MULTIVARIATE ANALYSIS OF VARIATION WITHIN THE *INSULARIA* COMPLEX OF THE MOTH *BISTON BETULARIA*

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SUMMARY

A multivariate analysis of the colour variation of the moth *Biston betularia* has been carried out. Previous workers have demonstrated that the black form, *carbonaria*, and the intermediate form, *insularia*, are controlled by alleles at the same locus. The results of the present analysis of the variation of the *insularia* phenotype in different populations and in 20 families show that there are at least three distinct *insularia* morphs and suggest that these are controlled by three different *insularia* alleles. There is evidence that the allele, which in the heterozygous state produces the palest *insularia* form, is incompletely dominant. For this reason an objective means of classification, considering several different variables, is required to distinguish the three *insularia* forms. The scoring method that was devised was used to classify *insularia* in samples from south Wales. In this area the darkest *insularia* morph reaches its highest frequencies in urban areas.

1. INTRODUCTION

THE colour morphs of the moth *Biston betularia* (L.) can be divided into three main categories: the black form, *carbonaria* (= *doubledayaria*); the intermediate form, *insularia* and the pale typical form. Several workers (*e.g.* Bowater, 1914) have shown that *carbonaria* is inherited as a single gene, dominant in expression to typical. Doncaster (1906) considered that the term *insularia* included several distinct forms and Kettlewell (1973) scores *insularia* into five categories. Clarke and Sheppard (1964) demonstrated that an *insularia* form was controlled by an allele at the same locus as *carbonaria* and that *carbonaria* was dominant to *insularia*, which in turn was dominant to typical. Lees (1968) showed that this was also the case for a darker *insularia* form.

These results suggest that the *insularia* complex may in fact include a range of phenotypes produced by several alleles. Evidence supporting this is found in the work of Bowater (1914), who in one family (11.20) seems to have obtained a clear segregation of a dark *insularia* and a paler *insularia* form. In addition, Lemche (1931) seems to have demonstrated allelism of two distinct *insularia* forms. However, Lemche scored all insects with completely black abdomens as *carbonaria* and so regarded the dark *insularia* form in his families as *carbonaria*, although the photographs given in his paper indicate that these were *insularia*.

There is general agreement (Kettlewell, 1973; Lees, 1974) that there are several distinct *insularia* forms but, as these phenotypes tend to intergrade with each other, some objective means of classifying the moths is required to compare the range of variation in different samples and families of moths. In this study a multivariate scoring method was used to examine the distinction between *carbonaria*, *insularia* and typical and then to examine the range of variation in the *insularia* progeny in families of known parentage.

2. THE SCORING PROCEDURE

For each moth, 13 areas of the dorsal surface of the wings and body, shown in fig. 1, were examined and scored on an eight-point scale for the
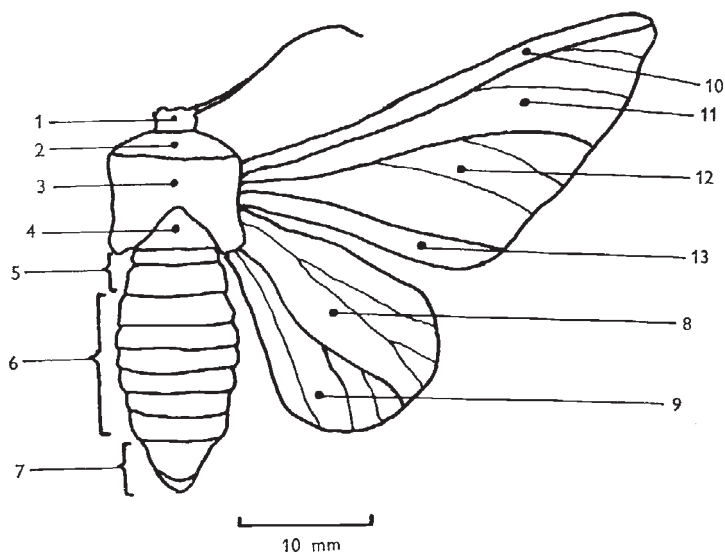

FIG. 1.—Diagram, showing the dorsal view of an imago of *B. betularia*, to indicate the 13 areas distinguished in the multivariate scoring system. The boundaries between the wing areas are the veins shown in thicker lines. Only the veins which are of importance in delineating the areas are represented.

relative amounts of black and white scales present in each area. The condition represented by the different values can be summarised as:

0 = No, or very few, black scales.
1 = Predominantly white, with small areas of black scales.
2 = White and black scales intermingled, approximately equal numbers of each.
3 = Predominantly black but a continuous " network " of white areas.
4 = Black, but with the whole area affected by small areas of white scales.
5 = Black, with a fine speckling of white scales over the whole area.
6 = Black, with white speckling affecting less than a third of the area.
7 = No white scales.

