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Dedicated to E. B. Ford

Edited by
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Preface

Without doubt the Lepidoptera are not only one of the largest Orders of insects, but also the most popular. While it must be admitted that much of this interest borders on the merely philatelic, a vast amount of amateur energy and professional time has been expended on the biology of these beautiful animals. It is curious, then, that the only readily available introductory texts on their biology remain E. B. Ford's outstanding *Butterflies* (1945) and *Moths* (1955).

The idea for a symposium on butterfly biology arose from a series of five one-day workshops, organised by the Butterfly Research Association, with the help of the Royal Entomological Society of London (see *Antenna* **1**:20-1, **2**:47-8, **3**:40-1, **4**:22-3, **5**:34-5). In June 1980 we were invited by the Society to organise such a symposium, to take place in September 1981. During preparations for this exhausting, but wholly stimulating affair (*Antenna* **6**:179-81; *Yadoriga* **109, 110**:15-20), it was realised that the meeting would take place during Professor Ford's 80th year. If an excuse was ever needed to dedicate the symposium to E. B. Ford, this lucky chance provided it. Those who attended the meeting were honoured not only by his acceptance, but also by his presence throughout.

Butterflies have featured in a wide range of experimental, observational and evolutionary studies, involving important work on biochemistry, physiology, embryology and parasitology. However, our intention was to organise a meeting which addressed butterflies as butterflies—in other words, butterflies as whole organisms, communicating with each other, interacting with their environment and evolving within our biosphere. It is in this very area, so well-fostered by Professor Ford, that butterflies have come into their own as challenging, fascinating and instructive creatures to study those most "biological" of all biological disciplines: ecology, genetics and behaviour. We hope this volume will help re-double efforts in these pursuits, and stimulate a wider appreciation of the successes and failures of attempts to understand the biology of butterflies.

February 1984

Dick Vane-Wright, Phillip Ackery

Acknowledgements

This volume comprises 31 of the 33 papers read at the 11th Symposium of the Royal Entomological Society of London, held at the British Museum (Natural History), 23-26 September 1981. Additional papers published here are by M. Parsons (not read at the Symposium: in absentia) and P. R. Ackery (specially prepared for this volume).

The Society is grateful to Professor Ford for graciously accepting the dedication, and his generous response in helping to defray the cost of the meeting. The cost of the colour plates was met by the Cyril O. Hammond Bequest (Royal Entomological Society of London).

The Editors, who were also the Symposium convenors, gratefully record their thanks to the Staff of the Royal Entomological Society, the Staff of the Summer Accommodation Centre of Imperial College, and the warding staff of the British Museum (Natural History) for their help and consideration during the hectic days of the meeting itself. Special thanks are also due to our BMNH Butterfly Section colleagues, Ramnik Arora and Robert Smiles, for invaluable help both during the meeting and with the subsequent editing of the volume. Cyril Simsa, Cindy North and Philip DeVries have also freely given much valuable assistance. Pamela Forey undertook the difficult task of preparing the indexes.

Additionally, we wish to thank Professor Ford, G. G. Bentley, Sam Bhattacharyya, Lincoln Brower, Luciano Bullini, Robert Campbell, Sir Cyril Clarke, Kit Cottrell, Robin Crane, Paul Ehrlich, Ian Gordon, John Huxley, Ian Kitching, Kevin Murphy, Charles Remington, Rachel Hampshire, Miriam Rothschild, Elly Scheermeyer, Ken Smith, Valerio Sbordoni and Osamu Yata, together with all the contributors to this volume, for their helpful co-operation. We are also indebted to all the delegates to the meeting for much lively discussion, and to Academic Press for undertaking the task of publication.

Finally, we are happy to record our grateful thanks to the Royal Entomological Society of London for the double honour of being asked to organise the Symposium and to edit this volume. We have grown older—and perhaps a little wiser—in the process!

February 1984

Dick Vane-Wright, Phillip Ackery

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16. *The Ecological Genetics of Quantitative Characters of* Maniola jurtina *and Other Butterflies*

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Ecological geneticists combine field and laboratory studies to investigate the dynamics of evolutionary processes. Their approach rests on the assertion that the causes of genetic variation cannot be understood without a knowledge of the ecology of the organisms concerned (Ford 1975*a*).

In genetics the term character is applied to any property of an organism for which individual variation, especially when of a heritable nature, can be recognized. A quantitative character normally varies in a continuous manner and its study depends on measurement. It is usually jointly determined by the interacting effects of a number of minor genes or polygenes. The same phenotype can be determined by different combinations of these polygenes. Selection on quantitative characters is usually recorded in terms of the population mean and/or variance since it cannot be represented in terms of gene frequency changes, as is possible for Mendelian characters controlled by major genes. However, the distinction between polygenes and major genes is not absolute (see also Ch.14). The relationship between these genes and the different types as recognized by molecular geneticists remains unclear (e.g. Mather & Jinks 1971, Cavalier-Smith 1978, Ayala & McDonald 1980).

Polygenic systems provide the basis for smooth adaptive change. Over 30 years ago Dowdeswell *et al.* (1949) chose to use variation in the number of small hindwing spots in the univoltine butterfly *Maniola jurtina* as an index of the fine adjustment and adaptation of populations. The field data now accumulated on this particular species represent the most extensive available on the evolution of quantitative characters in animal populations (spot-number variation reflects an underlying character whose variation is truly continuous). Indeed, spot patterns provide the most frequently studied examples of quantitative variation in butterflies. Unfortunately the genetic basis of such variation has only rarely been rigorously examined (see Robinson 1971). This must always be an initial aim in ecogenetical investigations. Furthermore, although differences among populations have sometimes been adequately quantified this has seldom led to the development of hypotheses regarding the specific nature of those factors influencing the observed variation. An important purpose of this contribution is to describe recent research on *M. jurtina* which seeks to expand on the questions of how the spot phenotypes are determined, and how their relative frequency within populations may be influenced by natural selection.

Spot Patterns

Nijhout (1978) reviews wing pattern formation in the Lepidoptera and develops a model for wing pattern determination based on the observation that the pattern in each wing cell is developed in a definite relation to a central focus. Experimental evidence for such a focus has been obtained in *Precis coenia* (Nijhout 1980*a*). Eyespots represent the simplest condition in which the pattern is laid down as a system of concentric circles around a focus. Modifications of this are envisaged by Nijhout as resulting from the interpretation process of the distribution of some form of gradient in positional values radiating from the focus. The position of a focus and hence of a spot may shift laterally along the cell midline. The pattern may be expressed to a different degree in each wing cell.

Schwanwitsch (1924, 1948; see also Süffert 1927, (1929) analysed the wing patterns of nine groups of Palaearctic Satyrinae. From each he selected a

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number of representative forms, and by combining all their pattern elements he constructed a prototype wing pattern. The group which includes the genus *Maniola* showed the presence of a submarginal series of 5 forewing and 6 hindwing spots.

Dowdeswell & McWhirter (1967) studied the geographic variation in spot-number of *M. jurtina* throughout the species' distribution. They also undertook a preliminary analysis of two other species of *Maniola* in west Asia. All three showed a similar pattern of individual variation in spot-number. They suggested that the genes were trans-specific, trans-generic and trans-familial and therefore of great antiquity ('paleogenes'). Frazer & Willcox (1975) extended this study of *Maniola* and also examined species of the closely allied genus *Pyronia*. Six out of seven species showed considerable individual and geographic variation in spot-number, often on both forewing and hindwing. Examples of intraspecific variation in spot pattern have been recorded in most families of butterflies.

In *M. jurtina* the black hindwing spots lie within a band of lighter pigmentation on the ventral wing surface. Electron micrographs of individual spots show that changes in the morphology and the orientation of wing scales occur between the spot and the surrounding wing surface (Brakefield 1979*a*; see also Caspari 1941 for review of gene effects on scale structure).

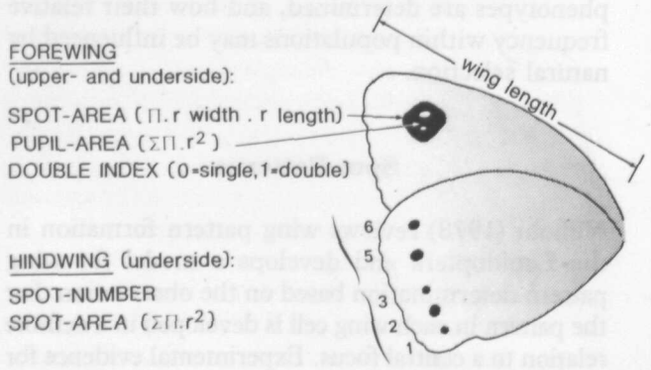


Fig. 16.1. Left wings of *M. jurtina* indicating the characters used in the spot pattern analysis. (Position numbers of hindwing spots are shown.)

The hindwing spots in *M. jurtina* occur at positions 1 to 6 of Schwanwitsch (1948) except that the spot at position 4 is rare or absent (Fig. 16.1; by convention only the left wings are scored). At each position the spots may be present or absent. Thus the range in spot-number is 0–5. Spots are usually encountered in only 13 out of 32 possible combinations (Table 16.1). I shall refer to these as spot types. McWhirter & Creed (1971) adopted a costality index to measure the spot-placing variation in populations. It is the percentage of the costal (positions 5 & 6) and anal (1 & 2) spots which are costally positioned. The neutral or centrally placed

Table 16.1. The spot-combination types of the hindwing spots of *M. jurtina*. (Modified from McWhirter & Creed 1971.)

Name	Spot position					Notation
	costal		median	anal		
	6	5	3	2	1	
Nought	—	—	—	—	—	0
Costal 1	—	●	—	—	—	C1
Anal 1	—	—	—	●	—	A1
Costal 2	●	●	—	—	—	C2
Splay 2	—	●	—	●	—	S2
Anal 2	—	—	—	●	●	A2
Costal 3	●	●	—	●	—	C3
Median 3	—	●	●	●	—	M3
Anal 3	—	●	—	●	●	A3
Costal 4	●	●	●	●	—	C4
Splay 4	●	●	—	●	●	S4
Anal 4	—	●	●	●	●	A4
All 5	●	●	●	●	●	all 5

The name and notation refer to both spot-placing and spot-number. ●, spot present; —, spot absent

spots at position 3 and individuals with all 5 spots are not included in the calculation. In other satyrine species which show a variable spot pattern the spots also only occur in certain combinations, e.g. *Coenonympha tullia* (Turner 1963, Dennis 1972*a*), *Aphantopus hyperantus* (Seppänen 1981) and *Pyronia tithonus* (Brakefield 1979*a*). Furthermore, individual spots usually only develop in the presence of a particular spot or group of spots.

The forewing spot pattern of *M. jurtina* is concentrated on the single spot at position 5. This forms an apical black eyespot (with a white pupil) on both the dorsal and ventral surfaces. It may be large enough to cover the area of position 4, when it usually becomes 'double' with an additional pupil at the centre of position 4 (Fig. 16.1; the form nomenclature of *M. jurtina* is described by Thomson 1973).

Morphometrics of Spot Pattern

Samples of *M. jurtina* were obtained from 13 populations (Fig. 16.2; details in Brakefield 1979*a*). Measurements of the characters shown in Fig. 16.2 were all made on left wings, using a binocular microscope fitted with a micrometer. Bipupillation of the forewing spot was not analysed for males (only 2.7% showed a double spot, cf. Frazer 1961).

The matrix of Pearson correlation coefficients was calculated for each sample. The common (or weighted mean) correlation coefficients for these population values are given in Table 16.2. All values ($n=57$) are positive and all except four are significantly different from zero. Furthermore, only three show a significant heterogeneity amongst the corresponding population values. They can

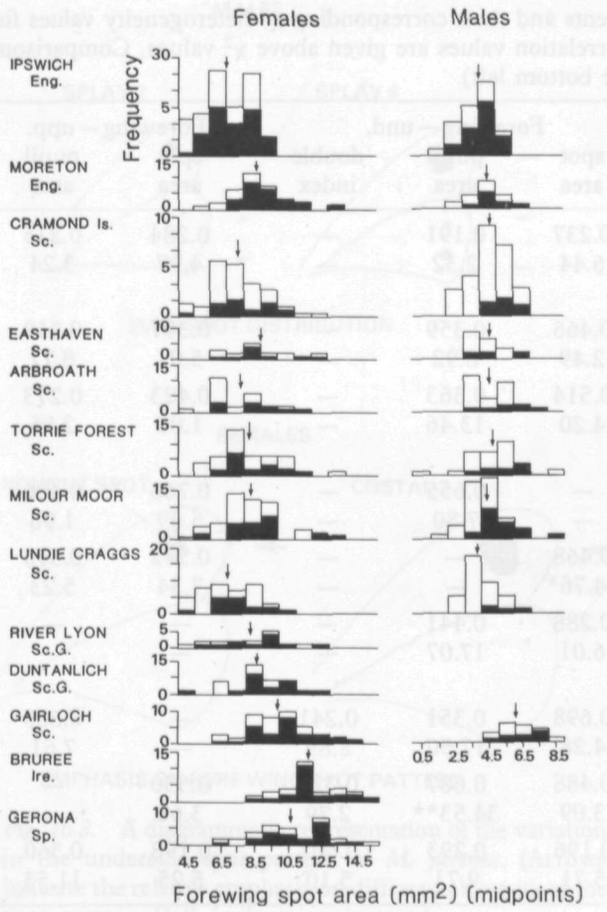


Fig. 16.2. Variation in underside forewing spot-size of *M. jurtina* in the populations indicated. (Unshaded areas of histograms show 0 spot females or 0–2 spot males. Shaded areas show all others. Arrows indicate mean spot areas. Abbreviations: Eng., England; Sc., Scotland; Sc.G., Scotland, Grampian Mountains; Ire., Ireland; Sp., Spain.)

therefore be considered to be good estimates for the species.

Table 16.2 groups the spot pattern characters by wing and additionally for the forewing by under- and upperside. The correlations for characters within these groups are consistently higher than those between the groups. There is a highly significant correlation between both the hindwing spot-number and spot-area and the forewing spot-area. In each population there is a more or less pronounced tendency for butterflies with relatively small forewing spots to be 0 spot females or low spotted (0–2) males (Fig. 16.2). Conversely, those with relatively large forewing spots tend to be spotted females or higher spotted males. Turner (1963) reported a 'good' correlation between the spot-number on the fore- and hindwings in a population of *Coenonympha tullia*. Using his data for the pooled sample the correlation amounts to +0.875 (n=73). Similar relationships may be a general feature in variable species of Satyrinae (Frazer & Willcox 1975).

