SPOT-DISTRIBUTION IN *MANIOLA JURTINA*: TUSCAN ARCHIPELAGO, 1968-1970

V. SCALI

*Institute of Zoology and Comparative Anatomy of the University, Pisa**

Received 25.ix.71

1. Introduction

The number of spots on the underside of the hind-wing of the Meadow Brown Butterfly, *Maniola jurtina* (Satyridae) is under polygenic control with high heritability (McWhirter, 1969) and the spot-distribution of many populations has been investigated over a period of 25 years in order to obtain quantitative information about micro-evolutionary changes occurring in nature. The principal findings have been summarised by Ford (1971, chs. 4 and 5) and a brief review of the subject has been given by Scali (1971*b*) and by Robinson (1971).

One of the outstanding features that have emerged from the extensive collecting is the occurrence of orderly spot-patterns and stabilisations overriding great environmental differences. Across Southern England the spot-distribution is stable and differently adjusted in the two sexes: males are unimodal at 2 spots, while females show a sharp single mode at 0 (Southern English pattern). This spot-distribution extends over the greater part of the range of this species in the Palearctic Region (Dowdeswell and McWhirter, 1967), but different stabilisations are quite common in the peripheral areas such as Cornwall, Isles of Scilly, Iberia and North-West Africa. In most of these stabilisations males are still unimodal at 2 spots; but a great amount of variability is found within this spot-distribution owing to the varying (relative) frequencies of the rare spot-classes. A general trend towards higher spotting is thus apparent in males when one passes from the northern parts of the range (Scotland, Ireland) to the southern ones (South Iberia, Sicily, North-West Africa), with some possible exceptions in mountain colonies.

On the other hand the female spotting is adjusted to various stable distributions and can be, for instance, unimodal at 0 or at 2, bimodal at 0 and 2, or show a " flat-topped " condition with equal numbers at 0, 1 and 2 spots; the female sex seems therefore to be the more sensitive to the varying ecological situations.

Within the framework of a high spotting stability, many instances of evolutionary changes have been witnessed and sometimes it has been possible to correlate them with ecological or climatic influences (Dowdeswell and Ford, 1955; Dowdeswell *et al.*, 1957, 1960; Creed *et al.*, 1959, 1964; Scali, 1971*b*). These spotting-shifts can affect many thousands of specimens over a large area within a single generation, of which there is one per year, showing that strong selective forces are operating in adjusting the spot-frequencies.

Investigations of the life-cycle and of spotting in this species have recently been extended to central Italy (Scali, 1971*a*, *b*) and the results obtained for

* This research has been carried out with the financial help of the Consiglio Nazionale delle Ricerche (Grant Nos. 1437 and 5030).

spot-distribution on the Tuscan Mainland over the period 1967-69 can be summarised as follows: males are unimodal at 2 spots while females tend to a " flat-topped " distribution. This spotting has retained a general stability and homogeneity for 1967 and 1968 in both sexes, but in 1969 a wave of higher spotting has affected males everywhere, producing heterogeneity with the previous years' collections. Over the same period, females have shown an intra-seasonal spotting shift with June-July samples unimodal at 1 but still homogeneous with 1967-68 collections, while September captures have proved to be unimodal at 0 and strongly heterogeneous with previous collections. In the spotting-shifts just indicated (as in two more instances of localised inter-seasonal changes which in 1968 have occurred in males) the selective forces involved have been of the same magnitude of those found at work in Southern England and the Isles of Scilly, reaching the 75 per cent. level (with ascertained confidence limits) against some phenotypes.

2. Collecting and reproductive biology

Collecting has been attempted on three islands: Elba, Giglio and Giannutri (see map, fig. 1). In spite of extensive and careful searches from May to September over a period of 3 years not a single specimen has been found on Giannutri where, therefore, the species seems to be absent (see also Scali, 1971*a*). It has therefore only been possible to obtain samples from Elba and Giglio. Emergence begins in late May and lasts only about 3 weeks; copulation takes place at this time, after which the males die. Females always undergo a long aestivation and from the second half of June until the end of August it is sometimes impossible to catch even an odd specimen in flight (Scali, 1971*a*).

The existence of this aestivation and its varying duration according to the weather conditions in each place, makes the collection of large female samples rather problematical each year; indeed, on Giglio no large collections have been obtained up to now during September, when the females should have appeared again after aestivation.

To these biological characteristics which make routine collecting not easy some more difficulties of a different kind have arisen: collecting sites on Elba have been disturbed by human interference (mainly building and agriculture), but on both Giglio and Elba worse ecological damage has been caused by repeated fires, which, especially in 1970, have destroyed several collecting areas.

