxy and allele frequencies from electrophoretic analysis proved conclusive. Examination of a long series of Chios *Maniola* in the Instituut voor Taxonomische Zoölogie, Zoölogisch Museum, Amsterdam, extended the morphological data and contributed to an overall picture of variation in the species.

Description

***Maniola chia*, new species** (Lepidoptera : Nymphalidae : Satyrinae)

Male

Wing length : mean 23.84 mm (±1.172 SD), largest 26.86 mm, smallest 21.10 mm.

Upperside forewing : ground colour uniformly blackish sepia. Fulvous around ocellus usually distinct, occasionally extending as a clear submarginal band to vein 2. Apical ocellus distinct, usually moderately large or large, frequently with a single white pupil, occasionally bipupilled. Androconial brand conspicuous, black, curved and tapering upwards, frequently extending beyond vein 3.

Upperside hindwing : uniformly blackish sepia.

Underside forewing : ground colour uniformly golden yellow-brown. Medial line, when present, slightly darker than ground colour. Outer margin mid grey-brown, about .1 wing length, widening towards the wing apex. Costa narrowly edged grey-brown. Apical ocellus distinct, usually single pupilled, occasionally bipupilled and surrounded indistinctly by light yellow-fulvous.

Underside hindwing : almost uniformly mid grey-brown, occasionally reddish grey-brown. Submarginal band slightly paler or indistinct. Ocelli

often large and distinct, 2 to 5 in intervenosa 2, 3, 4, 6 and 7, each surrounded by ochreous yellow, frequently white pupilled. Striae small and inconspicuous.

Female

Wing length : mean 25.87 mm (±1.745 SD), largest 28.06 mm, smallest 20.52 mm.

Upperside forewing : ground colour uniformly dark sepia, lighter than the ground colour of the male. Fulvous area extensive, always present in discal area and as a distinct but variable marginal band extending from vein 2 to vein 8. Discal and submarginal areas usually separated by a distinct, broad, dark medial line of the ground colour. Fulvous around apical ocellus paler. Apical ocellus large, frequently very large, usually distinct and with 1 or 2 white pupils.

Upperside hindwing : ground colour as forewing. Submarginal fulvous band usually clear and extensive.

Underside forewing : ground colour golden yellow-brown. Discal area paler, yellowish. Medial line inconspicuous, darker than ground colour. Outer margin grey-brown, almost .15 wing length, widening towards wing apex. Apical ocellus large, sometimes very large, distinct with 1 or 2 white pupils. Area around apical ocellus paler than the submarginal band.

Underside hindwing : ground colour variable, uniformly light grey-brown to mid sepia. Submarginal area shaded light violet-grey to fulvous, especially adjacent to the medial line. Ocelli 0 to 3. Striae conspicuous.

Genitalia

Male (figure 1, 1-4, figure 2, 2) : uncus long. Gnathos extending to more than .75 of the uncus length, gnathos base slightly dilated. Valve variable as with other *Maniola* species, significantly larger than *telmessia*, shaped differently from that of most *telmessia* but approximately similar to some individuals from Lesbos and Samos, distal process bluntly pointed, dorsal process (lobe) wide, less wide than in the eastern *jurtina* form (THOMSON, 1973) but wider than in western *jurtina*. Aedeagus broad and straight. Julien Organ thicker than that of *telmessia*, possibly slightly less so than in most eastern *jurtina*.

Female (figure 1, 5) : lamella postvaginalis large and broad, similar to that of *jurtina*, considerably larger than that of *telmessia*. Lamella antevaginalis variable, usually heavily sclerotised. Bursa occasionally with two signa of a length greater than those of most *telmessia* but much shorter and lighter than those in *cypricola*.

Ovum

Similar to that of *telmessia*, but shorter (less tall) and greater in diameter than most *telmessia* ova so far examined. Rib number 13 to 14, compared with 14 to 16 for *telmessia* and more than 18 for *jurtina*. Form symmetrical, unlike some *telmessia*.