Ehrlich & Mason (1966) and Mason *et al.* (1967, 1968) used morphometric techniques to study a large number of spot pattern characters in *Euphydryas editha*. The matrix of correlations suggested that the spots could be considered as being in several anterior-posterior columns affecting both wings. These results are consistent with what is known of the development of wing pattern in some Lepidoptera (see Sondhi 1963). Mason *et al.* (1968) suggested that changes in selected spot characters from each wing column in *E. editha* (which they called trend characters) could be considered to represent the temporal variation in the wing pattern as a whole. In *M. jurtina*, hindwing spot-number can be conveniently taken as a trend character because its expression is in terms of whole numbers. In contrast, spot dimensions or areas, which exhibit truly continuous variation, are more laborious to score. Furthermore, hindwing spot-area does not, in general, yield higher correlations with the other characters than does spot-number (Table 16.2).

The forewing eyespot of *M. jurtina* shows a wide variation in size (Fig. 16.2). The mean spot-area in females is larger by a factor of about 1.9. Significant heterogeneity of the population-means occurs in both sexes (Fig. 16.2; females—F = 26.54, d.f. 12 and 509; males—F = 9.19, d.f. 8 and 324, $P < 0.001$ for each value).

High positive correlations are found between the population-means of the sexes for each of the spot-number (cf. McWhirter 1957), hindwing spot-area and forewing spot-area characters (Brakefield 1979*a*). Furthermore, a female population with a high mean spot-number tends also to show a high mean for forewing spot-area (r = 0.93, d.f. = 11, $P < 0.001$). In males, for which fewer populations were analysed, the correlation is positive but not significant (r = 0.17, d.f. = 7). Each character shows a higher coefficient of variation for the population-means in females than males (Brakefield 1979*a*). This corresponds to the greater variability in spot frequency between female populations (see Ford 1975*a*).

Figure 16.3 illustrates the differences detected in the spot pattern both within and between the sexes. Individuals of two of the more abundant spot types in each sex which differ in spot-number are shown. In females there is a marked emphasis on the forewing spot pattern whilst males show a more even spot distribution between the wings. McWhirter & Creed's (1971) study of spot-placing showed that female populations tend towards a high costality index of about 65–85% whilst in males a value slightly lower than 50% is typical. Thus in females the hindwing spots tend to be more heavily expressed in the costal area of the wing which is closest to the apical forewing spot. My morphometric data show that the size of the individual hindwing spots

Table 16.2. The matrix of the common correlation coefficients and their corresponding χ^2 heterogeneity values for the wing-span and spot pattern characters in *M. jurtina* (correlation values are given above χ^2 values. Comparisons for males are given to the top right, and for females to the bottom left).

	Wing-span	Hindwing		Forewing—und.			Forewing—upp.	
		spot-number	spot area	spot area	pupil area	double index	spot area	pupil area
Wing-span	—	0.077	0.141	0.237	0.191	—	0.284	0.215
		6.53	6.86	6.44	2.32	—	4.97	3.24
Hindwing								
spot-number	0.099	—	0.706	0.466	0.359	—	0.397	0.319
	7.39	—	3.19	2.49	8.92	—	5.01	8.77
spot area	0.089	0.828	—	0.514	0.363	—	0.423	0.273
	12.76	35.73**	—	4.20	13.46	—	1.05	2.96
FW und.								
spot	0.365	0.307	0.322	—	0.655	—	0.760	0.486
area	7.18	11.12	13.41	—	7.80	—	5.09	1.96
pupil	0.193	0.175	0.139	0.468	—	—	0.552	0.576
area	12.81	16.12	17.53	24.76*	—	—	7.34	5.23
double index	0.044	0.173	0.126	0.288	0.441	—	—	—
	9.33	14.43	13.54	6.01	17.07	—	—	—
FW upp.								
spot	0.238	0.168	0.186	0.698	0.351	0.241	—	0.600
area	2.09	4.36	6.14	4.28	17.50	2.89	—	7.61
pupil	0.135	0.180	0.183	0.488	0.687	0.327	0.550	—
area	4.72	9.89	3.11	3.09	34.53**	2.79	3.66	—
double index	0.157	0.099	0.096	0.196	0.293	0.607	0.198	0.360
	2.96	5.92	8.24	5.71	9.71	5.10	5.25	11.53

Total sample size (n) for the comparisons involving upperside forewing characters are male = 239 and female = 320, for all others they are male = 333 and female = 522. Correlation coefficients, *except* those underlined, are significant ($P < 0.05$) when degrees of freedom of $n-2$ is considered. Degrees of freedom for the χ^2 values of the comparisons involving upperside forewing characters are male = 5 and female = 7, for all others they are male = 8 and female = 12.
For χ^2 values: *, $P < 0.05$; **, $P < 0.001$.

increases with spot-number and with the relative frequency of the individual spots. Spots 2 and 5 are the most frequent and usually the largest.

Heritability of Spot Pattern Characters

It is assumed that variation in a quantitative character results from a combination of genetic and environmental differences (Falconer 1981). The initial aim of a genetic investigation is to divide the total or phenotypic variance (V_P) into its components, the additive genetic variance (V_A) and the environmental variance (V_E). Heritability (h^2) is a parameter indicating the proportion of the total variance which is additive:

$$h^2 = V_A/V_P$$

The heritability can be estimated from the slope of the regression line of offspring on mid-parent value. It is a property not only of a character but also of the population and of the environmental conditions experienced by individuals. Therefore, a value for h^2 refers to a particular population under particular conditions. The principal use of h^2 is to predict response to directional selection. This is possible because h^2 gives the expected similarity between relatives.

The only available estimates of heritability for a quantitative character in butterflies are those obtained by McWhirter (1969) for spot-number in *M. jurtina*. McWhirter raised four broods of the Isles of Scilly race under temperature conditions fluctuating around 15°C. The brood sizes were 8, 9, 19 and 53. This limited material was analysed by linear regression of all individual offspring on mid-parent values (usually mean offspring values are used). The estimates for h^2 were 0.14 in males and 0.63 ± 0.14 in females. McWhirter also analysed the data by an analysis of variance of spot-number between and within broods. This yielded an estimate of h^2 based on double the full-sib contribution of 0.83 in females. McWhirter suggested that the latter estimate was more reliable because of the small broods, the difference in environment under which the parents (some collected *in copula*) and progeny developed,

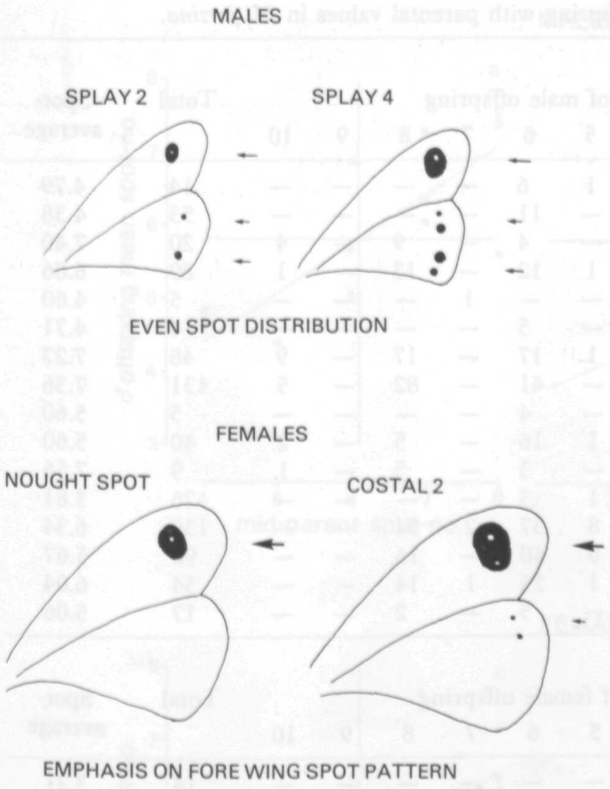


Fig. 16.3. A diagrammatic representation of the variation in the underside spot pattern of *M. jurtina*. (Arrows indicate the relative emphasis on different elements of the spot pattern. Full explanation in text.)

and the different estimates obtained for the sexes.

This suggestion of a difference in heritability between the sexes indicated the necessity of obtaining more complete breeding data (see Tudor & Parkin 1979). Furthermore, McWhirter's data provide no information about the inheritance of other spot pattern characters. In 1979, I obtained a sample ($n = 30$) of female *M. jurtina* from a population at Oude Mirdum in Friesland, N. Holland. The population is exceptionally high spotted (spot averages: 1979, female = 2.77, n = 35; male = 3.875, n = 62. 1980, female = 2.67, n = 9; male = 3.60, n = 35). The costality is similar to populations in S. England (costality index: female = 65.22%, male = 53.03%; cf. McWhirter & Creed 1971). In the early-mid flight period of 1980 a Lincoln Index estimate of daily population size for males of 55 ± 14 was obtained. This suggests a total population size in the order of several hundred insects (Brakefield 1982). More than 300 adults were raised from eggs, and crosses then set up between selected adults to provide material for the estimation of heritability. The regression of offspring on parents is not affected by the selection of parents (Falconer 1981).

Parents and offspring were reared in similar conditions. *M. jurtina* can easily be paired in net cages and females will lay readily in small plastic boxes covered with cotton net. Young larvae were raised on seedling grasses (from a lawn grass seed mix) sown in 20cm diameter pots. Mid to late instar larvae were fed on grass (mainly *Poa annua*) transplanted from outside into 45cm square boxes. Broods were kept in an unheated laboratory with dampened temperature fluctuations in comparison to outdoors. During the pupation period for the broods (59 days) the daily maximum and minimum temperatures were 20.5 ± 0.6°C (range 15–29.5°C) and 14.8 ± 0.4°C (9–21.5°C) respectively. In addition to conventional strip lighting (*ca* natural day length) the larvae were raised under 'gro-lux' lamps which emit UV light. Percentage mortality within broods was 42.65 ± 3.24% (measured from first/second instar larvae to adults). Eggs from English mainland stocks have proved difficult to raise because great mortality occurs from the third instar due to a bacterial pathogen (McWhirter 1965). Misyalyunene (1978) carried out experiments with *Pieris brassicae* which showed that irradiation of a bacterial pathogen with sunlight prior to inoculation of larvae reduced subsequent mortality to low levels in comparison to controls. Thus the 'gro-lux' lamps possibly act as an artificial bactericide. However, McWhirter & Scali (1966) found that larvae of *M. jurtina* were strongly selective as to their intestinal bacterial flora and that populations could show strongly distinctive gut floras. The Dutch stock may, like that from the Isles of Scilly (McWhirter 1969), be resistant to those pathogens which cause mortality in English mainland stocks.

Table 16.3 gives the breeding data for spot-number. Parents did not include 0 spot females or 0 and 1 spot females. Offspring included all spot classes. An analysis of variance shows that the male and female offspring are not equal in variance (F = 1.29, d.f. 654 and 684, $P < 0.001$). This means that, strictly speaking, h^2 must be estimated separately for each sex from the regressions on single parent values (Falconer 1981). For comparison, Fig. 16.4 shows the regressions for each sex of mean offspring values on both parent and mid-parent values. The corresponding estimates of h^2 are given in Table 16.4. The brood sizes varied widely (83.75 ± 19.2, range 8–260). Therefore Table 16.4 includes estimates of h^2 obtained by weighting the mean offspring values according to the number of offspring in each family (Kempthorne & Tandon 1953, Reeve 1955). This procedure has little effect on the estimates. Those obtained for each sex using single (same sex) parent values may be higher due to sex dependent expression. Estimates for hindwing spot-characters obtained using mid-parent values are intermediate between those for the same sex, and the opposite sex parent values (Brakefield & Noordwijk in prep.). The estimates of h^2 do not differ significantly

Table 16.3. Comparisons of hindwing spot-number of offspring with parental values in *M. jurtina*.

a) Males

Brood number	Parents m–f	Mid-parent	Spot-number of male offspring											Total	Spot-average
			0	1	2	3	4	5	6	7	8	9	10		
2	4–4	4	—	—	1	—	6	1	6	—	—	—	—	14	4.79
3	6–4	5	—	—	1	—	41	—	11	—	—	—	—	53	4.38
4	6–10	8	—	—	—	—	3	—	4	—	9	—	4	20	7.40
5	6–6	6	—	—	—	—	2	1	12	—	13	—	1	29	6.86
6	6–2	4	—	—	—	—	4	—	—	1	—	—	—	5	4.60
7	6–2	4	—	—	—	—	9	—	5	—	—	—	—	14	4.71
13	10–4	7	—	—	—	—	4	1	17	—	17	—	9	48	7.27
14	10–6	8	—	—	—	—	3	—	41	—	82	—	5	131	7.36
19	6–6	6	—	—	—	—	1	—	4	—	—	—	—	5	5.60
20	8–8	8	—	—	—	1	15	1	16	—	5	—	2	40	5.60
21	10–6	8	—	—	—	—	—	—	3	—	5	—	1	9	7.56
22	4–4	4	2	—	2	—	18	1	3	—	—	—	—	26	3.81
24	8–6	7	—	—	—	—	29	8	37	2	54	—	—	130	6.34
27	6–6	6	—	—	—	—	28	6	40	—	16	—	—	90	5.67
33	8–6	7	—	—	—	—	13	1	25	1	14	—	—	54	6.04
39	4–8	6	—	—	—	—	10	—	5	—	2	—	—	17	5.06

b) Females

Brood number	Parents m–f	Mid-parent	Spot-number of female offspring											Total	Spot-average
			0	1	2	3	4	5	6	7	8	9	10		
2	4–4	4	1	—	3	1	9	—	—	—	—	—	—	14	3.21
3	6–4	5	1	1	39	3	20	—	2	—	—	—	—	66	2.73
4	6–10	8	—	—	1	—	7	—	11	—	9	—	2	30	6.27
5	6–6	6	—	—	5	—	14	—	15	1	11	—	—	46	5.46
6	6–2	4	—	—	2	—	1	—	—	—	—	—	—	3	2.67
7	6–2	4	1	—	5	—	4	1	—	—	—	—	—	11	2.82
13	10–4	7	—	—	4	1	8	1	12	—	7	—	2	35	5.60
14	10–6	8	—	—	3	—	27	1	44	2	46	—	1	124	6.25
19	6–6	6	—	—	—	—	2	—	10	—	—	—	—	12	5.67
20	8–8	8	—	—	—	—	2	—	20	1	9	—	—	32	6.47
21	10–6	8	—	—	—	—	1	—	5	—	7	—	1	14	7.14
22	4–4	4	3	—	7	1	10	—	2	—	—	—	—	23	3.00
24	8–6	7	—	—	9	1	32	1	50	—	12	—	—	105	5.24
27	6–6	6	—	—	1	—	31	2	37	—	20	—	—	91	5.69
33	8–6	7	—	—	4	1	16	—	10	—	2	—	—	33	4.58
39	4–8	6	—	—	1	—	4	1	9	—	1	—	—	16	5.31

N.B. Spot-values in tables of heritability are for both wings and so are double those given in the usual tables of flying populations.

from unity with the exception of that for male offspring on mid-parent values. The heritable nature of the character is evident in Fig. 16.5. There is no evidence for a difference between the sexes.