3. Analysis of data

A. *Island of Giglio*

Males. Male samples are very highly spotted, their spot-averages ranging from 3·18 to 3·28 over the period 1968-70 (table 1). The male spot-distribution in 1968 and 1969 has retained a mode at 3 spots with the 2 and 4 spot-classes not much rarer; the 1970 sample shows a slightly different pattern with the 4-spotted specimens just outnumbering the 3-spotted ones (fig. 2). This spot-distribution provides a striking contrast with the populations unimodal at 2 spots found in continental Tuscany (Scali, 1971*b*) and so widespread in Europe (Dowdeswell and McWhirter, 1967).

FIG. 1.—Map of Tuscany indicating mainland and insular collecting sites. The largest island is Elba; Giglio is N.W. of Giannutri. The inset shows the position of Tuscany.

Samples taken in 1969 at L'Appiata and Le Porte (see map, fig. 1) are homogeneous ($\chi^2_{(3)} = 1{\cdot}92$; $0{\cdot}7 > P > 0{\cdot}5$, spotting classes being 0-2, 3, 4 and 5) and their sum is highly homogeneous with the 1968 collection ($P > 0{\cdot}9$). The slight increase of the 4-spotted specimens in 1970 has proved non-significant and a comparison of the 1968-69 collections with that of 1970 gives a $\chi^2_{(3)} = 2{\cdot}15$, with $0{\cdot}7 > P > 0{\cdot}5$, so demonstrating a great stability of spotting in this sex for the 3 years.

Females. Table 1 shows that female spotting is adjusted to different values from that of males. Females are highly spotted, their averages ranging from 1·65 for the June 1969 collections at L'Appiata to 2·15 for the June sample taken at Le Porte in 1970. These high values are expected,

TABLE 1

Spot-distributions and spot-averages of M. jurtina *from the Island of Giglio, 1968-70.*

Locality and date	Sex	Spots 0	1	2	3	4	5	Total	Spot-average ♂♂	♀♀	Remarks
Le Porte 8, 9 June 1968	♂	0	0	27	38	26	10	101	3·18		
	♀	1	1	4	3	0	0	9		—	
Le Porte 2, 14, 15 June; 24 July 1969	♂	0	1	22	49	32	11	115	3·26		
	♀	6	2	6	13	1	0	28		2·03	
19, 27, 28 Sept. 1969	♀	1	3	5	1	0	0	10		—	
L'Appiata 14, 15 June 1969	♂	0	0	20	25	20	8	73	3·22		Destroyed by a fire in Spring 1970
	♀	1	8	4	4	0	0	17		1·65	
19 Sept. 1969	♀	1	2	2	1	0	0	6		—	
Le Porte 16, 17 June 1970	♂	0	1	22	28	30	10	91	3·28		
	♀	3	8	24	12	5	0	52		2·15	
16 Sept. 1970	♀	0	3	2	1	0	0	6		—	
Total for homogeneous male samples	♂	0	2	91	140	108	39	380	3·24		

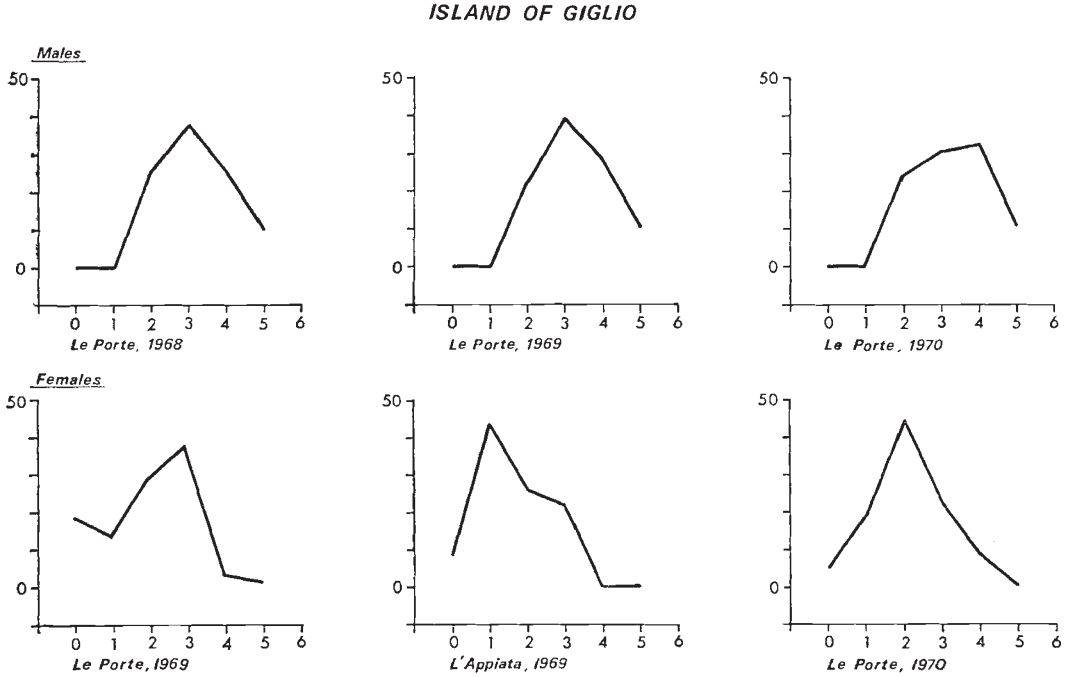

FIG. 2.—Spot-distributions of male and female *M. jurtina* from the island of Giglio in different years and colonies (percentages).