In the preliminary work the ventral surface of the moths was also scored but this was discontinued as the darkness of a given ventral area was found to be highly correlated with the darkness of the corresponding dorsal area. By scoring individual moths at intervals of several days, it was confirmed that this method of scoring was consistent for the same observer.

3. THE DISTINCTION BETWEEN *carbonaria*, *insularia* AND TYPICAL

The principal components of the 13 variables were calculated, using a computer programme in the University of California BMD series, for

carbonaria, insularia and typicals in random samples from localities in England and Wales. Plotting the individuals' scores on the first two principal components indicated that *carbonaria* and typical are clearly distinct from the range of forms labelled *insularia*. The criteria used for distinguishing these three main categories are discussed below. Only the results for the analysis of the variation within the *insularia* phenotype are presented in detail in this paper but many other analyses were carried out which confirmed the distinction between *carbonaria, insularia* and typical.

Previous workers have shown that *insularia* is inherited as a single gene. Therefore, if an *insularia*/typical heterozygote is crossed with a typical, then all the *insularia* progeny must be carrying the same *insularia* allele and so the range of variation within this family represents the effects of the genetic and external environments on the expression of the *insularia* allele. In order to investigate the number of distinct phenotypes included within *insularia*, the ranges of variation in different family groups of *insularia* were compared.

4. THE FAMILIES OF *insularia* ANALYSED

The majority of the moths were from 14 families of *B. betularia* reared in this study (table 1). All the *insularia* progeny (except for crippled moths) and at least half of the *carbonaria* and typical progeny of these families, were

TABLE 1

Origin of the parents of the families of
B. betularia *reared in this study*

Family	Male parent	Female parent
B/10/72L	*insularia* ex Wytham (Oxford)	typical ex stock
B/3/73	typical ex Beddau	*insularia* ex stock*
B/6/73	typical ex Cardiff	*insularia* ex B/10/72L
B/7/73	*carbonaria* ex Beddau	*insularia* ex B/10/72L
B/8/73	*insularia* ex stock*	*insularia* ex B/10/72L
B/13/74	*insularia* ex Beddau	typical ex B/6/73
B/14/74	*insularia* ex Beddau	*insularia* ex stock*
B/16/74	*insularia* ex Beddau	*insularia* ex stock*
B/18/74	*insularia* ex B/7/73	typical ex B/6/73
B/19/74	*insularia* ex B/7/73	typical ex B/6/73
B/21/74	*carbonaria* ex B/7/73	*insularia* ex B/7/73
B/23/74	*insularia* ex B/3/73	typical ex B/2/73
B/26/74	*insularia* ex B/6/73	*insularia* ex B/6/73
B/29/74	(light) *carbonaria* ex B/4/73	*insularia* ex B/3/73

* Indicates that the parent originated from a family reared by Dr D. R. Lees, involving a cross between a typical and a melanic parent, so that any melanic offspring are known to be heterozygous. In Dr Lees' crosses a melanic male captured at Beddau was mated with a female from laboratory stock (ex Oxford).

scored for all 13 variables on the day of emergence. The families were all reared under the same conditions except that, whereas most families were fed on sallow (*Salix* spp.) foliage collected at Cardiff, some families were fed on sallow foliage collected at a locality where air pollution levels are higher. There was no evidence that this difference in larval treatment had any effect on the colour of the adult progeny. Of the 14 families, the moths of B/10/72L were part of a large family resulting from a cross set up by Dr D. R. Lees. To avoid confusion in referring to families, those crosses set up by Dr Lees are marked " L ".

A random selection of the *insularia* from five families (B/1/67L, B/5/67L, B/8/72L, B/9/72L and B/8/73L), reared by Dr D. R. Lees, were also scored. The details of the parentage of B/1 and B/5/67L are given in Lees (1968). B/8/72L, B/9/72L and B/8/73L resulted from crosses between male *insularia* from south Wales samples and typical females from stock. The other *insularia* were from a family (B/MT/25) reared at Merthyr Tydfil. This consists of 50 *insularia* and 47 typicals with about equal numbers of males and females and is laid out in a separate storage case in the " Fleming Duplicate " Lepidoptera Collection in the Welsh National Museum at Cardiff. All the moths of this family emerged within a 3-week period in May 1925. No parents for this family could be found but the segregation indicates that the progeny resulted from a cross between an *insularia*/typical heterozygote and a typical.