Jarvis and Høegh-Guldberg have made detailed investigations of the genetic relationships between two European lycaenids, *Aricia agestis* and *A. artaxerxes*. They demonstrated a genetic basis for a number of quantitative characters separating the species and the subspecies/races of each (Jarvis 1966, Høegh-Guldberg 1968, Høegh-Guldberg & Jarvis 1970). The characters studied in adults included underside spot variation (spot-number and size), upperside orange lunulation, wing size and ground colour. An intermediate or heterotic distribution of phenotypes relative to the parental stocks was evident for each trait in the crosses.

Early experiments on several species of Nymphalinae and Lycaenidae demonstrated that the adult phenotype could be altered by subjecting the pupae to extremes of temperature (Süffert 1924, Kühn 1926, Köhler & Feldotto 1935, Krodel 1904). Every pattern element was found to have its particular sensitive period. Lorković (1938, 1943) and Høegh-Guldberg (1971) produced effects on underside spot pattern in two lycaenids by prolonged pupal cooling. Høegh-Guldberg & Hansen (1977), working on *A. artaxerxes*, found that a lower spot-number was sometimes produced by subjecting insects just before or after pupation to one or more

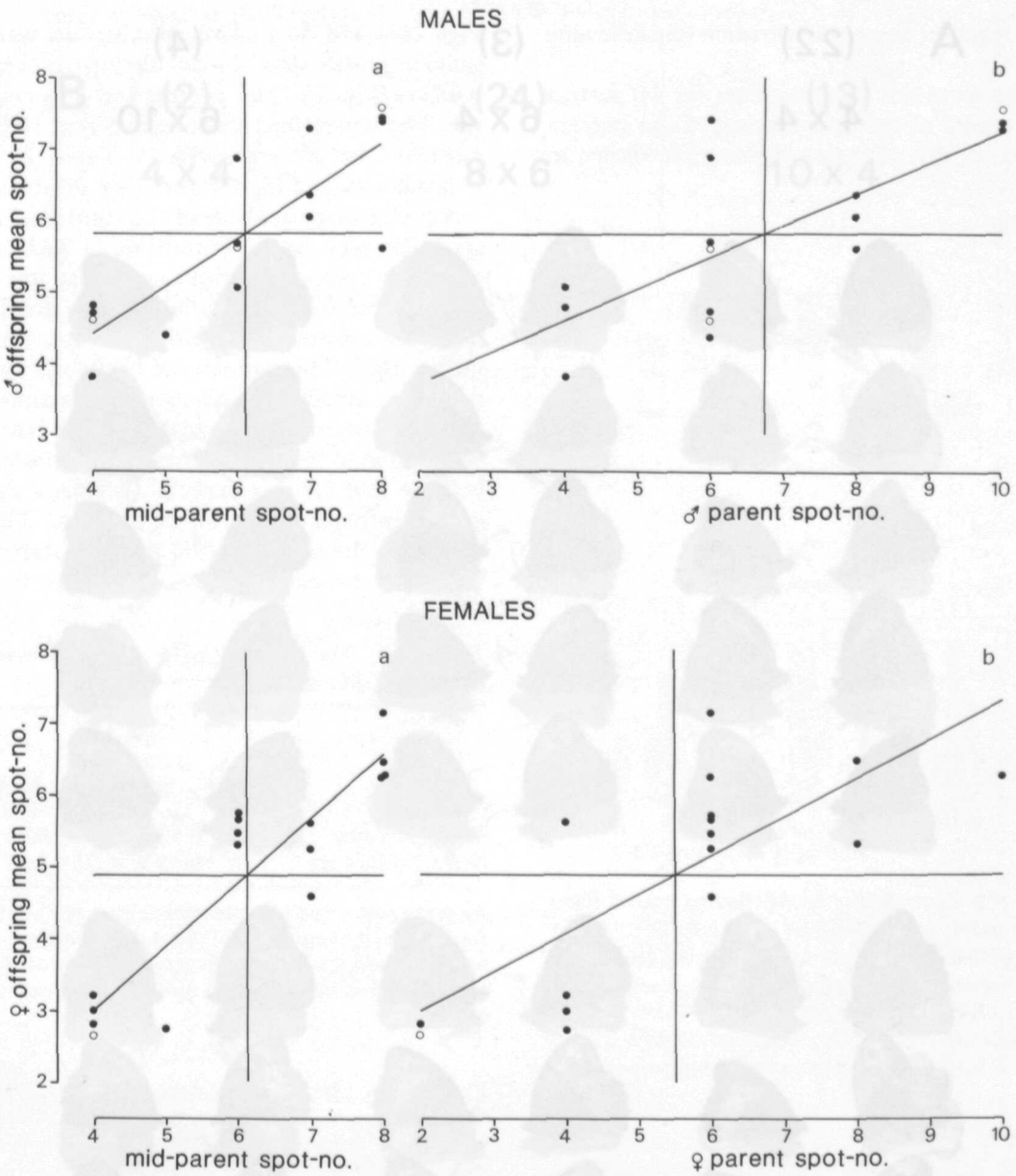


Fig. 16.4. Heritability of hindwing spot-number in *M. jurtina*. (Circles show mean offspring values in broods plotted against (a) mid-parent value and (b) parent value. Open circles indicate a brood size of <10. Note, in (b) slope of regression $= 0.5h^2$.)

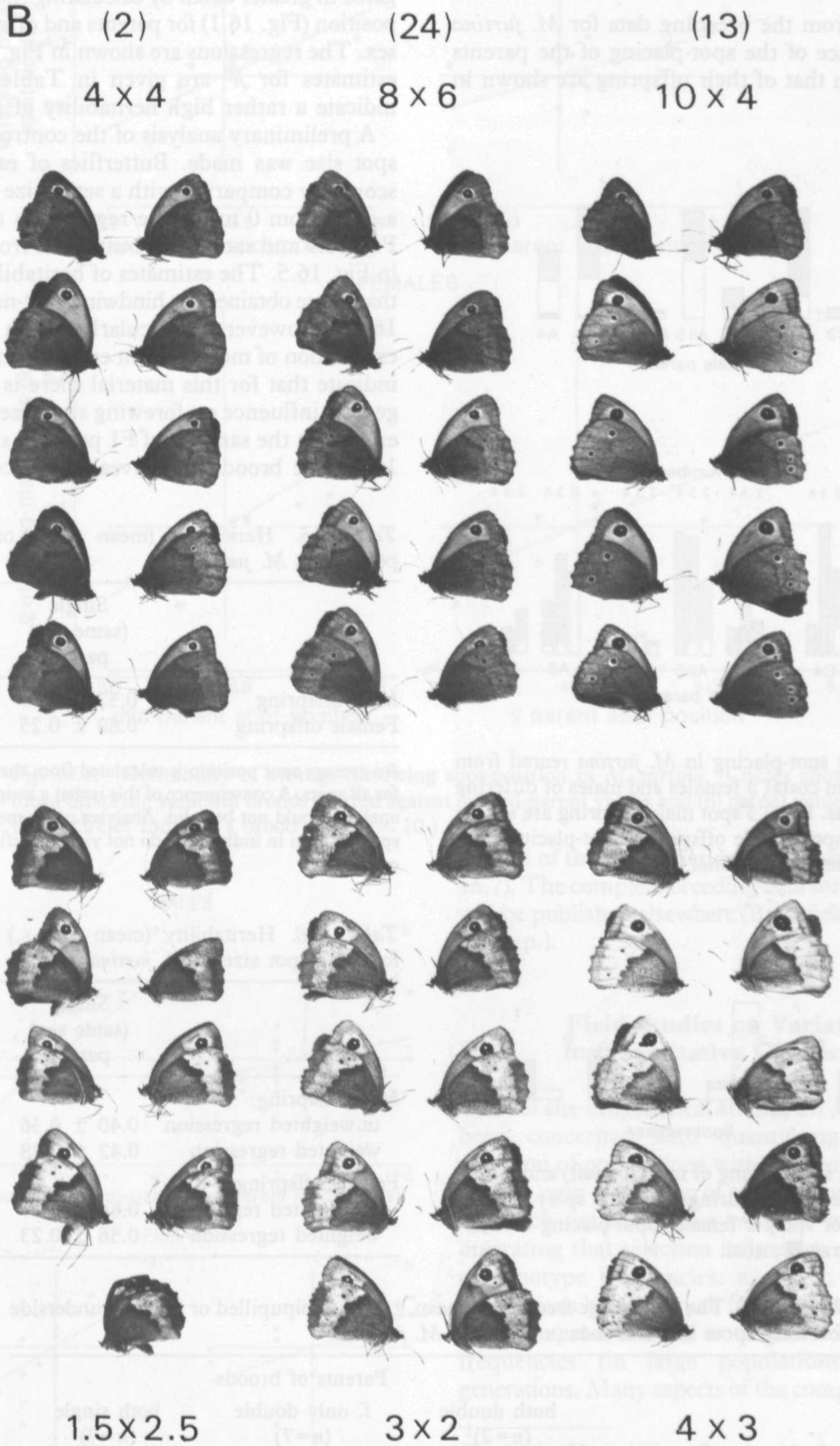
Table 16.4. Heritability (mean ± S.E.) of spot-number in *M. jurtina* (see text for details of method of analysis).

	Single (same sex) parent	Mid-parent
Male offspring:		
unweighted regression	0.88 ± 0.21	0.66 ± 0.11[1]
weighted regression	0.87 ± 0.22	—
Female offspring:		
unweighted regression	1.08 ± 0.25	0.89 ± 0.11[1]
weighted regression	1.05 ± 0.20	—

[1]These estimates are not significantly different ($t = 1.43$, d.f. $= 28$).

periods of cooling at +2–5°C for 9–12 hours. The experimental pupae produced some rare forms. The authors suggest that their results may be a possible explanation for such aberrations in nature. Similar experiments to those of Høegh-Guldberg & Hansen on material from a single brood of *M. jurtina* did not detect an environmental effect on spot phenotype. Relative differences in spot variation between populations of *M. jurtina* are maintained when samples of wild-collected larvae are reared under similar conditions in the laboratory (see below). This observation and the finding of a high heritability for spot-number indicates that differences between, or changes within populations in the frequency distribution of spot-number (= spot frequency) can more confidently be interpreted as due to the action

Fig. 16.5. Spot pattern variation in F1 broods of *M. jurtina*. In each section (A and B) a random sample of 10 males (above) and females (below) are shown in columns for each of three broods (reference numbers given in brackets, see Table 16.3). The hindwing spot-number and the forewing spot size index for the male × female parents are indicated above and below each brood respectively.



Within each section parents showed a trend of increasing spot expression from left to right. The heritable nature of the spot characters (see text) is then evident in the corresponding trend amongst the illustrated offspring. Brood 13 includes some butterflies in which the usually rare hindwing spot at position 4 is expressed.

of natural selection on genetic variation (see following section).

Examples from the breeding data for *M. jurtina* of the influence of the spot-placing of the parents of each sex on that of their offspring are shown in Figs 16.6 and 16.7. This relationship was investigated in greater detail by calculating the average spot position (Fig. 16.1) for parents and offspring of each sex. The regressions are shown in Fig. 16.8 and the estimates for h^2 are given in Table 16.5. They indicate a rather high heritability of about 0.6.

A preliminary analysis of the control of forewing spot size was made. Butterflies of each sex were scored by comparing with a set of size standards on a scale from 0 to 5. The regressions are shown in Fig. 16.9 and samples of butterflies from six broods in Fig. 16.5. The estimates of heritability are lower than those obtained for hindwing spot-number (Table 16.6). However, particularly when the higher expectation of measurement error is considered, they indicate that for this material there is a significant genetic influence on forewing spot size. This is also evident in the samples of F1 progeny shown in Fig. 16.5. The broods also reveal evidence for genetic

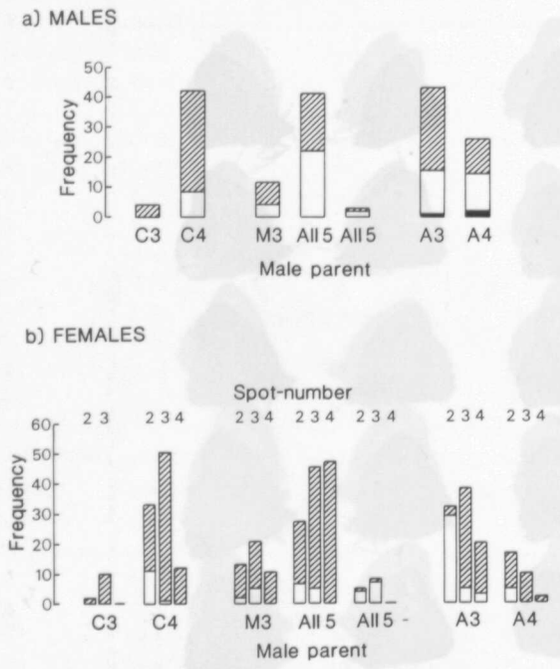


Fig. 16.6. The spot-placing in *M. jurtina* reared from pairings between costal 3 females and males of differing spot-placing bias. In (a) 3 spot male offspring are shown and in (b) 2–4 spot female offspring. Spot-placing bias: ▨, costal; □, neutral; ■, anal.