however, because there is a correlation between male and female spotting, the highest female spot-averages usually being found together with the most spotted males (McWhirter, 1957; Scali, 1971*b*).

Only in 1969 and 1970 are the samples large enough for useful comparisons; they are representative of pre-aestivation populations, the September collections having up to now yielded inexplicably poor results in spite of careful search. Large female collections, though variable, clearly suggest a unimodal spot-distribution (fig. 2): in 1969 they show unimodality at 3

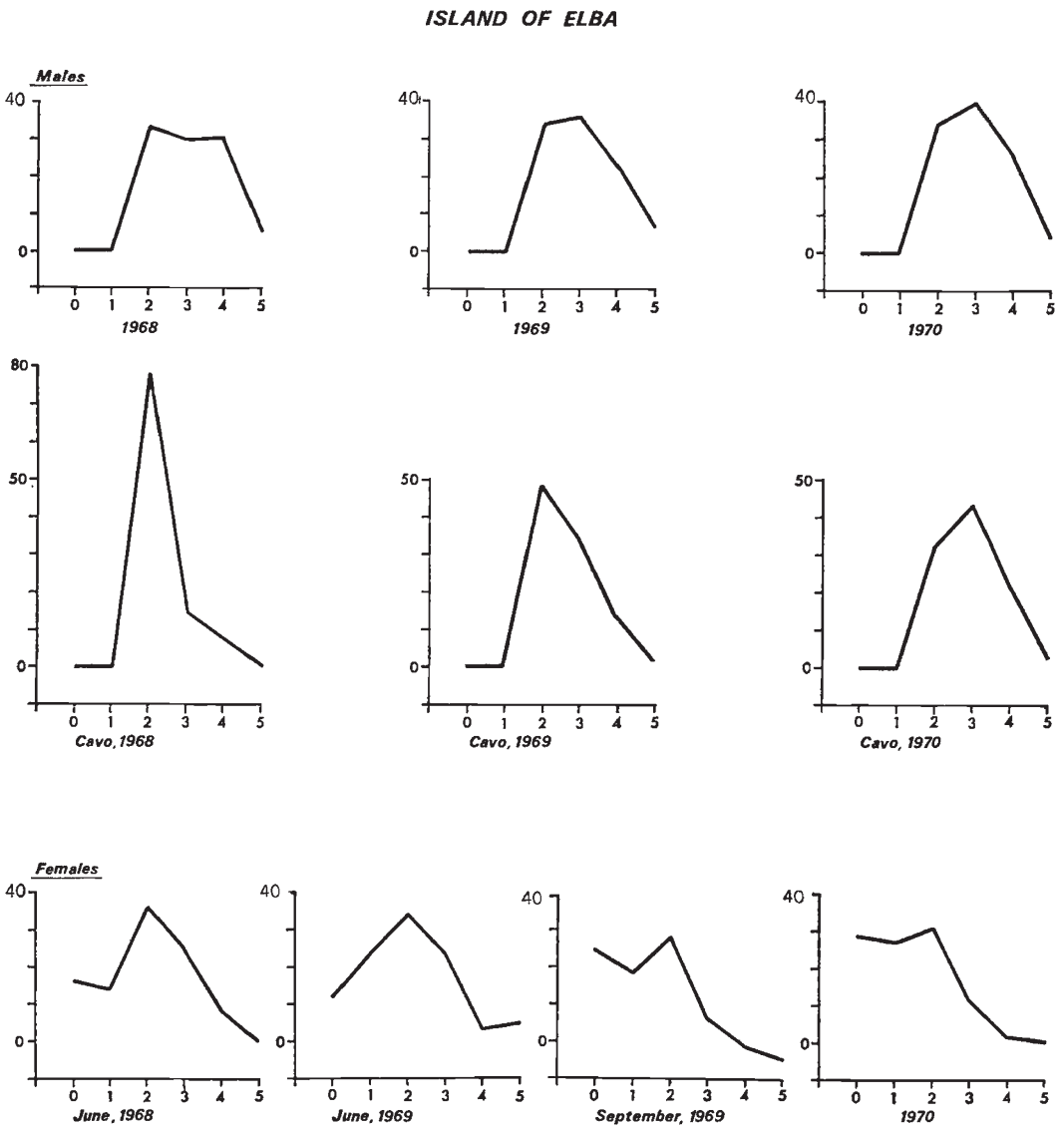

FIG. 3.—Percentage spot-distributions of male and female *M. jurtina* from the island of Elba in different years and colonies. Unless differently stated the diagrams refer to the sum of homogeneous samples for each year.