5. ANALYSIS OF VARIATION IN FAMILY GROUPS OF *insularia*

(i) *Principal component analysis*

The principal components of the 13 variables were calculated for *insularia* from eight families. The scores of the individuals on the first two components are shown in fig. 2. In six of the families (B/1, B/3, B/5, B/6, B/7, B/MT) all of the *insularia* progeny within a given family must have been carrying the same *insularia* allele. The *insularia* progeny within any one of these families have similar scores on the two components. The *insularia* from B/6/73 and B/7/73, which were pale forms of *insularia*, show a similar range of scores on the two components and have large positive scores on component 1, while the dark *insularia* from B/3/73 have large negative scores on component 1. The *insularia* from B/1/67L, B/5/67L and B/MT/25 tend to cluster together and have intermediate scores on component 1 and, although their scores on this component overlap to a certain extent with *insularia* from B/3, B/6 and B/7, if the scores on component 2 are also taken into account there is good separation of the " clusters " of *insularia* families.

The clustering of family groups of *insularia* suggests that there are three distinct *insularia* alleles involved, each producing a different range in variation. Further evidence of this comes from the scores of the *insularia* progeny of B/8/72L and B/9/72L. The male parents for these two crosses were homo-zygous for *insularia*, although both individuals may have been carrying two different *insularia* alleles. It is likely, therefore, that in both families two different *insularia* alleles were segregating. For each family, fig. 2 shows that the progeny are partitioned between the two groups at either end of component 1; none occurs in the " intermediate " group.

The results of the analyses summarised in fig. 2 and many similar analyses suggest that *insularia* within a given family, in which only one allele can be segregating, show only a limited range in variation, compared with that of the whole *insularia* complex, and that families involving the same *insularia* phenotype show a similar range in variation. From these analyses, it seems that the pale phenotypes in B/8/72L, B/9/72L and the *insularia* progeny of B/6/73 and B/7/73 are all representatives of the range in one phenotype, designated P *insularia*, while the dark phenotypes of B/8/72L and B/9/72L and the *insularia* progeny of B/3/73 represent the range in expression of the darkest *insularia* phenotype, here labelled D *insularia*. A
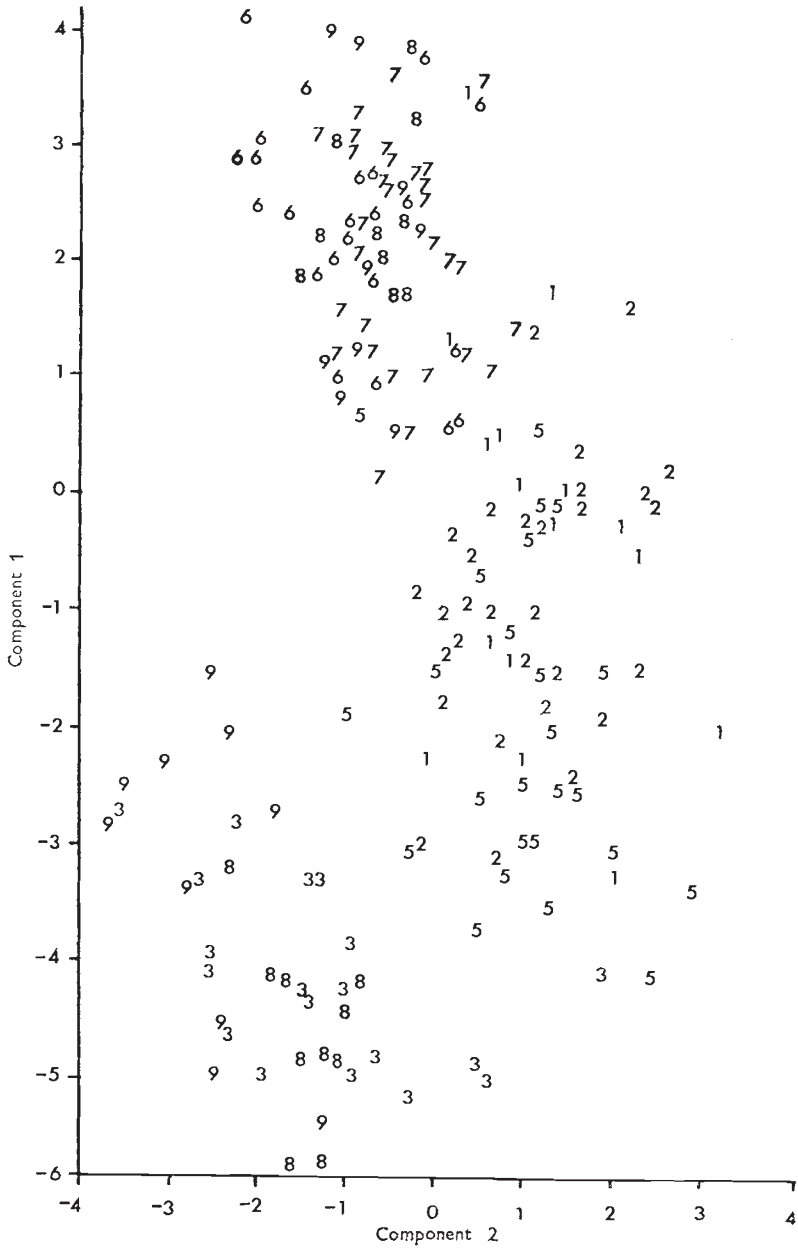
FIG. 2.—Principal component analysis of the *insularia* progeny of several families. A plot of the individuals' scores on the first two principal components is shown. On component 1, paler *insularia* have large positive scores and dark *insularia* have large negative scores. To distinguish the families: 1 = B/1/67L, 2 = B/MT/25, 3 = B/3/73, 5 = B/5/67L, 6 = B/6/73, 7 = B/7/73, 8 = B/8/72L, 9 = B/9/72L.