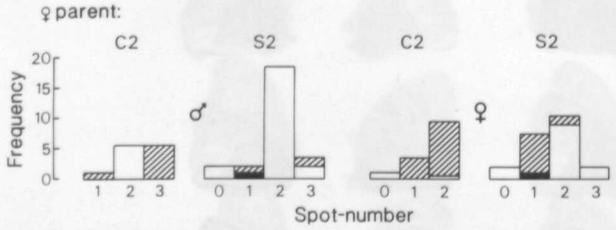


Fig. 16.7. The spot-placing of male (on left) and female *M. jurtina* reared from pairings between splay 2 males and a costal 2 or splay 2 female. Spot-placing bias: ▨, costal; □, neutral; ■, anal.

Table 16.5. Heritability (mean ± S.E.) of average spot position in *M. jurtina*.

	Single (same sex) parent	Mid-parent
Male offspring	0.57 ± 0.19	0.46 ± 0.12
Female offspring	0.80 ± 0.25	0.61 ± 0.20

An average spot position is calculated from the combined data for all spots. A consequence of this is that a weighted regression analysis would not be valid. Analyses using means for average spot position in individuals do not yield significantly different estimates.

Table 16.6. Heritability (mean ± S.E.) of underside forewing spot size in *M. jurtina*.

	Single (same sex) parent	Mid-parent
Male offspring:		
unweighted regression	0.40 ± 0.36	0.80 ± 0.21
weighted regression	0.42 ± 0.28	—
Female offspring:		
unweighted regression	0.66 ± 0.27	0.59 ± 0.20
weighted regression	0.56 ± 0.23	—

Table 16.7. The percentage frequency (mean ± S.E.) of bipupilled or 'double' underside forewing spots in F1 broods (n given) of *M. jurtina*.

	Parents of broods		
	both double ($n=2$)[1]	f. only double ($n=7$)	both single ($n=7$)
Male offspring	68.63 ± 3.25	16.79 ± 4.33	5.49 ± 2.24
Female offspring	98.57 ± 1.43	83.98 ± 7.57	68.59 ± 8.88

[1]Brood nos 13 ($n=83$) and 24 ($n=235$)

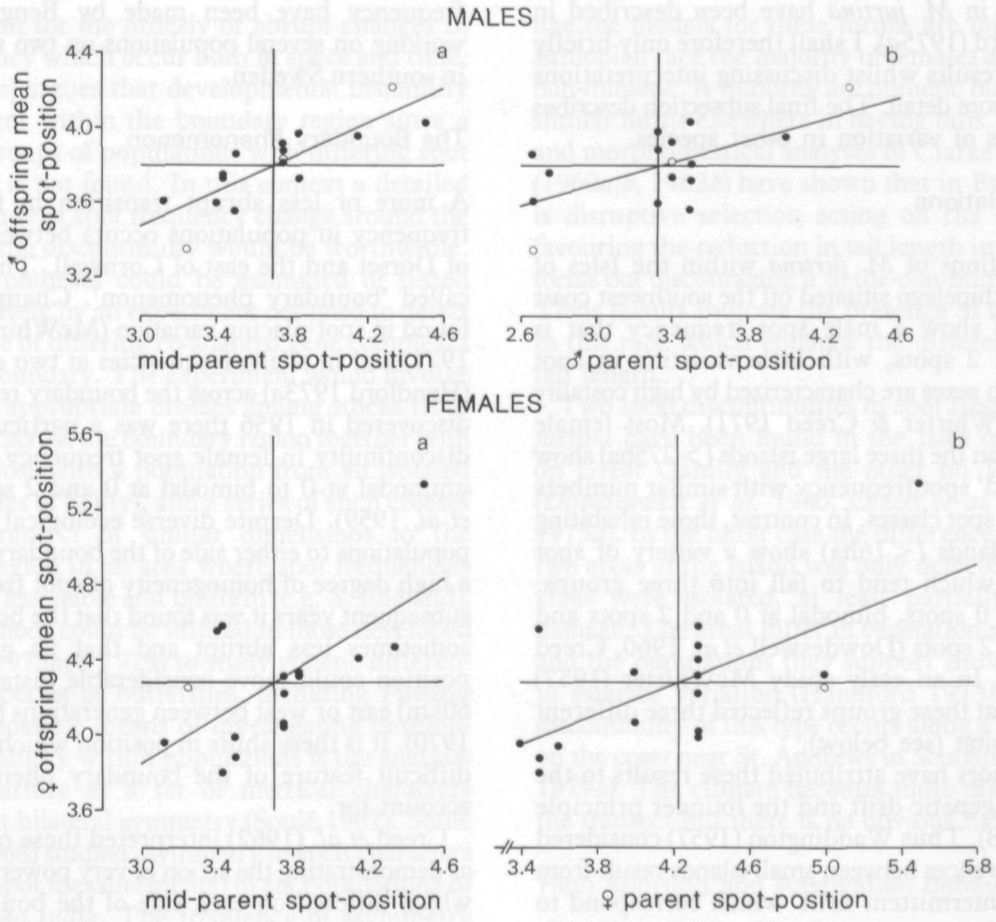
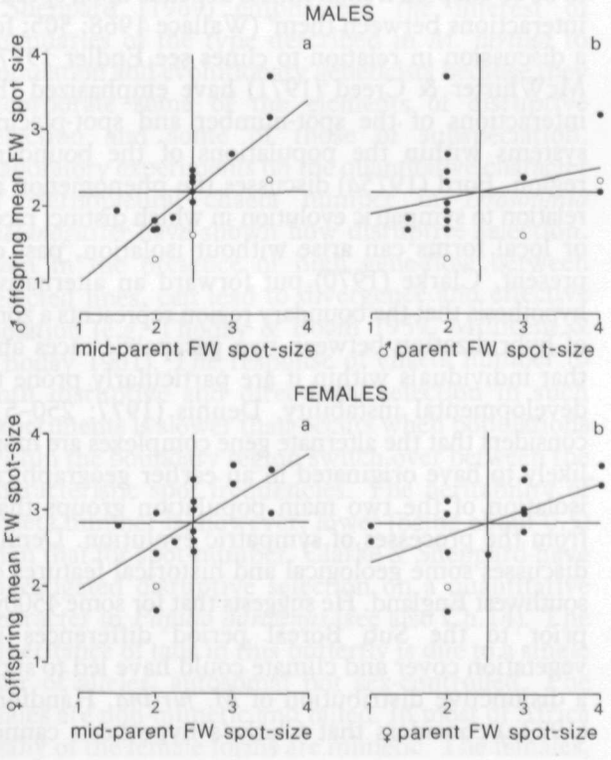


Fig. 16.8. Heritability of average hindwing spot-position in *M. jurtina*. (Circles show mean offspring values in broods plotted against (a) mid-parent values and (b) parent value. Open circles indicate a brood size of < 10.)



control of the bipupillation of forewing spot (Table 16.7). The complete breeding data for each character will be published elsewhere (Brakefield & Noordwijk in prep.).

Field Studies on Variation in Quantitative Characters

Most of the ecogenetical studies on *M. jurtina* have been concerned with quantifying spot-number variation of populations within a geographical area, usually over a number of years (generations). This approach embraces two possible means of demonstrating that selection influences specific morph or genotype frequencies: to search for consistent correlations between such frequencies and particular environmental factors, or to follow changes in the frequencies (in large populations) over many generations. Many aspects of the comparative studies

Fig. 16.9. Heritability of underside forewing spot size in *M. jurtina*. (Circles show mean offspring values in broods plotted against (a) mid-parent value and (b) parent value. Open circles indicate a brood size of < 10.)

of variation in *M. jurtina* have been described in detail by Ford (1975*a*). I shall therefore only briefly outline the results whilst discussing interpretations of them in more detail. The final subsection describes some studies of variation in other species.

Island Populations

The populations of *M. jurtina* within the Isles of Scilly (an archipelago situated off the southwest coast of England) show a male spot frequency that is unimodal at 2 spots, with little variation in spot average. Both sexes are characterized by high costality indices (McWhirter & Creed 1971). Most female populations on the three large islands (>275ha) show a 'flat-topped' spot frequency with similar numbers of 0, 1 and 2 spot classes. In contrast, those inhabiting the small islands (<16ha) show a variety of spot frequencies which tend to fall into three groups: unimodal at 0 spots, bimodal at 0 and 2 spots and unimodal at 2 spots (Dowdeswell *et al.* 1960, Creed *et al.* 1964). In an early study McWhirter (1957) suggested that these groups reflected three different types of habitat (see below).

Some authors have attributed these results to the workings of genetic drift and the founder principle (Wright 1948). Thus Waddington (1957) considered that the differences between small islands result from periods of 'intermittent drift' which correspond to bottle-necks in population size. Again, Dobzhansky & Pavlovsky (1957) suggested that the small island populations were derived from founder groups with differing gene frequencies from which relatively stable but different gene pools were developed. Ford and his co-workers disagree (and see MacArthur & Wilson 1967). Ford (1975*a*) discusses the hypothesis that those populations which occupy the large (and diverse) islands result from natural selection producing a gene complex simultaneously adapted to a wide range of environments. In contrast, populations occupying the small islands (and isolated, small areas on the large islands) tend to be dissimilar to each other, being closely adjusted to particular and different environments. In this context Ford argues that the following observations are of particular significance. On several occasions populations have been found to pass through an extreme bottle-neck in size with no subsequent change in spot frequency, although this may be different in the period of low numbers (Creed *et al.* 1964). On the other hand, some changes in spot frequency have not been associated with periods of low numbers but rather with a change in habitat, for example when grazing by cattle ceased on Tean (Dowdeswell & Ford 1955; Dowdeswell *et al.* 1957) and when exceptional drought occurred on St Martin's and Tresco (Dowdeswell *et al.* 1960). Similar observations of a coincidence of an unusually warm and dry summer with a change in spot frequency have been made by Bengtson (1978) working on several populations on two small islands in southern Sweden.

The Boundary Phenomenon

A more or less abrupt transition in female spot frequency in populations occurs between the west of Dorset and the east of Cornwall. This is the so-called 'boundary phenomenon'. Changes are also found in spot-placing variation (McWhirter & Creed 1971) and in allelic frequencies at two esterase loci (Handford 1973*a*) across the boundary region. When discovered in 1956 there was a particularly sharp discontinuity in female spot frequency from being unimodal at 0 to bimodal at 0 and 2 spots (Creed *et al.* 1959). Despite diverse ecological conditions, populations to either side of the boundary itself show a high degree of homogeneity of spot frequency. In subsequent years it was found that the boundary was sometimes less abrupt and that its geographical position could move considerable distances (up to 60km) east or west between generations (Creed *et al.* 1970). It is these shifts in position which is the most difficult feature of the boundary phenomenon to account for.

Creed *et al.* (1962) interpreted these observations as demonstrating the action of very powerful selection which differed on each side of the boundary. The nature of these forces remains unknown. Others, such as Handford (1973*a*), have elaborated on this interpretation: for example invoking a switch over between two co-adapted genetic systems at a critical point in an environmental gradient. 'Genes are said to be co-adapted if high fitness depends upon specific interactions between them' (Wallace 1968: 305; for a discussion in relation to clines see Endler 1977). McWhirter & Creed (1971) have emphasized the interactions of the spot-number and spot-placing systems within the populations of the boundary region. Ford (1975*a*) discusses the phenomenon in relation to sympatric evolution in which distinct races or local forms can arise without isolation, past or present. Clarke (1970) put forward an alternative hypothesis that the boundary region represents a zone of hybridization between two parapatric races and that individuals within it are particularly prone to developmental instability. Dennis (1977: 250–51) considers that the alternate gene complexes are more likely to have originated in an earlier geographical isolation of the two main population groups than from the processes of sympatric evolution. Dennis discusses some geological and historical features of southwest England. He suggests that for some 4500yr prior to the Sub Boreal period differences in vegetation cover and climate could have led to such a disjunctive distribution of *M. jurtina*. Handford (1973*a*) considers that Clarke's hypothesis cannot

alone account for the orderly or abrupt changes in spot frequency which occur both in space and time. Ford (1975*a*) argues that developmental instability is not evident within the boundary region since a mosaic of groups of populations with differing spot frequencies is not found. In this context a detailed and local study of spot frequency change around the position of the discontinuity would be worthwhile.

These hypotheses could be examined or tested more objectively by an experiment designed to detect the existence of differing co-adapted gene complexes across the boundary. The experiment would involve making the appropriate crosses among stocks from populations across the boundary region together with a control cross with a population from an isolated area (e.g. Isles of Scilly), and a series of such crosses within a transect of similar dimensions to the boundary region in an area where spot frequencies are more or less stable and uniform (e.g. S. England). Similar methods could be utilized to those developed by Oliver (1972*a*,*b*, 1979) to study genetic differentiation in species of butterflies, including *Pararge megera*. A possible means of investigating developmental instability within populations is the analysis of the departure of a set of metrical characters from perfect bilateral symmetry (Soulé 1967). Soulé & Baker (1968) studied asymmetry in such characters (including spot measurements) in six populations of *Coenonympha tullia*. The frequency of asymmetry of spot-number in the broods of *M. jurtina* (Table 16.3) was 3.5%. Asymmetry of spot-size is more frequent. Mason *et al.* (1967) found that up to 25% of the size variation in pattern elements of *Euphydryas editha* was due to asymmetry.

Sheppard (1969) emphasized the interest of boundaries of the type described in *M. jurtina* to population and evolutionary geneticists because they incorporate some of the elements of disruptive selection and some of those of subspeciation. Laboratory experiments on the quantitative character of sternopleural chaeta number in *Drosophila melanogaster* have shown how disruptive selection, even in the presence of high gene-flow between selected lines, can lead to divergence and effective isolation (e.g. Thoday & Boam 1959, Millicent & Thoday 1961). The response of chaeta number to both disruptive and directional selection in such experiments is slower than occurs when populations within the boundary region switch-over between the characteristic spot frequencies. The heritability of chaeta number is, however, lower (being about 0.5) than that for spot-number. Clarke & Sheppard have investigated disruptive selection on a quantitative character in *Papilio dardanus* (see also Ch.14). The inheritance of tails in this butterfly is due to a single pair of alleles, autosomal but sex-controlled. The males are non-mimetic and tailed. In most of Africa many of the female forms are mimetic. The females, like the models for these forms, are tailless. In the Ethiopian race the majority of females are tailed and non-mimetic. A minority are mimetic but differ from similar forms elsewhere in having tails. The genetic and morphometrical analyses of Clarke & Sheppard (1960*a*,*b*, 1962*b*) have shown that in Ethiopia there is disruptive selection acting on the females and favouring the reduction in tail length in the mimetic forms but discouraging it in the non-mimetic females. Their results indicate the presence of modifier loci in this race which enhance the difference in mean tail length.