(Le Porte) and at 1 (L'Appiata), while in 1970 they are unimodal at 2 (Le Porte).

Calculations carried out with the combined samples for each year show that the 1969 and 1970 collections at Le Porte are homogeneous ($\chi^2_{(3)} = 6{\cdot}00$; $0{\cdot}2 > P > 0{\cdot}1$ spotting classes being 0, 1, 2, 3-5), while a comparison between Le Porte and L'Appiata in 1969 gives a difference bordering on significance, being $\chi^2_{(3)} = 7{\cdot}62$ and $0{\cdot}1 > P > 0{\cdot}05$, with the same spotting classes. If we perform the χ^2 test using 3 spot-classes (0-1, 2, 3-5) we find a $\chi^2_{(3)}$ value of 2·95 for which $0{\cdot}3 > P > 0{\cdot}2$.

B. *Island of Elba*

Males. On this island most samples show very high spotting (spot-averages from 2·86 to 3·15) and a characteristic distribution with approximately equal numbers at 2, 3 and 4 spots (table 2, 3, 4 and fig. 3). Where

TABLE 2

Spot-distributions and spot-averages of M. jurtina *from the Island of Elba, 1968*

Locality and date	Sex	Spots 0	1	2	3	4	5	Total	Spot-average ♂♂	♀♀
Mt. Perone 2, 16 June	♂	0	0	28	19	27	5	79	3·11	
	♀	0	2	3	4	0	0	9		—
Mola 1, 6 June	♂	0	0	9	12	8	3	32	3·15	
	♀	2	2	2	2	0	0	8		—
S. Giovanni 1, 2, 16 June	♂	0	0	27	26	24	3	80	3·04	
	♀	3	1	7	2	2	0	15		1·93
Cavo 16 June	♂	0	0	21	4	2	0	27	2·25	
	♀	1	0	1	1	1	0	4		—
Total for homogenous male samples	♂	0	0	64	57	59	11	191	3·09	
Total for females	♀	6	5	13	9	3	0	36		1·94

useful comparisons could be made, such samples have proved homogeneous within each season ($\chi^2_{(6)} = 4{\cdot}04$ with $0{\cdot}7 > P > 0{\cdot}5$ for 1968; $\chi^2_{(6)} = 7{\cdot}28$ with $0{\cdot}3 > P > 0{\cdot}2$ for 1969; $\chi^2_{(3)} = 1{\cdot}22$ with $0{\cdot}8 > P > 0{\cdot}7$ for 1970) and over the 3 years ($\chi^2_{(6)} = 10{\cdot}1$; $0{\cdot}2 > P > 0{\cdot}1$). A major exception, however, has been found at Cavo. This colony, facing the continent (see map, fig. 1), is at present completely isolated by woods from any other localities suitable for *M. jurtina* and in 1968 it had an extreme type of spot-distribution with a sharp mode at 2 spots (fig. 3), so proving strongly heterogeneous with the other Elban samples ($P < 0.001$), while homogeneous with continental collections ($\chi^2_{(1)} = 2{\cdot}05$ using Yates' correction, with $0{\cdot}2 > P > 0{\cdot}1$).

This situation was again found in 1969, the Cavo collection still being unimodal at 2, heterogeneous with the other Elban samples ($\chi^2_{(2)} = 8{\cdot}54$ with $0{\cdot}02 > P > 0{\cdot}01$) and homogeneous with the continental populations ($\chi^2_{(2)} = 0{\cdot}69$, $0{\cdot}8 > P > 0{\cdot}7$). This close similarity with the mainland collections in 1969 is due to the large increase of highly spotted specimens (3-5); this change has paralleled the wave of high spotting which affected the Continent that year (Scali, 1971*b*). The shift towards higher spotting has produced heterogeneity between the 1968 and 1969 Cavo collections: $\chi^2_{(1)} = 6{\cdot}28$ (accumulating 0 to 2 and 3 to 5 spots and using Yates' correction)

TABLE 3

Spot-distributions and spot-averages of M. jurtina *from the Island of Elba, 1969*