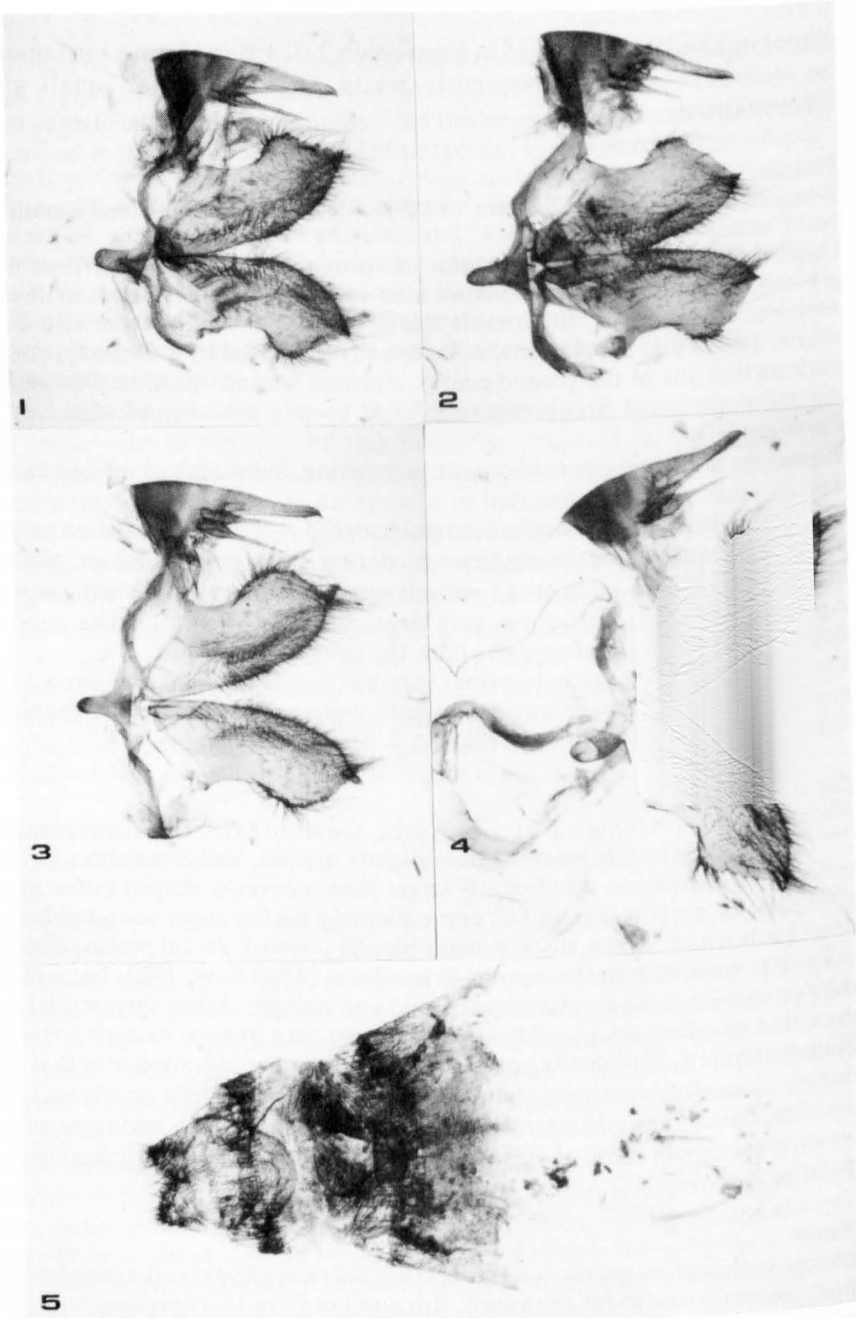

Figure 1 : 1-4 *Maniola chia* n.sp., Nea Moni, Chios, 30 May 1986 - male genitalia; 5 *Maniola chia* n. sp., female genitalia - data as male (magnification x10)

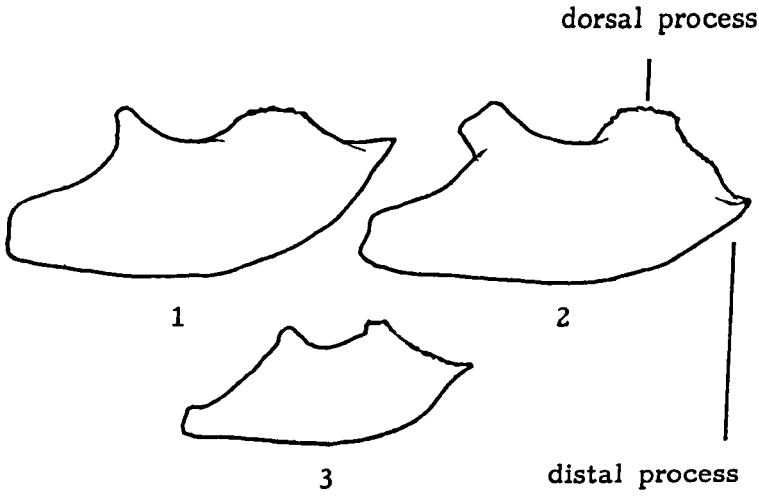

Figure 2 : Shape of typical male genital valves - 1 *Maniola jurtina* ('eastern' form, Istanbul, Turkey), 2 *Maniola chia* n.sp. and 3 *Maniola telmessia* (Samos, Greece).