Two local discontinuities in spot frequency in *M. jurtina* have been found in the Isles of Scilly, one on Great Ganilly and the other on White Island (Dowdeswell *et al.* 1960, Creed *et al.* 1964, Ford 1975*a*). In the latter case the difference between the two areas of the island was only detected after these areas were isolated, at least partially, by storm damage. The areas differ in vegetation and exposure and the populations they support show differences in esterase variation (Handford 1973*b*). A further discontinuity of this type occurs along a 5km transect on the coast near St. Andrews in Scotland (Brakefield 1979*a*). The climate becomes more maritime along the transect but there is no obvious habitat change.

High Spotting and Aestivation Behaviour

Scali and co-workers have sampled many populations of *M. jurtina* in Tuscany, central Italy. High spot averages prevailed. In mainland populations females tend towards the 'flat-topped' spot frequency whilst males are unimodal at 2 spots with very few lower spotted individuals (Scali 1971*a*). In populations on two large offshore islands even higher spot averages were found (Scali 1972). The male populations did not exhibit their usual uniformity. Significant changes between generations occurred in two male populations with an estimated 72.7% elimination of high spotted phenotypes in one. This latter population showed some evidence of a reverse change in the following year. A climatic factor may have caused the initial changes since they coincided with those in several mainland populations. Scali pointed out their parallel in the widespread spotting shifts in populations in S. England and the Isles of Scilly which were associated with unusual climatic conditions in 1955–57 (Creed *et al.* 1959, 1962, Dowdeswell *et al.* 1960). Two earlier examples of intergeneration changes in male populations in mainland Tuscany coincided with habitat changes. Nearby colonies remained unaffected (Scali 1971*a*).

The reproductive biology of *M. jurtina* in Tuscany has been studied in detail (Scali 1971*b*, Masetti & Scali 1972, Scali & Masetti 1975). At lower altitudes adult emergence occurs over a short period of about three weeks from late May. Copulation takes place,

after which the males die. Females then always undergo a long aestivation during the hottest season. In late August and September the eggs which have matured during aestivation are fertilized from stored sperm and laid. A difference in spot frequency has consistently been found in populations between females flying before and after aestivation. The spotting shifts always tend towards lower values with usually a change from a 'flat-topped' or unimodal at 2 spots distribution to one unimodal at 0 spots. The calculated selection against high spotted phenotypes (2–5 spots) amounts in many instances to 65–70%. In mountain populations aestivation does not occur and the adult flight period extends from late June until early September. A mixed strategy is found in a population at intermediate altitude with some butterflies emerging early, followed by aestivation of females, and others late with no subsequent aestivation. An investigation of the control of aestivation behaviour would be most interesting.

Spot Stabilizations

Dowdeswell & McWhirter (1967) examined museum samples of *M. jurtina* from throughout its distribution. They described a system of stabilization areas characterized by populations with particular spot frequencies. The largest of these, called the General European, extended from Britain (except the southwest) through much of continental Europe. Here the spot frequency is unimodal at 2 spots in males and at 0 spots in females. Dowdeswell & McWhirter considered that changes between stabilizations were sharp and resulted from prolonged and violent alterations in selection (cf. the boundary region).

The type of data analysed by Dowdeswell & McWhirter and the distribution of their samples suggests that distinctions between stabilizations are somewhat imprecise. This is supported by additional samples. In several stabilization areas enclaves of populations with distinctive spot frequencies are found. In Scotland, females in the Grampian Mountains tend towards a bimodal spot frequency whilst the spot variation of neighbouring populations is typical of the General European area (Table 16.8; Forman *et al.* 1959). Samples obtained from Ireland up to 1967 consistently showed very low spot averages (Dowdeswell & McWhirter 1967, Frazer & Willcox 1975) but much higher frequencies of spotted females were later found in two populations in a different region (Table 16.8). Unusually high spotted populations have sometimes been found in central and southern England and in coastal regions of the continental General European area (Frazer & Willcox 1975, Brakefield unpublished).

Populations of *M. jurtina* tend to show one of a limited number of types of spot frequencies. Thus whilst females are often unimodal at 0 or 2, or bimodal at 0 and 2 and may change from one to the other, they are very rarely unimodal at 1 spot. This feature has been attributed to the occurrence of co-adapted gene complexes (McWhirter & Creed 1971, Handford 1973*a*). However, the probability of a mode occurring at 1 spot may be less than at 0 or 2 spots. The set of spot frequencies which will result from selective processes will depend on the fitness relationships between spot genotypes and on the developmental relationship between genetic variation and spot phenotype. At the simplest level, the spot-number classes include differing numbers of spot types (Table 16.1). Within each type of spot frequency there is some variability in the height of the mode(s) in populations.

Sometimes a general change in spot variation has been detected in populations within part of a stabilization area (see above). The recent samples from Gairloch in northwest Scotland and from northwest England (Table 16.8) suggest that a change to high spotting has occurred in these areas in the last 25 years (early samples in Creed *et al.* 1959, 1962, Dowdeswell & McWhirter 1967; comparison—Gairloch $\chi^2_3 = 10.89$; West Kirby area $\chi^2_3 = 9.89$, $P < 0.05$ for each value). When considered together, the samples of *M. jurtina* indicate that the distribution map for stabilization areas given by Dowdeswell & McWhirter is an oversimplification and that the different types of spot frequencies may not represent such a discontinuous nature of variation as has been supposed.

Dowdeswell & McWhirter showed that a number of different stabilizations occur around the periphery of the species' distribution. They considered that populations in such areas are adjusted to specialized environments. I have analysed the change in spot-number variation between generations in samples from three areas collected over five-year periods (Brakefield 1979*b*). A greater constancy of female spot average between generations was found both within the ecologically more marginal populations of central-eastern Scotland and the geographically peripheral populations of the Isles of Scilly than within those more centrally located in southern England. The results were consistent with the hypothesis that adaptive specialization and selection favouring a relative homozygosity predominate in marginal populations of *M. jurtina*.

Variation in Other Species

Discontinuities involving characters supposed to have a polygenic basis have been described in some other butterflies. Owen & Chanter (1969) found differences between adjacent populations of the African nymphalid *Acraea encedon* in the underside hindwing

Table 16.8. Spot variation in some recent samples of female *M. jurtina* from the British Isles (full data given in Brakefield 1979*a*).

Locality	Year	Spot-number 0	1	2	3	4	5	Total	Spot average	Costality Index (%)
CE Scotland:										
Grampian Mts	1973–77	280	144	190	47	18	8	687	1.13	38.6
All others	1973–77	1597	600	309	122	24	2	2654	0.64	48.2
CW Ireland:										
Co. Limerick	1976	77	58	57	18	2	1	213	1.12	58.4
NW Scotland:										
Gairloch	1976–77	18	12	19	7	4	0	60	1.45	53.0
NW England:										
West Kirby	1975	43	24	24	10	1	0	102	1.04	62.3
Hightown	1976	95	78	131	78	17	1	400	1.62	69.6
Whitchurch	1976	28	20	24	12	1	1	86	1.31	66.7

'Grampian Mts' and 'All others' comprise 3 and 12 populations respectively. Each group of populations gave homogenous samples in each year.

pattern of 20 spots (but see also Ch.18). The smaller spots, which tended to be closer to the thorax, were the most frequently absent. A low rate of adult movement was detected between some populations. Lucas (1969) investigated clinal variation in wing pattern characters in populations of *Tisiphone abeona* along the southeast coast of Australia. Preliminary evidence was presented for a simple genetic control for some characters. Clines in several characters showed local steepenings which, with one exception, coincided in geographical position. Endler (1977: 76) discusses the possibility that this may be an effect of a partial barrier to gene-flow. The variation in underside spot-number and spot-size found in British populations of *Coenonympha tullia* represents a complex pattern of clines and races associated with isolated distributions (Dennis 1977, Porter 1980).

Ehrlich & Mason (1966) and Mason *et al.* (1968) in a morphometric analysis of spot pattern characters in two Jasper Ridge populations of *Euphydryas editha* found that changes in trend characters occurred in a uniform and concurrent manner over a period of eight generations (years). They suggested that such changes were caused by strong fluctuating selection, but the possibility that they were due to a complex penetrance system could not be excluded. The characters studied in *E. editha* affected the lightness of wing coloration (see also Le Gare & Hovanitz 1951, on *E. chalcedona*). Descimon & Renon (1975) showed that in France, *Melanargia galathea* becomes blacker in warm-dry areas. The interaction between temperature, insolation and pigment is complex (Papageorgis 1975, Turner 1977*a*, Watt 1968, 1969). The common occurrence of seasonal forms which differ in extent of melanin deposition is discussed by Shapiro (Ch.27).

Geographical and seasonal differences in wing size are found in many butterfly species (e.g. Baker 1972*b*, Ishii & Hidaka 1979). The influence of polygenic systems on wingspan has been demonstrated in crosses between subspecies of *Aricia* spp. (Høegh-Guldberg & Jarvis 1970) and of *Anthocharis cardamines* (Majerus 1979). The existence of genetic variability within populations of a species has not been rigorously examined. Population studies in species of moths have shown examples of both environmental and genetical influences on pupal or adult size (e.g. Danthanarayana 1976, Lorimer 1979, Richards & Myers 1980, Myers & Post 1981). Dempster *et al.* (1976) investigated size variation of *Papilio machaon* in England. A comparison of museum samples showed that insects from the isolated Wicken Fen had a longer wing relative to body size and a narrower thorax relative to body length than those from more continuous habitat in Norfolk. Examination of temporal changes showed that the butterflies from these areas were similar until 1880 when those from Wicken declined in size (wing shape remained similar). After 1920 the difference diminished, largely because Norfolk butterflies became smaller. Experiments indicated that a functional relationship might exist between body shape and flight speed. The changes at Wicken could be interpreted as due to selection against mobility associated with a reduced area of habitat. The later changes in Norfolk were less easy to explain because a change in habitat was gradual. An interesting parallel distribution of dwarf forms of *Hipparchia semele* and *Plebejus argus* occurs on a small isolated peninsula of carboniferous limestone in north Wales (Thompson 1944, Ford 1975*b*, Dennis 1972*b*,*c*, 1977). The forms also show differences in wing pattern characters and phenology to adjacent (for *H. semele*) or nearby populations. Dennis (1977:

253–56) suggests that a calcareous landscape and a warmer and drier microenvironment have been responsible for the evolution of these forms.

Geographical variation in the number of annual generations occurs in many species of butterflies in temperate zones (refs. to examples in Britain in Dennis 1977). Ecological aspects of differences between species are discussed by Gilbert & Singer (1975; see also Chs 2, 3) and Watt *et al.* (1979). Lees (1962*a*) showed that differences between stocks of *Coenonympha pamphilus* from two English populations with predominantly univoltine and bivoltine strategies respectively were maintained in identical laboratory conditions. The population origins differed in latitude and altitude. Lees (1965) was able to selectively increase the proportion of non-diapausing larvae in the normally univoltine stock from 23.7 to 46.3% over two years. The response to selection was not as rapid as that reported for the Gypsy moth (Hoy 1977). Some additional evidence has been obtained for genetic differentiation within butterfly species in response to the environmental factors determining diapause (e.g. Petersen 1949, Danilevski 1965, Jarvis 1966).

Selection on Spot Variation in *M. jurtina* during Development

The surveys of spot variation in *M. jurtina* in combination with the demonstration of high heritability provide strong evidence for selection, even though the specific factors involved remain unidentified. Examples of rapid changes in this variation have indicated that sometimes such selection can be very powerful. In this section I examine evidence that selection operates during pre-adult development.

Rearing Experiments

Dowdeswell (1961, 1962) reared samples of late instar larvae from two Hampshire populations collected by sweeping. The population at Middleton East was sampled in each of four years. Dowdeswell showed that sweeping probably samples larvae at random in relation to spot-number. Larvae collected before and after late May were treated as early and late samples respectively. Females emerging from the early samples consistently yielded a higher spot average than those from the late samples. Larval mortality was higher in the late samples mainly due to much heavier parasitism by *Apanteles tetricus* (about 25% parasitized). Dowdeswell noted that parasitism probably takes place during the first instar. He suggested that *A. tetricus* was the principal agency responsible for a selective elimination amounting to about 70% of 2–5 spot females in the late samples. However, if parasitism was not restricted to spotted genotypes (which seems unlikely) it must have caused a considerably higher mortality in the field than that detected in the laboratory to fully account for the differences between early and late samples. No causal relationship has been established between parasitism and spot variation. An experiment in which mid-instar larvae from a Scillonian population were introduced into experimental grass enclosures near Liverpool provided no evidence for selective elimination by birds or small mammals (Brakefield 1979*a*).

In three of the four years at Middleton East the combined reared females and the samples of flying adults showed significantly different spot frequencies. Table 16.9 shows that the relative fitness of the female spot classes declines with increasing spot-number. Although differences were not detected in individual years for males, the combined data do suggest that a similar relationship between fitness and spot-number occurs (Table 16.9). When the other Hampshire population is considered these conclusions are only supported for females (Dowdeswell 1962).

McWhirter (1965) reported that in the Isles of Scilly a powerful selective elimination of high spotted males occurs in the late-larval and pupal stages. McWhirter's records of the braconid parasites of *M. jurtina* in the Isles of Scilly do not include *A. tetricus*. I obtained comparable data to those of Dowdeswell for three local sites on St Martin's Island. Table 16.9 shows that a similar relationship between fitness and spot-number is found in this population as at Middleton East. The estimates for intensity of selection are similar for females in these populations but the intensity is much higher for males on St Martin's Island (Table 16.9). If selection takes a similar form in the populations it may have largely operated on males at Middleton East by the time of larval sampling. This is more likely when the faster development of males is considered.

Larvae have also been obtained from Cramond Island in eastern Scotland and Buckley in N. Wales (Brakefield 1979*a*; total sample size = 340). The larvae from each population were roughly comparable in development to early and late samples from Middleton East respectively. Only 1 of 116 deaths was due to a parasite. There was no evidence for selection in either population since the spot variation in the reared and flying samples was similar. The wide difference in spotting between the populations was maintained in the reared material.

Endocyclic Selection.

The results of the rearing experiments have been interpreted as due to a powerful selective elimination of high spotted genotypes in late

Table 16.9. The intensity of selection and the relative fitness of spot-number classes estimated from mid-late instar larvae to adults in two populations of *M. jurtina*.