Locality and date	Sex	Spots 0	1	2	3	4	5	Total	Spot-average ♂♂	♀♀	Remarks
Mt. Perone	♂	0	0	33	37	13	4	87	2·86		
8 June	♀	2	0	0	1	0	0	3		—	
10, 21 Sept.	♀	17	15	24	8	3	0	67		1·47	
Mola	♂	0	0	13	11	12	4	40	3·17		
9 June	♀	1	1	5	3	1	1	12		(2·41)	
11, 21 Sept.	♀	6	5	3	3	1	0	18		1·33	
S. Giovanni	♂	0	0	1	1	0	0	2	—		The colony has been
8 June	♀	0	0	0	0	0	0	0		—	disturbed by
11 Sept.	♀	3	3	5	2	0	0	13		(1·46)	agriculture
Porto Azzurro	♂	0	0	20	24	19	5	68	3·06		The colony has been
7, 9 June	♀	4	10	9	6	0	1	30		2·03	destroyed by a fire during August 1969
Cavo	♂	0	0	53	38	16	2	109	2·70		
8, 9 June	♀	3	5	9	7	1	1	26		2·04	
9, 12, 20 Sept.	♀	18	12	16	3	1	0	50		1·14	
Total for homogeneous samples	♂	0	0	66	72	44	13	195	3·02		
June	♀	8	16	23	16	2	3	68		1·95	
September	♀	44	35	48	16	5	0	148		1·34	

which gives $0{\cdot}02 > P > 0{\cdot}01$. The trend towards higher spotting values continued in 1970 as well (table 4): males were for the first time unimodal at 3 spots (reaching a spot-average of 2·94, fig. 3) and thus fully homogeneous with the 1970 sample of Monte Perone ($\chi^2_{(3)} = 1{\cdot}22$; $0{\cdot}8 > P > 0{\cdot}7$).

The inter-seasonal change which has occurred at Cavo between 1969 and 1970 is bordering on significance ($\chi^2_{(2)} = 4{\cdot}92$; $0{\cdot}1 > P > 0{\cdot}05$) and it is

TABLE 4

Spot-distributions and spot-averages of M. jurtina *from the Island of Elba, 1970*

Locality and date	Sex	Spots 0	1	2	3	4	5	Total	Spot-average ♂♂	♀♀	Remarks
Mt. Perone	♂	0	0	38	47	32	6	123	3·05		During July the colony
9, 12, 15 June	♀	5	3	3	1	0	0	12		(1·00)	has been almost
3, 7 Sept.	♀	9	6	7	1	0	0	23		1·00	destroyed by fires
Mola											Inexplicable extreme
10, 14 June;	♂	0	0	1	6	7	0	14	(3·43)		reduction in
5 Sept.	♀	1	3	0	0	0	0	4		—	numbers
S. Giovanni	♂	0	0	1	0	0	0	1	—		During June no collec-
4, 7 Sept.	♀	13	15	18	5	2	0	53		1·40	tion was possible because of human interference
Cavo	♂	0	0	24	32	16	2	74	2·94		On the 2nd and 7th of
3, 10, 14 June	♀	3	3	3	2	0	0	11		(1·36)	Sept. no specimens
23 Sept. 1 Oct.	♀	3	4	4	4	0	0	15		1·60	were found
Totals for homogeneous samples	♂	0	0	63	85	55	8	211	3·04		
	♀	33	31	35	13	2	0	114		1·39	

of course, heavily significant if the 1968 and 1970 collections are compared. Over the 3 years now considered, the overall selection against the 2-spotted phenotypes, and in favour of the 3-5-spotted specimens, evaluated according to the method used by Woolf (1954), amounts to 86·2 per cent. with 95 per cent. confidence limits at 93·8 per cent. and 61·6 per cent.

The combined samples from places which were themselves homogeneous in 1968, 1969 and 1970, also prove to be homogeneous when compared with each other, so indicating a general spotting stability. It must be noticed, however, that in 1969 Mt. Perone has shown a remarkable reduction in 4-spotted individuals (table 3 and fig. 3). This did not occur anywhere else on the island, nor on the Continent (Scali, 1971*b*), and it evidently constitutes a response to local and unknown factor(s). This change is formally significant when a comparison is made between the 1968 and 1969 samples ($\chi^2_{(3)} = 10{\cdot}78$ with $0{\cdot}02 > P > 0{\cdot}01$); the selective elimination of 4- and 5-spotted phenotypes contrasted with the 3-spotted ones in this case amounts to 72·7 per cent., with 95 per cent. confidence limits at 87·5 per cent. and 38·8 per cent.

By 1970 the frequency of 4-spotted specimens had risen again and almost returned to its former level; the 1970 collection is actually homogeneous with both the 1969 and the 1968 samples ($\chi^2_{(3)} = 3{\cdot}89$ with $0{\cdot}3 > P > 0{\cdot}2$ for the former and $\chi^2_{(3)} = 1{\cdot}71$ with $0{\cdot}7 > P > 0{\cdot}5$ for the latter). Together with the increase of the 4-spotted specimens in the 1970 sample the frequency of the 3-spotted ones has risen, showing that two spot-classes have been involved in bringing the colony back to the former spot-distribution.