First instar larva

Superficially similar to *telmessia*. Position of spines and setae identical with *telmessia*, but spine lengths differ markedly between the species.

Genetics

Allele frequencies suggest a relationship distinct from other *Maniola* species. A single monomorphic locus is fixed for a mobility different from that of both *jurtina* and *telmessia*. This dimeric locus is diagnostic for the *jurtina* and *telmessia* groups.

Variation

The range of variation in wing markings is similar to that in other *Maniola* species. Variation occurs principally in the size of the apical ocellus, the extent of fulvous on the upperside, the degree of melanism on the underside and the number and position of the underside hindwing ocelli. Three males and one female of a form homologous with form *addenda* MOUSLEY (THOMSON, 1969) of *M. jurtina* and three males with an ocellus on the upperside hindwing are included in the material studied. The range of variation in size is considerable, particularly in the males (figure 3).

Distribution and range

Known only from the island of Chios where it is, apparently, widespread and common, flying to at least 500 m.

Habitat

Flies in similar situations to *jurtina* and *telmessia* in south-eastern Europe, garigue, cultivated areas and less open habitat where the adults can find shade

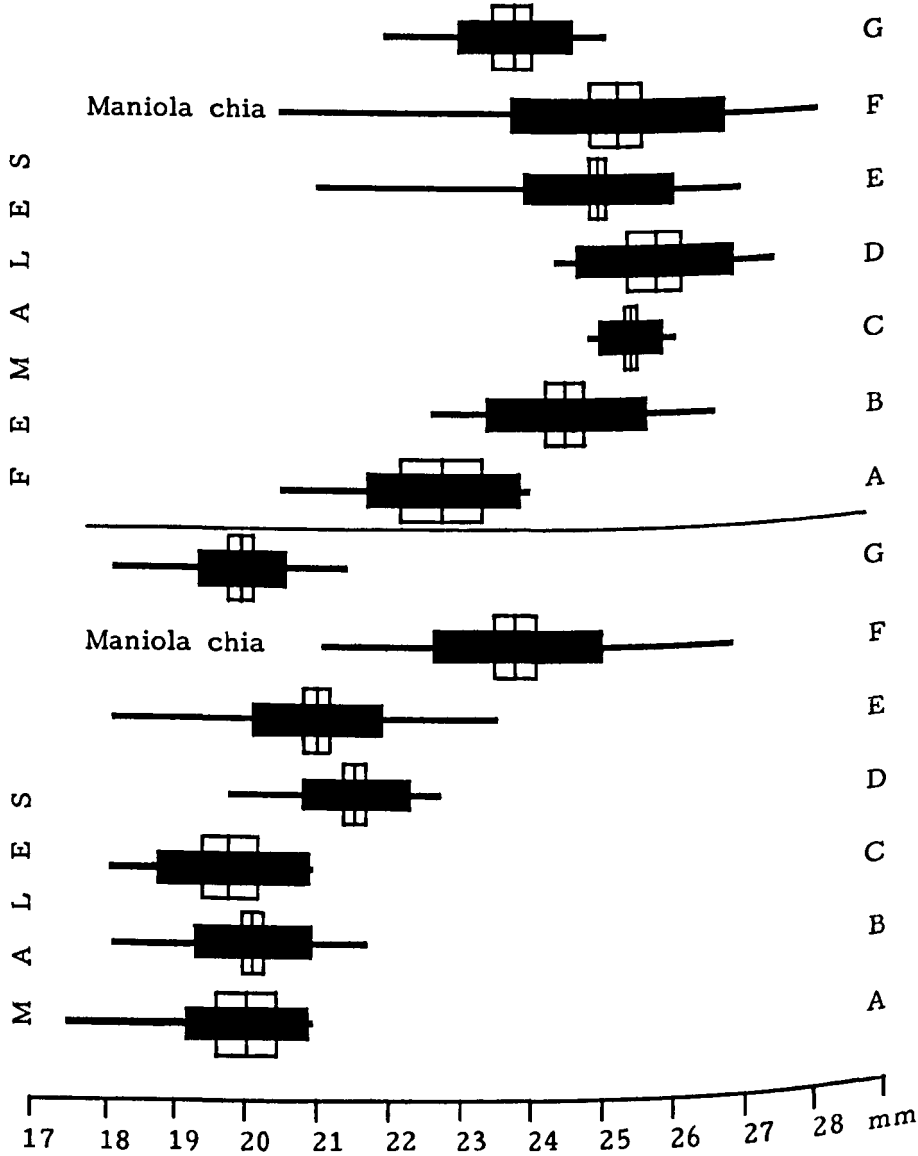

Figure 3 : Wing lengths of male and female *Maniola telmessia* and *M. chia* n.sp. from the Greek islands of Karpathos (A), Rhodes (B), Symi (C), Kos (D), Samos (E), Chios (F) and Lesbos (G); the thin line represents the range, the thick line 1 standard deviation each side of the mean and the open box 2 standard errors on each side of the mean.

including small pine woods with undergrowth and in particular near bushes especially *Quercus ilex* and *Rubus* sp. (OLIVIER, pers. comm.).