Spot-number class	Females		Males	
	Middleton East	St Martin's Island	Middleton East	St Martin's Island
0	1.000	0.708		
1	0.513	1.000	1.000	
2	0.469	0.668	0.641	1.000
3	0.364	0.513	0.725	0.186
4	0.064	0.079	0.590	0.189
5	—		0.212	0.032
Intensity of selection	0.294	0.360	0.252	0.697
Sample sizes:				
before selection:	339	229	240	216
after selection:[1]	929	69	1148	32

Data: Middleton East (1957-60) from Dowdeswell (1961): St Martin's Island (1976-77) from Brakefield (1979*a*).
Method of calculation given by O'Donald (1971). Maximum fitness is defined to be $w = 1$. Where the number before or after selection = 0, braces indicate some combining of spot classes. A dash is used when both numbers = 0. Intensity of selection, which equals − mean w, is calculated directly using estimates of relative fitness. For the combined 1959-60 female data from Middleton East, O'Donald showed that the estimates obtained by the direct method and using an unbiased quadratic model were similar.
[1]The data for St Martin's Island were kindly supplied by Professor K. G. McWhirter.

pre-adult development. To account for this it is necessary to predict an earlier counterbalancing selection favouring these genotypes (McWhirter 1967, Ford 1975*a*). A small sample of adults reared with heavy mortality from larvae collected on St Martin's Island in November before the winter period of slow growth suggested high spot averages (males = 3.69, $n = 13$; females = 2.47, $n = 17$; Brakefield 1979*a*). Therefore the initial period of directional selection may occur in early development.

One-generation cyclical selection has been called endocyclic by McWhirter (1967). The basic models of population genetics developed by Fisher, Haldane and Wright assume that the selective forces which act on individuals are constant. Sheppard (1953), Sheppard & Cook (1962), Kojima (1971) and others have pointed out that this assumption is invalid. Pasteur (1977) has suggested that endocyclic selection is widespread (also see Bishop 1969, Dowdeswell 1971). He indicates that as such selection helps to maintain the genetic variability and evolutionary potential of the species, it should be common in eukaryotes. Goux (1978) developed a simple model of a single diallelic locus which shows that an endocyclic pattern of selection can produce a stable polymorphism if the mean fitness of the heterozygotes over the whole life cycle is greater than that of either homozygote.

Some further examples of endocyclic selection in *M. jurtina* have been proposed. Masetti & Scali (1978) and Scali & Masetti (1979) found that adult allelic frequencies at the phosphoglucomutase locus remained homogeneous through two successive generations in two Italian populations, whilst the intervening larval populations showed different frequencies from the adults. Scali & Masetti (1973) showed that in certain Italian populations a sex ratio in favour of females (up to 5.4: 1) at the embryonic stage became sequentially reduced until adults showed a probable excess of males. The relevance of Scali & Masetti's results to other populations is uncertain. The broods of *M. jurtina* (Table 16.3) show no evidence for heterogeneity of sex ratio ($\chi^2_{15} = 21.86$) or for an overall departure from a ratio of unity ($\chi^2_1 = 0.62$).

Effects of Spot Genes on Development

Intraseasonal changes in spot frequency have been detected in adult populations (Creed *et al.* 1959, Dowdeswell 1962, Beaufoy *et al.* 1970, Scali 1971*a*, Brockie 1972, Scali & Masetti 1975, Brakefield 1979*a*). They do not occur in all populations (Tudor & Parkin 1979). When a shift has been detected it is nearly always towards lower spot averages in the later sample(s). These observations could be due to differential development rates (Scali 1971*a*).

The date of emergence in the broods of *M. jurtina* was recorded. Analysis of the male data provides evidence for genetically determined differences in the rate of development of the spot-number classes. Figure 16.10 shows that in four of the seven larger broods the early and later portions of the emergence differed in spot frequency. In each example the spot average is lower in the later portion. A similar difference is only found in one of the six larger broods of females (brood no. 13:$\chi^2_2 = 13.04$, $P < 0.001$). The evidence in females is therefore inconclusive. Differences in development time between spot classes could readily affect their relative fitness. That major

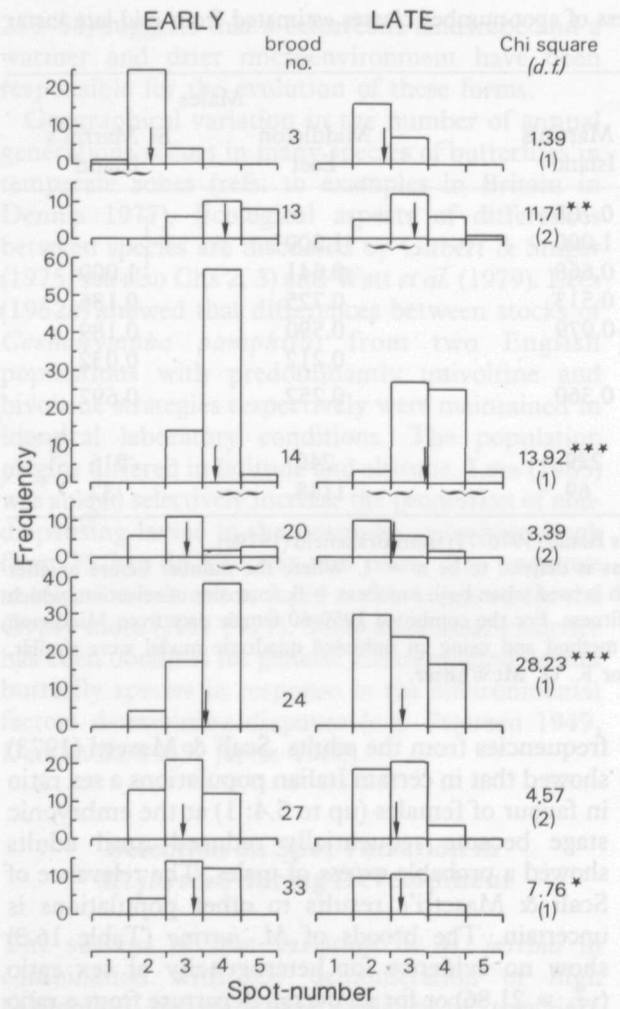


Fig. 16.10. Timing of emergence of male spot-number classes in broods of *M. jurtina*. (Each brood with $n > 35$ was divided as closely as possible into two with respect to date of emergence to give an early (first) and a late half. Arrows show mean spot-number. Spot frequencies are compared by χ^2 (chi-square) with braces indicating groupings where necessary: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.)

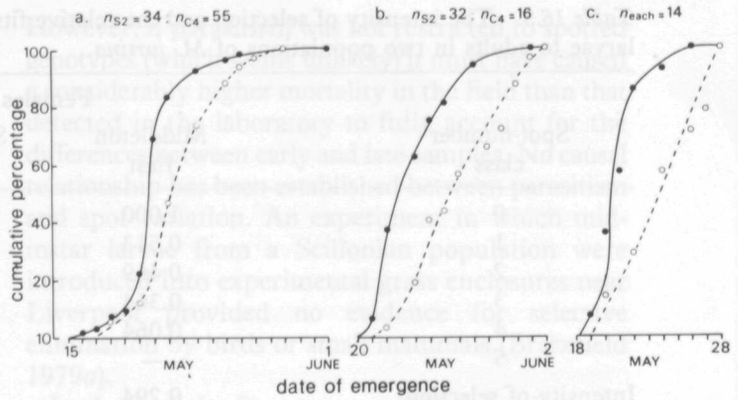


Fig. 16.11. Cumulative emergence curves for splay 2 (○) and costal 4 (●) males in broods of *M. jurtina* in which each spot type comprised >15% of all males.

genes determining adult phenotypes in Lepidoptera can influence development time has been suggested by studies on *Colias* butterflies (Graham *et al.* 1980) and on several melanic moths (Bishop *et al.* 1978).

Differences in the timing of emergence of two male spot types were detectable in three broods (Fig. 16.11). Unfortunately the data could not distinguish between spot types within any of the spot-number classes. A difference of this form was evident in an earlier emergence of anal-3 than costal-3 males in two populations near Liverpool which were sampled daily during the emergence period in 1978 ($P = 0.04$ and $P < 0.001$; from Brakefield 1979*a*). A difference was not found in one of these populations in 1977. The spot-placing of female populations in Scotland becomes more anal with increasing altitude (Fig. 16.12). Males also show higher frequencies of anal-spot types (particularly anal-3) at higher altitudes. McWhirter & Creed (1971) have shown that the anality of populations increases northwards through Britain. These observations suggest the hypothesis that anal spot types are favoured at high altitudes and in northern latitudes because of a faster development rate. A relationship might also exist between spot variation and the variability which occurs in the timing and length of the flight period in British populations. This is sometimes associated with differences in soil type and habitat (Pollard 1979*a*; and see Thomson 1971, Ford 1975*a*, Brakefield 1979*b*).

It is unlikely that differential development rates could fully account for Dowdeswell's (1961, 1962) results, since the differences he found between early and late samples of larvae were not always paralleled by intraseasonal changes of spotting in the flying populations. Such changes have also not been recorded in the Isles of Scilly. The reared and flying samples of males from St Martin's Island differed particularly widely (spot average = 3.69 and 2.50).

Does Visual Selection Influence Spot Variation?

The hindwing spots of *M. jurtina* have been described as trivial or unimportant to the individual (Ford 1955: 220, 1973, Sheppard 1969, Scali 1971*b*). I suggest that this may be an invalid assumption. No reference has been made to the prominent and variable forewing eyespot in connection with the significance of the hindwing spot variation. In this section a model is developed to account for the phenotypic variation in spot pattern in terms of visual selection.

Functions of Spot Patterns

Beak-damage patterns suggest that small eyespot markings on butterfly wing margins direct the attacks of birds away from the vulnerable body (Marshall & Poulton 1902, Swynnerton 1926, Carpenter 1941,

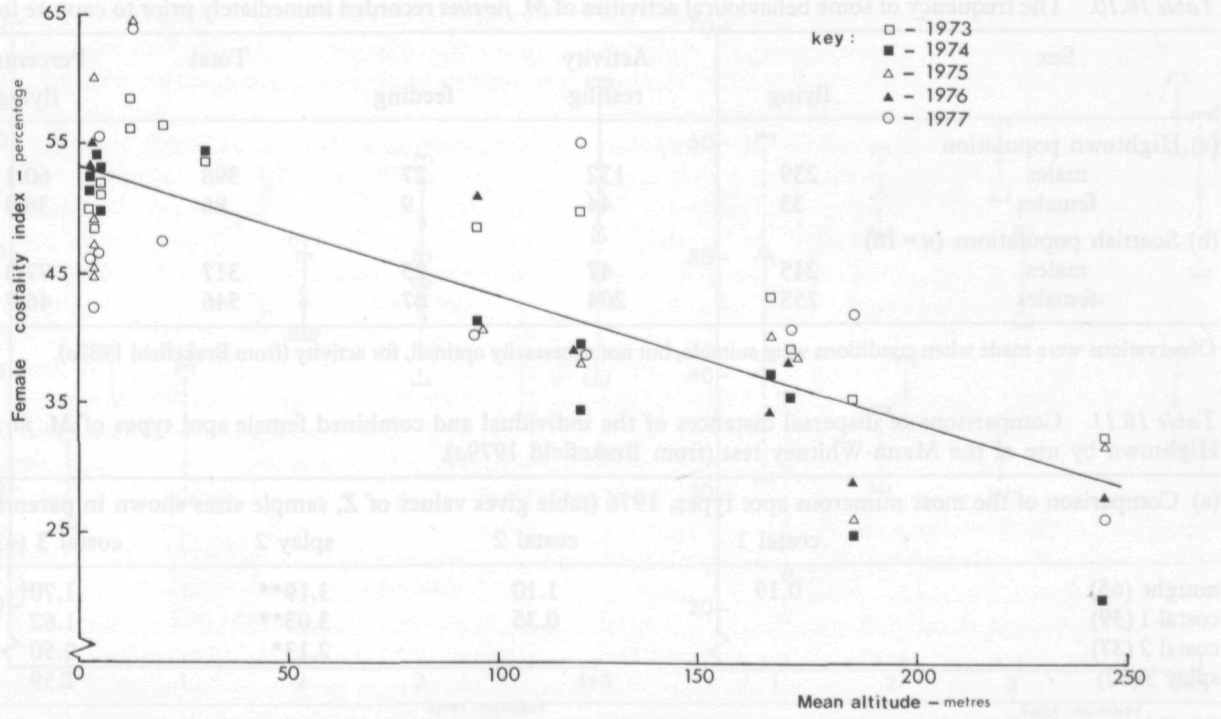


Fig. 16.12. The relationship between altitude and female spot-placing in populations of *M. jurtina* in central-eastern Scotland (from Brakefield 1979*a*). Fitted regression line: $g = 53.19 - 0.0987\,x$.

Blest 1957; also see Robbins 1980, 1981, Larsen 1982*b*). Similar observations have been made in relation to predation by lizards (Van Someren 1922, Brockie 1972, Ford 1975*b*). Experimental evidence was obtained by Blest (1957) working with painted mealworms and captive birds. Additional experiments supported the hypothesis that whilst a weak stimulus (e.g. small undifferentiated eyespots) evokes an approach from a bird, a strong stimulus (large solid-coloured eyespots) causes withdrawal (Schneirla 1965, Coppinger 1969, 1970). These responses have been attributed to anti-predator mechanisms of deflecting and startling respectively (Cott 1940, Edmunds 1974*a*). The effect of startling (or confusing) may be enhanced by a form of flash-coloration in which the stimulus is rapidly exposed in response to disturbance (Blest 1957).

Most organisms exhibit some form of cryptic coloration (Cott 1940, Edmunds 1974*a*). The evolution of spot patterns in butterflies must have been closely integrated with that of such coloration (see Schwanwitsch 1948). The adaptive significance of cryptic coloration has been demonstrated by numerous experimental studies (refs. in Endler 1978, 1980; examples involving butterfly pupae in Baker 1970, Wiklund 1975*a*). Endler (1978) provides a useful amplification of the term cryptic: 'a pattern is cryptic if it resembles a random sample of the background perceived by the predator at the time and age, and in the microhabitat where the prey is most vulnerable to visually hunting predators'. The matching of pattern and background must extend to features of grain, geometry, contrast and colour. The visibility of an organism's colour patterns is also influenced by predator vision and hunting tactics and by prey behaviour. The effects of these factors are interdependent and may vary from place to place. The optimum cryptic pattern is determined by their interaction (Endler 1978).