Although the Giglio and Elba populations have shown a similar spot-average in 1968, in subsequent years there has been an increase in spotting on the former and, on the whole, a trend toward lower values on the latter. Actually in 1968 the Le Porte collection and the combined homogeneous samples from Elba proved homogeneous with one another ($0{\cdot}3 > P > 0{\cdot}2$); in 1969, differences in spot-distributions between the combined samples of the two islands bordered on significance and finally they became significant in 1970 ($\chi^2_{(3)} = 8{\cdot}99$; $0{\cdot}05 > P > 0{\cdot}02$) owing mainly to opposite shifts of 4-spotted specimens.

Females. Table 2 shows that during June 1968 very few females were found in flight; in no samples, therefore, can a clear distribution pattern be made out and it is necessary to sum all these small collections to get a reasonably large total. The spot-average is rather high (1·94) and the distribution is unimodal at 2 spots (fig. 3).

In 1969 (table 3) females have been collected both during June (pre-aestivation populations) and September (post-aestivation populations). June collections are highly spotted (average 1·95) and mainly unimodal at 2; the samples are large enough for a comparison to be made (Mola, Cavo, Porto Azzurro) and they have proved to be homogeneous ($\chi^2_{(6)} = 3{\cdot}80$; $P \simeq 0{\cdot}7$).

The grand totals for June 1968 and for June 1969 are remarkably homogeneous ($\chi^2_{(4)} = 1{\cdot}56$; $0{\cdot}9 > P > 0{\cdot}8$), so demonstrating spotting stability for early collections over the 2 years.

In 1969, the September collections are also homogeneous ($\chi^2_{(9)} = 5{\cdot}65$, with $0{\cdot}8 > P > 0{\cdot}7$) but they are no longer unimodal at 2 as they were during June because they show a flat-topped distribution with the 1-spotted specimens slightly rarer than the unspotted and 2-spotted ones; their spot-average

is accordingly lower (1·34). If we compare the homogeneous June samples with those caught in September we find them significantly different ($\chi^2_{(3)} = 12{\cdot}95$ with $0{\cdot}01 > P > 0{\cdot}001$). This demonstrates that a selective elimination of high-spotted females has occurred during the imaginal aestivation; an overall estimate of selection against 3-5-spotted phenotypes compared with the unspotted ones gives a figure of 81·8 per cent. with 95 per cent. confidence limits at 93·0 per cent. and 52·2 per cent. A similar calculation can be made comparing the 0- and 1-spotted females with the 3-5-spotted ones for the single colony of Cavo; selection against the high-spotted specimens of 88·1 per cent. (with 95 per cent. confidence limits at 97·1 per cent. and 51·3 per cent.) can be assessed.

Samples of females caught in June 1970 have been very small owing, no doubt, to human interference at S. Giovanni and Cavo, and to an inexplicable reduction in numbers at Mola (see table 4). None of the samples, however, suggests the unimodal distribution at 2 spots which had been found in the June collections in both 1968 and 1969. The combined samples for June, on the contrary, indicates a " flat-topped " distribution.

September collecting has produced better results; the three colonies which can usefully be compared (table 4) show a " flat-topped " distribution for 0, 1 and 2 spots and are homogeneous ($\chi^2_{(6)} = 5{\cdot}27$ with $0{\cdot}7 > P > 0{\cdot}5$). The grand total for the September collections is homogeneous with the sum of June samples ($0{\cdot}8 > P > 0{\cdot}7$) so demonstrating that no intra-seasonal shift has occurred during aestivation this year. The general spot-average for females is 1·29 and the spot-distribution found throughout 1970 is very similar to that encountered in post-aestivation females in 1969 ($P > 0{\cdot}9$) while, of course, strongly heterogeneous with that shown by pre-aestivation samples of that year.

It is finally to be noticed that at Cavo the females have always shown the same spotting pattern, and spotting-changes, as the females of the other colonies on the island, while the males in 1968 and 1969 showed the continental spot-distribution and only in 1970 have become consistent with the insular samples.

4. Discussion

A. *The male spot-distribution*

The pattern found on the Tuscan Mainland (Scali, 1971*b*) and, with minor differences, over the greater part of the species' range suddenly breaks down on Giglio and Elba in the Tuscan Archipelago; here new and characteristic stabilisations with very high spotting are encountered. Within the framework of a basic spotting similarity, however, these two islands began to diverge in 1969 and have become heterogeneous in 1970 owing to an increase of 4-spotted specimens on the former and to a decrease of the same phenotype on the latter. The findings show that on these islands the normally extremely stable male spot-system readily responds to the local ecological needs.