Flight

Earliest 23 May, latest 20 September. The adults are presumed to aestivate in the summer months like all southern *Maniola* species.

Types

Holotype male (figure 4, 1, 3) : «Nea Moni (Hios, Griekenland) (500 m) / 30-V-1986 / coll. Alain OLIVIER» (Nea Moni, Chios, Greece, 500 m, 30 May 1986, leg. Alain OLIVIER), deposited in the Instituut voor Taxonomische Zoölogie, Zoölogisch Museum, Amsterdam.

Allotype female (figure 4, 2, 4) : «Nea Moni (Hios, Griekenland) (500 m) / 27-V-1986 / coll. Alain OLIVIER» (Nea Moni, Chios, Greece, 500 m, 27 May 1986, leg. Alain OLIVIER), deposited in the Instituut voor Taxonomische Zoölogie, Zoölogisch Museum, Amsterdam.

Paratypes : 11 males, 20 females deposited in the Instituut voor Taxonomische Zoölogie, Zoölogisch Museum, Amsterdam, 28 males, 28 females (A. OLIVIER collection), 5 males, 5 females (author's collection), 1 male, 1 female (D. VAN DER POORTEN collection), 1 male, 1 female (J. DILS collection), 1 male, 1 female (A. RIEMIS collection), 1 male, 1 female (W.O. DE PRINS collection).

Material examined : 10 males, 26 May 1986, 5 km north-west of Chios, 300 m, leg. A. OLIVIER; 1 male, 28 May 1986, 5 km north-west of Chios, 300 m, leg. A. OLIVIER; 1 male, 29 May 1986, 5 km north-west of Chios, 300 m, leg. A. OLIVIER; 16 males, 11 females, 25 May 1986, Nea Moni, 500 m, leg. A. OLIVIER; 1 male, 26 May 1986, Nea Moni, 500 m, leg. A. OLIVIER; 8 males, 12 females, 27 May 1986, Nea Moni, 500 m, leg. A. OLIVIER; 16 males, 16 females, 30 May 1986, Nea Moni, 500 m, leg. A. OLIVIER; 5 males, 13 females, 30 May 1986, Nagos, 50 m, leg. A. OLIVIER; 12 females, 26 May 1986, 7 km north-west of Chios, 500 m, leg. A. OLIVIER; 1 female, 29 May 1986, 7 km north-west of Chios, 500 m, leg. A. OLIVIER; 3 males, 5 females, 23-28 May 1984, Kardamila, leg. D.E. GASKIN; 3 females, 16-20 September 1985, E. Marmaron, leg. D.E. GASKIN; 1 female, 28 May 1984, Marmaron, leg. D.E. GASKIN.

Discussion

Chios is one of the large group of islands which lies a short distance from the Turkish mainland. On the other islands which have been studied, Karpathos, Rhodes, Symi, Kos, Samos and Lesbos, *telmessia* is the only *Maniola* which has been found. The wing length of male *Maniola chia* should distinguish it from most *telmessia* (figure 3). Female size overlaps with that of *telmessia* on the other islands in the eastern Aegean and dissection of genitalia is essential for identification.

Although the large size of *chia* contrasts greatly with the small *telmessia* on