Adult Behaviour and Survivorship of *M. jurtina*

M. jurtina is a resident species which typically shows a low rate of movement from favourable areas of grassland habitat (Dowdeswell *et al.* 1949, Brakefield 1979*a*, 1982*a*, Tudor & Parkin 1979, Pollard 1981*b*). Males and females within a population show similar survivorship curves. The available estimates for English populations indicate that the adult life expectation is 5–12 days. I have estimated that adult mortality, on average, accounts for 50–60% of a female's potential egg supply of about 180 eggs. The dispersal rates for the sexes are similar (Brakefield 1979*a*, 1982*a*). However, males fly more often than females (Table 16.10; see also Baker 1978, Pollard 1981*b*). Males show a slow exploratory flight with frequent changes in direction when searching for mates. Females rest on vegetation for long periods. They fly to lay eggs and to feed. My field observations and Baker's tracking experiments show that in general males are more active and make more frequent changes in behaviour than females. In common with many species of butterflies, male *M. jurtina* are selected to maximize the number of

Table 16.10. The frequency of some behavioural activities of *M. jurtina* recorded immediately prior to capture in 1977.

Sex	Activity			Total	Percentage
	flying	resting	feeding		flying
(a) Hightown population					
males	239	132	27	398	60.1
females	33	44	9	86	38.4
(b) Scottish populations ($n=16$)					
males	245	47	25	317	77.3
females	255	204	87	546	46.7

Observations were made when conditions were suitable, but not necessarily optimal, for activity (from Brakefield 1982*a*).

Table 16.11. Comparisons of dispersal distances of the individual and combined female spot types of *M. jurtina* at Hightown by use of the Mann-Whitney test (from Brakefield 1979*a*).

(a) Comparison of the most numerous spot types, 1976 (table gives values of Z, sample sizes shown in parentheses).

	costal 1	costal 2	splay 2	costal 3 (43)
nought (65)	0.19	1.10	3.19**	1.70†
costal 1 (39)		0.35	3.03**	1.62
costal 2 (37)			2.13*	0.50
splay 3 (32)				1.59

(b) Comparison of nought, costal 1 and costal 2 spot types combined (n_1) with all other spot types (n_2)

Year	Sample sizes		Z	U
	n_1	n_2		
1976	141	95	3.57***	
1977	18	22		331.5***
1978	43	18	2.36*	

2-tailed significance level: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $\dagger 0.1 > P > 0.05$.

matings whilst females mate only once (Scali 1971*b*), thus allowing maximum time for nectar feeding, egg maturation and locating breeding microhabitats (cf. Wiklund & Åhrberg 1978). The sexes in a population at Hightown near Liverpool showed different microdistributions over a grid of 7.5m squares (Brakefield 1979*a*, 1982*a*). This difference and temporal changes in microdistribution were correlated with the distribution of the adult resources of each sex. These observations confirm Handford's (1973*a*) suggestion that males and females occupy differing ecological niches.

Tudor & Parkin (1979), in a population study of *M. jurtina*, found changes between generations in the relative rate of recapture of different groups of spot classes. Some observed changes in spot frequency in males could have had a selective basis if differences in fecundity were associated with those in recapture rate. The results of applying Leslie's (1958) test and of a comparison of recapture rates with the Poisson distribution suggested that males within some populations do not have an equal probability of capture (Brakefield 1979*a*, 1982*b*). The survivorship curves of the most numerous spot types in each sex were similar. However, there is evidence that differences in dispersal behaviour occurred between some female spot types (Table 16.11). Figure 16.13 suggests that dispersal increased with increasing spot-number and that a similar but less marked relationship may be found in some male populations. There was also evidence for differences in microdistribution between some male spot types.

Additional intensive capture-recapture experiments are necessary to more firmly establish the relationship between dispersal and spotting. However, some support comes from another source. Bengtson (1981) investigated wing damage which resulted from attacks by birds, especially *Lanius collurio*, in five populations on two islands in Sweden. He found that spotted and unspotted females showed significant differences in beak-damage frequencies. This result can be accounted for if differences in behaviour influence the exposure and conspicuousness of these groups to avian predators. Such differences could also be a factor in the observed selective elimination of spotted females during aestivation in central Italy (e.g. Scali 1971*a*). Bengtson found that males and females showed different frequencies of beak-damage (13.3 and 8.5%). This could be due to various factors including differences in behaviour, age structure or

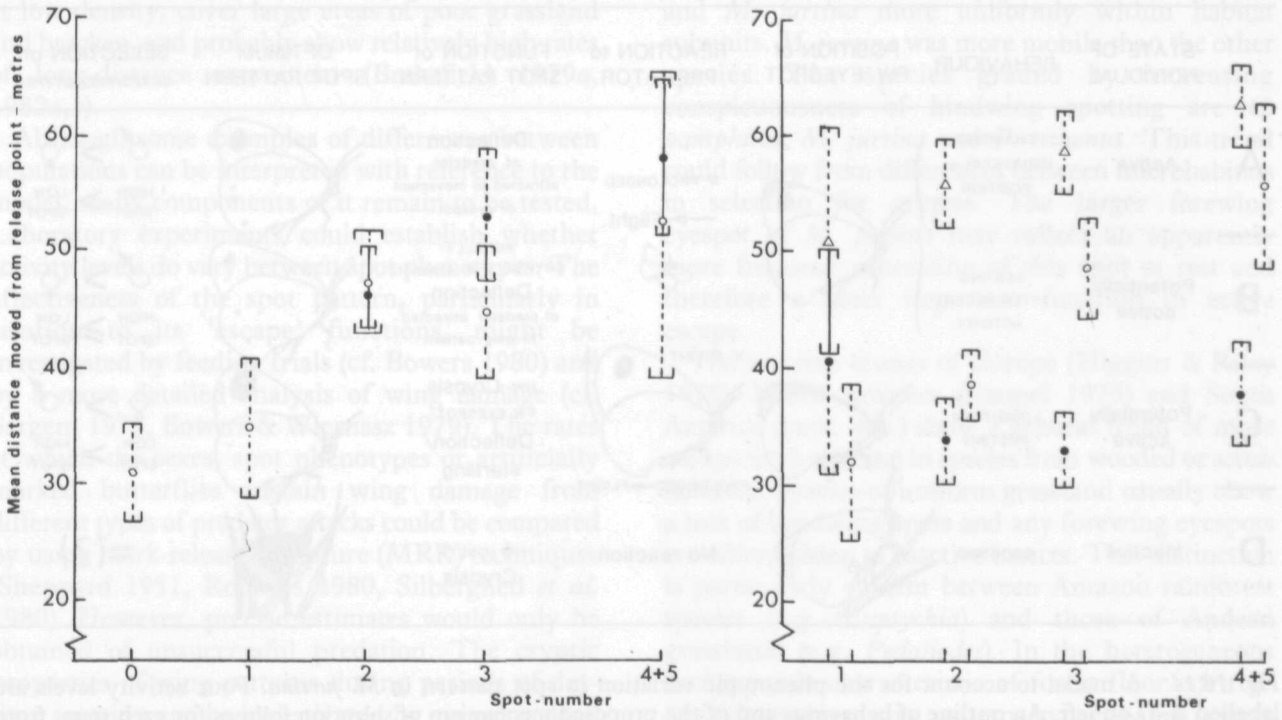


Fig. 16.13. Mean distance moved (± S.E.) by *M. jurtina* in relation to spot-number for the populations indicated (full details are given in Brakefield 1982*a*,*b*). a) Hightown 1976: ● males; ○ females. b) Hightown: ● males 1977; ○ females 1978. Hall Road: ▵ males 1978.

palatability (see Edmunds 1974*b*). Lane (1957) presented adults to a captive Shama bird. In early trials it showed an apparent preference for males. Thereafter the sexes were taken indiscriminantly. Frazer & Rothschild have found more histamine-like substances in females (Rothschild pers. comm.). These observations merit further investigation. However, the sexes show a similar survivorship and Lane found that their taste is similar. Bowers & Wiernasz (1979) showed that the satyrine *Cercyonis pegala* is palatable. Samples from two populations showed similar overall frequencies of wing damage to those found by Bengtson for *M. jurtina*.

A Model for *M. jurtina*

The components of the model are outlined in Fig. 16.14. The behaviour of an adult *M. jurtina* can be related to four activity states (A-D). Details of behaviour, wing-positioning and reaction to predators differ between states. It is proposed that a spot pattern is both most likely to be effective in deflection (with subsequent escape) and most frequently elicited when a butterfly is changing its position or behaviour (state A, also B). Movement increases the likelihood of attracting a predator. At such times the butterfly usually has its wings closed above its body with the submarginal ring of spots displayed. Males are more likely to be encountered in these circumstances than females. It may then be more effective to have several spread out spots than the single large eyespot which is the effect of the female pattern (Fig. 16.3). The latter could increase the initial likelihood of a predator being attracted.

Females are more likely to be encountered whilst resting between bouts of activity (state C). The forewings are often withdrawn between the hindwings. Prominent hindwing spots may then reduce the effectiveness of crypsis (see below). If slightly disturbed, such a butterfly will frequently raise its forewing rapidly so exposing the eyespot (cf. Tinbergen 1958 and Ford's 1975*b* observations for the Grayling, *Hipparchia semele*). The trend in females towards a large eyespot with no alternative stimuli may then be advantageous and could be associated with both deflecting (Lane 1957) and startling anti-predator mechanisms. The effect of a single large stimulus is accentuated in females by a greater contrast between the eyespot and surrounding wing pigmentation, a more marked costal positioning of any hindwing spots, and a 35% larger white pupil area relative to eyespot area than in males (Fig. 16.3).

Whilst in an inactive state the forewings are withdrawn (state D). Therefore visual selection will only act on the hindwings and will then only favour cryptic properties. In uniform grassland habitats of predominantly linear backgrounds, prominent hindwing spots are probably disadvantageous (Cott 1940, Wickler 1968, Edmunds 1974*a*). This selection pressure will differ between males and females if they show differences in roosting behaviour.

I have outlined the model in relation to the sexual

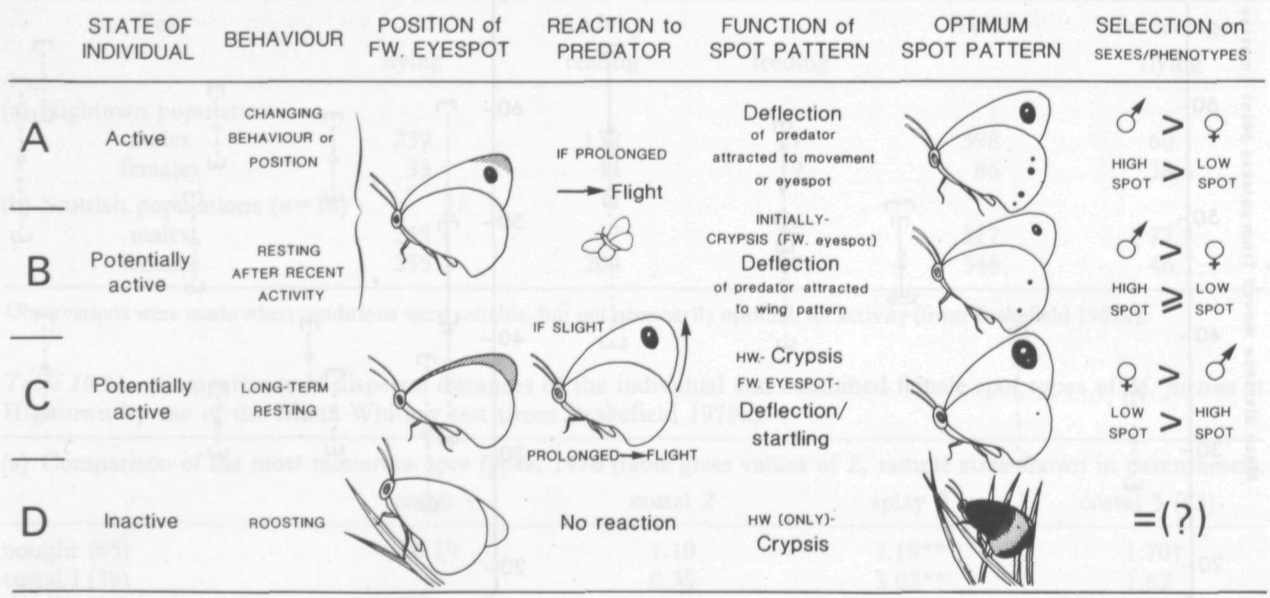


Fig. 16.14. A model to account for the phenotypic variation in spot pattern in *M. jurtina*. Four activity levels are labelled A–D on left. An outline of behaviour and of the proposed mechanism of selection follows for each state, from left to right. The final column indicates the predicted relative fitness of the sexes and hindwing spot phenotypes in relation to the model. (See text for full explanation.)

dimorphism in *M. jurtina*. It can also be applied to the spot variation within each sex. Males and females with larger and more numerous hindwing spots tend to show relatively large forewing eyespots (Fig. 16.3). This relationship is also found at the population level. The model proposes that the fitness of a given phenotype will depend on the relative probabilities of it being encountered by a predator whilst in each activity state (Fig. 16.14). This set of probabilities will vary between phenotypes if the positive relationship indicated between spot-number and dispersal rate is a real one. If it is assumed that dispersal is positively related to the level of activity, then the relationship between spotting and movement can be accounted for in the general prediction of the model that it is advantageous for a more active butterfly to show a heavier spot pattern. An anomaly is evident since in situations of long-term resting the model predicts that a large forewing eyespot and a lack of hindwing spots is favoured (state C). However, this disadvantage of hindwing spots could be outweighed by their advantage in complementing the forewing eyespot in other circumstances (especially A). A further complicating factor is that there may be some threshold of hindwing spot size (or contrast) below which visual selection is ineffectual.

Bengtson's (1978, 1981) studies of avian predation and spot variation in island populations of *M. jurtina* in Sweden were made over a five-year period. He found that a change in one year in the relative frequency of beak-damage in spotted and unspotted females coincided with changes in spot frequency, adult numbers and overall beak-damage frequency. These observations are consistent with a selective influence of avian predation on spot variation (Bengtson 1981).