Significant inter-seasonal changes have been witnessed in the males on Elba at Mt. Perone and Cavo. The first instance constitutes a case of selective elimination of high-spotted phenotypes amounting to 72·7 per cent., very similar in magnitude, though opposite in effect, to those shifts noticed in two continental colonies (Scali, 1971*b*); the same colony showed a reverse

change, though of lesser magnitude, the following year. This increase of high-spotted specimens has brought the spotting back almost to its original level: this can be taken as a further demonstration that the spot-system in each place is very stable and tends to counteract forces producing different distributions.

The spotting changes seen at Cavo tell us something more about the adjustment of spotting to new distributions. The inter-seasonal change noticed in 1969 was not related to any obvious ecological event and it can be thought of as climatic in origin because it reproduced the same features which occurred that year on the Continent (Scali, 1971*b*). There has, however, been a striking difference between Cavo and the mainland colonies in 1970. While continental populations have again shown the well-known spot-distribution unimodal at 2 (Scali, unpublished data), this insular colony has further increased its spotting average and jumped to a new spot-distribution, unimodal at 3; this is heterogeneous with the continental collections (Scali, unpublished data), and fully homogeneous with the only other large sample obtained on the island for that year (table 4). The two combined samples of Cavo and Mt. Perone are homogeneous with 1968 and 1969 Elban collections ($\chi^2_{(6)} = 10{\cdot}10$; $0{\cdot}2 > P > 0{\cdot}1$), so demonstrating that the Cavo colony has really reached the spot-distribution characteristic for this island. The prolonged increase in spotting at Cavo over the 3 years investigated has resulted in an elimination of the 2-spotted specimens, compared with the 3- to 5-spotted ones, of 86·2 per cent.

B. *Female spotting*

This needs further investigation on Giglio so that comparisons of the pre- and post-aestivation spot-distributions may be made at each colony to obtain full data on spot-stabilisations, possible intra-seasonal shifts and " endocyclic selection ". From the information now available it seems, however, quite clear that on Giglio the spotting is high and probably unimodal at 2 during June. The sample taken at Le Porte in 1969 has shown a very high proportion of 2-spotted specimens (higher than any other sample both on the islands and Mainland) so that it is likely that this mode will be retained in post-diapause populations unless a very strong intra-seasonal shift takes place. Furthermore, there are some suggestions that the colony at L'Appiata may be adjusted to different values from that at Le Porte.

Samples taken on Elba during June have a spot-distribution unimodal at 2 in both 1968 and 1969, while (although nothing can be said for 1968) September captures for 1969 have given a " flat-topped " spot-distribution at 0, 1 and 2 spots; in 1970 both June and September samples are " flat-topped " and in fact homogeneous with the 1969 September collections. This means that in 1969 a significant intra-seasonal shift toward lower spotting occurred in the females which, on the other hand, have not shown any change throughout the season following.

Two main points must be considered here:

(1) Since an intra-seasonal shift has occurred in 1969 when populations started as unimodal at 2 while no shifting has been detected in 1970 when populations showed a " flat-topped " distribution from the beginning, it seems, as far as can be judged at present, that on Elba the spot-system is adjusted in females so as to provide egg-laying populations with equal numbers at 0, 1 and 2. On both Elba and the Tuscan Mainland, spotting

shifts have always brought about a lowering of the spotting (Scali, 1971*b*); however, on the Continent this has often resulted in a population unimodal at 0, while on the island the lowest spot-distribution reached a flat-topped type with equal numbers at 0, 1 and 2. This clearly demonstrates a different level of spot-stabilisation between the Continent and Elba.

(2) In all colonies on Elba, females hatch in a short time mainly during the first half of June and undergo aestivation, after which egg-laying starts (Scali, 1971*a*).

As we have already seen, if we compare June and September samples for 1969 we find an 81·8 per cent. selection against the 3- and 5-spotted specimens compared with the spotless ones; this figure reaches the 88·1 per cent. level when 0- and 1-spotted females are contrasted with the 3- to 5-spotted ones for the single colony of Cavo. This remarkable spotting shift occurring in populations which undergo an imaginal aestivation must be due to a selective elimination of adult females with respect to their spot-phenotype; this is a clear indication of the intensity of natural selection, even within one season. The directional selection occurring at this one stage of the life-cycle is a clear indication of " endocyclic " selection; the repeated occurrence of such spotting adjustments in subsequent years will provide a good proof of reversing selection within the life span of this butterfly.

5. Summary

1. The number of spots on the underside of the hind-wings of *Maniola jurtina*, a character polygenically controlled with high heritability, has been used as an index of variation relative to the evolutionary adjustments of this species.

2. Investigations on the Tuscan Mainland show that males are unimodal at 2 (which is the spot-distribution commonly found in the general European stabilisation), while females tend to a " flat-topped " spot-frequency with about equal numbers at 0, 1 and 2 during June and July, sometimes with a reduction in spotting as the season proceeds (intra-seasonal shifts). Inter-seasonal changes have been observed in males. Selection pressures involved are very strong and could be of the same magnitude in the two sexes.