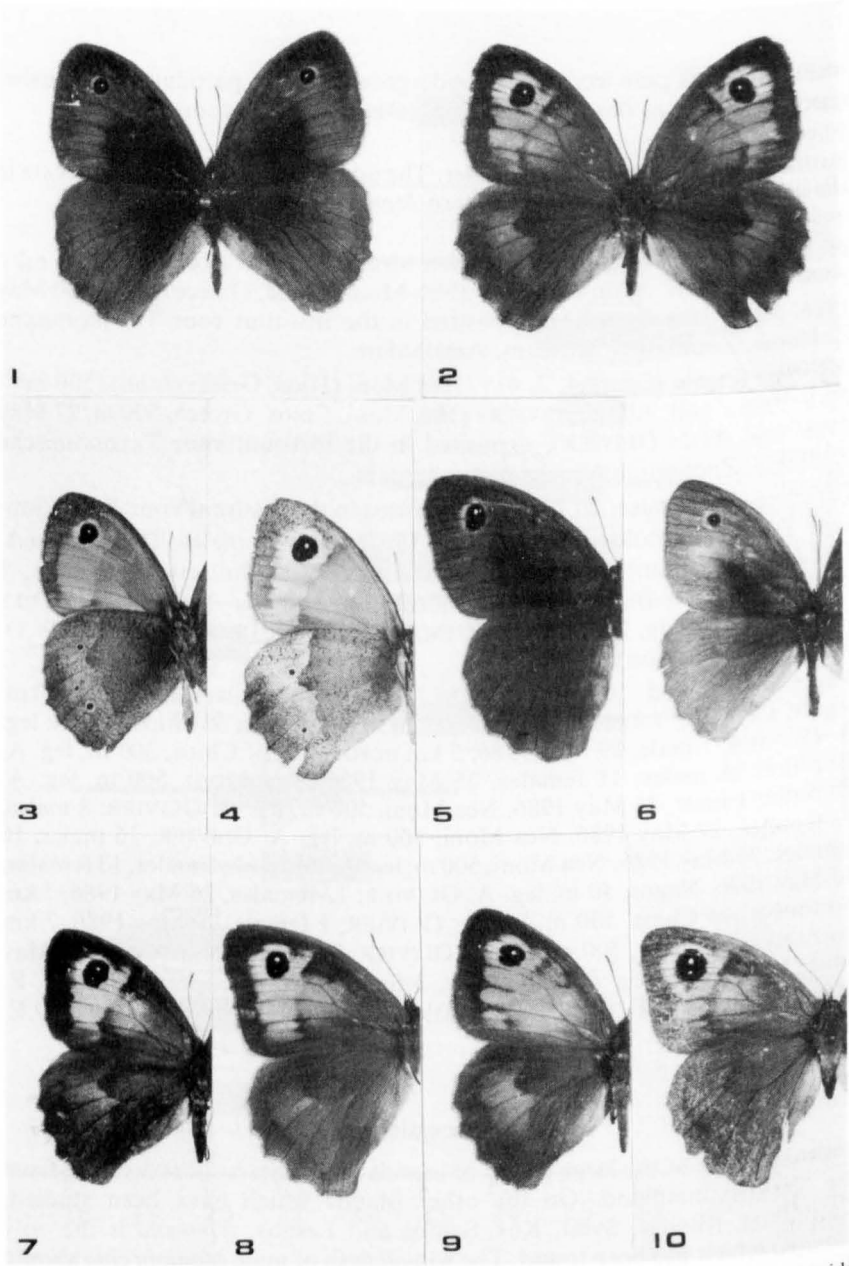

Figure 4 : *Maniola chia* **n. sp.** 1. holotype male; 2. allotype female; 3. holotype male (underside); 4. allotype female (underside); 5. male, Kardamila, Chios, 28 May 1984; 6. male, Nea Moni, Chios, 30 May 1986; 7. female, Nea Moni, Chios, 30 May 1986; 8. female, Marmaron, Chios, 28 May 1984; 9. female, Nea Moni, Chios, 27 May 1986; 10. female, E. Marmaron, Chios, 20 September 1985.

the adjacent islands, especially Lesbos, neither morphological nor electrophoretic data indicate character displacement. The affinities which this species displays with both *jurtina* and *telmessia* in the form of the genital armature and immature stages could suggest hybrid origin. However, electrophoretic analysis do not support this. It is likely that *Maniola chia*, an insular endemic species, is the consequence of founder effect during a period of rapid postglacial colonization, subsequent genetic drift and later consolidation by stabilising selection.

Acknowledgements

I am grateful to Professor David GASKIN for generously donating specimens and bringing this *Maniola* to my notice, A. OLIVIER for the supply of living material for electrophoresis and livestock as well as introducing me to the material in the Instituut voor Taxonomische Zoölogie, Zoölogisch Museum, Amsterdam. Thanks are due to the curators of the Zoölogisch Museum, Amsterdam for allowing access to their collections. I must also thank H. VAN OORSCHOT, H. VAN DEN BRINK, W. DE PRINS and A. RIEMIS and other members of the Instituut voor Taxonomische Zoölogie for giving much of their time and commenting constructively on the manuscript.

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Author's note

The analysis leading to the conclusions detailed in this paper form part of a major study of *Maniola* and related genera. It is not appropriate that details of electrophoretic techniques and analysis or larval chaetotaxy should be published here.

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