The model in Fig. 16.14 predicts that relationships will occur between spot variation and habitat. In particular, it predicts that a high level of spotting will be found in types of habitat which favour high mobility and activity, or where the background is less uniformly linear and visual selection against large spots in relation to crypsis is weaker. These factors may coincide in mixed habitats of grass and scrub. Some authors have suggested that examples of differences in spot variation between populations of *M. jurtina* and of changes between generations were due to selective forces associated with differences in habitat (e.g. Dowdeswell & Ford 1955, Dowdeswell *et al.* 1957, 1960, Scali 1971*a*). McWhirter (1957) proposed that three types of female spot frequencies characteristic of populations in the Isles of Scilly might reflect differences in habitat. In particular, amongst the small islands areas of open, often exposed grasslands are usually associated with females unimodal at 0 spots whilst luxuriant habitats with patches of scrub tend to show high spot averages. This difference is therefore consistent with the prediction. It is well illustrated by the discontinuity found between the ends of White island (Ford 1975*a*). A similar association is found in the Grampian Mountains in Scotland. Here a high spotting on both hindwing and forewing (Table 16.8, Fig. 16.2) is found in three populations which are

at low density, cover large areas of poor grassland and bracken, and probably show relatively high rates of long-distance movement (Brakefield 1979*a*, 1982*a*,*b*).

Although some examples of differences between populations can be interpreted with reference to the model, many components of it remain to be tested. Laboratory experiments could establish whether activity levels do vary between spot phenotypes. The effectiveness of the spot pattern, particularly in relation to its 'escape' functions, might be investigated by feeding trials (cf. Bowers 1980) and by a more detailed analysis of wing damage (cf. Sargent 1973, Bowers & Wiernasz 1979). The rates at which the sexes, spot phenotypes or artificially marked butterflies sustain wing damage from different types of predator attacks could be compared by using mark-release-recapture (MRR) techniques (Sheppard 1951, Robbins 1980, Silberglied *et al.* 1980). However, precise estimates would only be obtained of unsuccessful predation. The cryptic properties of wing patterns during periods of daytime roosting can be investigated by exposing dead insects placed in natural positions to predation (Kettlewell 1955, 1956).

Relevance to Other Species

Differences in type of grassland habitat are associated with the groups of populations or races of *Coenonympha tullia* in Britain which show striking differences in spot development (Brakefield 1979*a*, Porter 1980). The races of European and N. African species of this genus (including *tullia*) at higher altitudes and latitudes tend to show smaller spots (see Higgins & Riley 1975). Cooler environments are likely to result in less adult activity. The model (Fig. 16.14) would then predict more emphasis on cryptic properties and lower spotting. The effect would interact with variation in habitat.

The model can also be applied to the common incidence of seasonal forms in species of Satyrinae in the Old World tropics. Such forms frequently show striking differences in spot development (Owen 1971*a*) which are consistent with selection favouring crypsis in the dry season forms which aestivate and are relatively immobile and the active anti-predator functions of the spot pattern in the wet season forms (Brakefield & Larsen 1984).

Some examples of interspecific variation in spot pattern in the Satyrinae are consistent with a relationship between habitat and visual selection. Three species inhabit a meadow study site near Liverpool (Brakefield 1979*a*). These showed different microdistributions which could be summarized as follows: *Coenonympha pamphilus* in open, short vegetation; *Pyronia tithonus* along grass/scrub edges; and *M. jurtina* more uniformly within habitat subunits. *M. jurtina* was more mobile than the other species. The species graded by increasing conspicuousness of hindwing spotting are *C. pamphilus*, *M. jurtina* and *P. tithonus*. This trend could follow from differences between microhabitats in selection for crypsis. The larger forewing eyespot of *M. jurtina* may reflect an apparently more frequent concealing of this spot at rest and therefore a more important function in active escape.

The satyrine faunas of Europe (Higgins & Riley 1975), North America (Emmel 1975) and South America (pers. obs.) show a general trend of more conspicuous spotting in species from wooded or scrub habitats. Species of uniform grassland usually show a lack of hindwing spots and any forewing eyespots are often hidden in inactive insects. This distinction is particularly evident between Amazon rainforest species (e.g. *Euptychia*) and those of Andean grasslands (e.g. *Pedaliodes*). In the heterogeneous backgrounds characteristic of forest floor environments prominent spot patterns may enhance crypsis except at close predator-prey distances when they can function in deflecting or startling predators (see Barcant 1970, Stradling 1976).

The model for *M. jurtina* shows some features in common with the hypothesis developed by Young to account for interspecific differences in eyespot development in *Morpho* species. Young (1975, 1979*b*) studied the habit of some species in Neotropical forests of feeding on rotting fruit falls. He proposed that the prominent underside eyespot patterns in these butterflies afford some protection from opportunistic vertebrate predators in the vicinity of the food sources. Young (1980) classified species of *Morpho* into: (1) primarily low flying ground feeders with large eyespots and (2) partially (a) or occasionally (b) ground feeders, flying at intermediate to high levels, and having small eyespots. In museum samples a high proportion of the primarily ground feeders showed evidence of unsuccessful predator attacks whilst at rest than of the partially or occasionally ground feeders ($\chi^2_1 = 68.62$; no difference between groups 2a and 2b). This supports the hypothesis that the large eyespots have evolved in response to the relatively high intensity and particular nature of predation at fruit falls. Selection may have operated in a similar way in some species of Satyrinae and Brassolinae (Young 1980). In some ground feeders (especially *M. peleides*) considerable intraspecific variation occurs in spot size (Young pers. comm.). The significance of this variability in relation to Endler's (1978) prediction that pattern diversity among morphs or species subject to predation on the same background should decrease with increased visual selection intensity merits further investigation.

Concluding Remarks

Although some trends in spot development between butterfly communities are consistent with an influence of visual selection, a detailed ecogenetical investigation is necessary to properly assess its role at the population, species or community level. It is my bias that the possibility of visual selection on even 'minor' pattern variation should not be discounted (e.g. Bowden 1979) without an adequate investigation (see Cain 1977). The model developed for *M. jurtina* is not proposed as a unifying explanation for the spot variation. Rather it should be considered in addition to the non-visual effects of the spot genes of which differential development rates may be one consequence. The model may indeed have an ability to explain by virtue of the input of many variables. However, it may be of value in the absence of alternative hypotheses providing a mechanistic connection between selective factors and the observed phenotypic variation. A more rigorous testing of the model's components may lead to more specific predictions. It is possible to predict the direction of changes in spot variation in response to general changes in habitat in natural (e.g. Tean) or experimentally manipulated populations. The apparent examples of associations between habitat differentiation and spot variation in *M. jurtina* have been found in peripheral or ecologically marginal areas. In centrally located areas populations usually show a similar spot variation. The hypothesis that co-adapted gene complexes are characteristic of these areas needs to be tested. Moreover, co-adaptation is an effect of differentiation not a cause or maintenance mechanism (see Endler 1977).

Ford & Ford's (1930) observations on phenotypic variation and population fluctuations in a colony of *Euphydryas aurinia* were consistent with a relationship between intensity of selection and population size. They found that a period of declining and then expanding population size was accompanied by a decrease and an increase in variation respectively. In view of the ultimate importance to ecological geneticists of understanding the control of population size in terms of genetic variation within a population, it is surprising that these observations were not followed by attempts to quantify similar phenomena using more refined techniques. Such a study could usefully be combined with surveys of enzyme variation. Various species of *Euphydryas*, for which some understanding of population dynamics has been gained, could provide study material (see Ch.2). Since these species lay egg masses, genetic analyses might be facilitated by using Lorimer's (1979) technique of comparing variance in quantitative characters between and among sibling groups.

Ecogenetical studies in quantitative characters in butterflies have been almost entirely limited to morphological traits in adults. Studies involving the pre-adult stages and also on fitness components such as fecundity and longevity would be valuable. The work on *M. jurtina* and some observations made on *E. editha* (Gilbert & Singer 1973, Singer 1971) suggest that the investigation of genetic variance for behavioural traits within and between populations, including dispersal, provides an exciting possibility for future research.

17. *Enzyme Variation Within the Danainae*

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Electrophoretic studies on Lepidoptera enzymes have mainly addressed two problems. Many (e.g. McKechnie *et al.* 1975, Brittnacher *et al.* 1978, Menken *et al.* 1980) have used electrophoresis as a tool in population studies, together with mark-release-recapture (MRR) and other ecological techniques. Quite often (e.g. Brussard & Vawter 1975) attempts have been made to detect selection on enzyme loci and hence provide evidence in the selectionist-neutralist debate. The other main use of electrophoresis has been taxonomic, for the detection or identification of sibling species (e.g. Jelnes 1975, Hudson & Lefkovitch 1980, Suomalainen *et al.* 1981, Geiger 1981). In some cases, certain allozymes (*sensu* Prakash *et al.* 1969) have proved diagnostic.

The only published study on the application of electrophoresis at the suprageneric level in butterflies is that of Geiger (1981). However, he did not have a defined model for the interrelationships of the pierid genera under consideration and thus, although his results are very similar to intuitive estimates based on morphological data (R. I. Vane-Wright, pers. comm.), it is difficult in this case to assess the utility or validity of the method.

However, for the Danainae such a model is available. Ackery & Vane-Wright (in press *a*) have assessed the cladistic relationships of the genera, based on over 200 adult morphology characters. To test both this classification and a variety of classificatory techniques, new information has been collected on immature stage morphology and adult allozymes (Kitching 1983). This paper briefly summarizes some results from the latter, the starch gel electrophoresis of enzymes.

Initially, 33 enzymes were resolved, of which only the 17 most consistently typable, representing 22 zones of activity, were used routinely. Preliminary analysis was performed using the genetic distance function of Cavalli-Sforza & Edwards (1967), followed by a principal coordinate analysis. The most striking result is that the generic groupings *Tirumala*, *Danaus (Danaus)*, *D. (Salatura)* and *D. (Anosia)* form distinct clusters. The absence of overlap is due to several enzymes within each group being represented by a band or bands restricted to that group. For example, in *D. (Salatura)* (of which *D. genutia*, *D. melanippus*, *D. philene* and *D. affinis* have been studied) the enzyme aspartate aminotransferase-1 consists of three bands present in all species, but not found elsewhere. The general result has been corroborated by further analyses using four other genetic distance functions and weighted, pair-group hierarchical cluster analyses (Kitching 1983).

Tirumala is a complex of sibling species. Unfortunately, large samples were not obtained, and five of the six samples consisted of less than ten individuals. Despite this, the species cluster together. Disagreement only occurs when the relationship of *Tirumala* with other genera is considered. According to Ackery & Vane-Wright (in press *a*), *Tirumala* is the sister-group of *Danaus*, while the enzymes appear to favour a relationship with *Parantica*. However, this is largely a result of clustering by overall similarity. If a Wagner tree is constructed, in which the allozyme bands are coded as present or absent and the hypothetical ancestor is allocated state 0 for all bands, then the pattern of relationships is similar to that given by Ackery & Vane-Wright—although the root occurs in a different position. Work is in progress, using distance Wagner techniques (Farris 1970) to investigate this discrepancy.

D. plexippus is the only danaid that has been investigated previously. Eanes & Koehn (1979) were interested in population structure, and they concluded that in the eastern USA the Monarch was panmictic when considered on an annual basis. Importantly for this study, the band frequencies they obtained, using samples of 75 or more individuals, are very similar to those that I have obtained using eastern USA samples of 40 or less. Only phosphoglucomutase showed a significant disparity—due largely to a higher frequency in one of the three

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bands. The frequency rank-order was the same. This similarity suggests that allozyme frequencies obtained for other species are reasonable estimates of the population values, despite a number of small samples.

D. plexippus from Brisbane, Australia, was found to have only a few differences from the eastern USA samples, with extra bands in four of the enzymes occurring at reasonable frequencies (>10%). If the Californian population were screened, it could shed some light on the origin of the Australian Monarch, which only reached the continent about 150 years ago (Walker 1914).

Danaus (Anosia) contains four species, of which *D. gilippus* and *D. chrysippus* could be considered conspecific (Vane-Wright 1978). Unfortunately, the principal coordinate analysis is equivocal. Kenyan *chrysippus aegyptius* is as distant from *gilippus* as it is from Australian *chrysippus petilia*, while a sample of nominotypical *chrysippus* from Thailand and a Nigerian sample of *c. aegyptius* are much closer to the Kenyan sample than the Australian. The allozyme data would thus appear to suggest that if the American *D. gilippus* is given specific status, then *D. chrysippus petilia* should be also. This contradicts Pierre (1980), who considered *D. chrysippus* to be monotypic.

Average heterozygosity ($\overline{H}$) depends on both the number of allozymes (or alleles) and their frequencies. The $\overline{H}$ values for most of the 26 danaine samples investigated fell into the range 10–25%, similar to most lepidopterans so far studied. However, there was one exception. Three populations of the *D. philene/D. affinis* complex were sampled: *D. p. ferruginea* from Papua New Guinea, *D. a. affinis* from Australia and *D. a. malayana* from Malaysia. Their $\overline{H}$-values are 15% (n= 35), 11% (n=2) and 4% (n=52) respectively.

The sample of *D. a. malayana* was collected from mangrove swamp near Kuala Selangor. Although small in area, the swamp supported a population of hundreds of adult butterflies. There does not appear to be a similar habitat in the vicinity (this requires confirmation). Twenty of the 22 enzymes were monomorphic (in other species the number is usually 10–15), and this even included phosphohexose isomerase, highly polymorphic in other danaines, including the closely related *D. genutia*. The two enzymes found to be polymorphic are NAD^+-dependent malate dehydrogenase (MDH) and an esterase (EST-A).

EST-A in *D. a. malayana* comprises two bands, with a distribution strongly suggestive of X-linkage. Females only possess one or other band, while in males, double-banded, putative heterozygotes also occur. This proved to be widespread throughout the Danainae studied (although one double-banded *Euploea tulliolus* was detected). MDH is more or less monomorphic throughout the Danainae sampled; variant bands detected occur at frequencies of less than 5%. However, in *D. a. malayana* there are two bands, the one occurring at a frequency of about 0.6 being the widespread allozyme. The other band is apparently unique.

Thus the Kuala Selangor population of *D. affinis* is probably isolated, it is reasonably large and has a very unusual enzyme profile. It may have passed through a population bottleneck (as might occur if the mangrove largely dried out at some period), or it may have (relatively recently?) been founded by one or a very few individuals. Whatever the reason, this population ought to be studied more thoroughly.

Enzymes can be seen to have potential in helping to elucidate both inter- and intra-specific relationships in butterflies. Investigation of further danaine populations could clarify some of the points raised above, but would no doubt raise an equal or even greater number of new questions.