3. On two islands of the Tuscan Archipelago (Giglio and Elba) males show a much higher spotting than that found in continental Tuscany; it is clear that the European spot-stabilisation breaks down on these islands.

4. On Giglio the males had a spot-distribution unimodal at 3 in 1968-69 while in 1970 the 4-spotted specimens just outnumbered the 2- and 3-spotted ones. The samples, however, are homogeneous over the 3 years.

5. On Elba, male spotting has approached a " flat-top " type at 2, 3 and 4 in most colonies. In 1969, however, one of them (that on Mt. Perone) shows a significant lowering of the 4-spotted class, but in 1970 it has gone back to almost the former spot-distribution.

6. The Elban colony of Cavo constitutes an exception to the insular spot-pattern: the males started as unimodal at 2 spots in 1968 and, although retaining that mode, they were affected by a wave of higher spotting in 1969, showing a change similar to that experienced by mainland colonies, with which Cavo was homogeneous in both years. The 1970 collection was unimodal at 3 and fully homogeneous with insular samples. Selection against the 2-spotted specimens amounts to 86·2 per cent. over the 3 per cent. years.

7. On both Giglio and Elba, females always undergo aestivation after which they lay eggs.

8. On Giglio, pre-aestivation females have shown various unimodal spot-distributions for different years and colonies.

9. On Elba, pre-aestivation females were unimodal at 2 spots in 1968 and 1969; post-aestivation samples in 1969 have shown a " flat-topped " distribution at 0, 1 and 2, indicating a significant intra-seasonal shift with 81·8 per cent. selection against high-spotted phenotypes. In 1970 both pre- and post-aestivation populations show a " flat-top " spot-frequency that is homogeneous with the post-aestivation females of 1969.

10. In populations having a short emergence spell and undergoing aestivation, intra-seasonal shifts are brought about by selection operating on adult females. The importance of such a device for adjusting the spot-distribution of laying females relative to " endocyclic selection " is pointed out.

Acknowledgments.—I would like to thank those who have helped me with the capturing of the specimens: Dr L. Scali, Dr L. Bussotti, and Mr A. Campo. Professor E. B. Ford, F.R.S. and Dr E. R. Creed have read the manuscript and given valuable suggestions.

6. References

CREED, E. R., DOWDESWELL, W. H., FORD, E. B., AND MCWHIRTER, K. G. 1959. Evolutionary studies on *Maniola jurtina*: the English Mainland, 1956-57. *Heredity, 13,* 363-391.

CREED, E. R., FORD, E. B., AND MCWHIRTER, K. G. 1964. Evolutionary studies on *Maniola jurtina*: the Isles of Scilly, 1958-59. *Heredity, 19,* 471-488.

DOWDESWELL, W. H., AND FORD, E. B. 1955. Ecological Genetics of *Maniola jurtina* in the Isles of Scilly. *Heredity, 9,* 265-272.

DOWDESWELL, W. H., FORD, E. B., AND MCWHIRTER, K. G. 1957. Further studies on isolation in the butterfly *Maniola jurtina* L. *Heredity, 11,* 51-65.

DOWDESWELL, W. H., FORD, E. B., AND MCWHIRTER, K. G. 1960. Further studies on the evolution of *Maniola jurtina* in the Isles of Scilly. *Heredity, 14,* 333-364.

DOWDESWELL, W. H., AND MCWHIRTER, K. G. 1967. Stability of spot-distribution in *Maniola jurtina* throughout its range. *Heredity, 22,* 187-210.

FORD, E. B. 1971. *Ecological Genetics,* 3rd Ed. Chapman and Hall, London.

MCWHIRTER, K. G. 1957. A further analysis of variability in *Maniola jurtina* L. *Heredity, 11,* 359-371.

MCWHIRTER, K. G. 1969. Heritability of spot-numbers in Scillonian strains of the Meadow Brown butterfly (*Maniola jurtina*, L.). *Heredity, 24,* 314-318.

ROBINSON, R. 1971. *Lepidoptera Genetics,* 1st Ed. Pergamon Press, Oxford.

SCALI, V. 1971*a*. Imaginal diapause and gonadal maturation of *Maniola jurtina* (Lepidoptera: Satyridae) from Tuscany. *Jour. Animal Ecology, 40,* 435-440.

SCALI, V. 1971*b*. Spot-distribution in *Maniola jurtina* (Lepidoptera, Satyridae): Tuscan Mainland 1967-1969. *Italian Jour. Zool.* (in press).

WOOLF, E. 1954. On estimating the relation between blood group and disease. *Ann. Hum. Gen., 19,* 251-253.