third phenotype, labelled M *insularia*, seems to be present in B/MT/25, B/1/67L and B/5/67L.

To examine whether a similar grouping of the phenotypes would result from a different form of analysis, a random selection of the *insularia* from the families was classified into three groups using the " Classify " Computer programme in the Genstat series. This programme finds an optimum grouping for a given number of categories. No assumptions about the relationship of the individuals are made at the start of the analysis. During the analysis, individuals are moved between groups so that the variance within each group is minimised. Table 2 indicates that the three groups formed in the analysis correspond to P *insularia*, M *insularia* and D *insularia*.

TABLE 2

Results of using Genstat classify program for classification into three groups

Family	Number of cases	Group 1	Group 2	Group 3
(Pinsul)				
B/8/72L	10	10	0	0
B/9/72L	21	20	1	0
B/6/73	24	23	1	0
B/7/73	30	30	0	0
(Minsul)				
B/MT/25	30	2	28	0
B/1/67L	17	5	12	0
B/5/67L	25	1	22	2
(Pinsul + Minsul)				
B/8/73L	52	38	14	0
(Dinsul)				
B/8/72L	12	0	0	12
B/9/72L	10	0	0	10
B/3/73	21	1	2	18

(ii) *Discriminant function analysis*

Representatives of P *insularia* from B/8/72L, B/9/72L and B/7/73, of M *insularia* from B/MT/25, and of D *insularia* from B/8/72L, B/9/72L and B/3/73 were used to calculate discriminant functions for the three *insularia* phenotypes, using the University of California Computer programme, BMD 07M. The discriminant functions (linear functions of the 13 original variables) that were calculated were used to classify the *insularia* progeny and *insularia* parents of the families (table 3).

For the 12 families in which only one *insularia* allele can have been segregating (Group 1, table 3), all the *insularia* progeny of a given family tend to be classified into one of the three categories and the scoring of the *insularia* progeny agrees with that of the *insularia* parent (where available). These results suggest that the variation in the *insularia* complex is determined by three different *insularia* alleles, each of which has a limited range in variation, which only overlaps to a small extent with the " neighbouring " phenotypes. On this basis only 75 out of the 598 *insularia* progeny of the 12 families are mis-classified in table 3. The suggestion that the dark *insularia* and pale *insularia* segregating in B/8/72L and B/9/72L are controlled by two allelomorphs inherited at the *carbonaria* locus has been confirmed by rearing investigations carried out by Dr D. R. Lees (Lees and Creed, 1977).

TABLE 3

Results of using discriminant analyses to classify insularia *families into three phenotypes*

Family	Classification of Parents		Classification of *insularia* Progeny		
	Male	Female	Pinsul	Minsul	Dinsul
1. *Families in which only one* insularia *allele should be segregating*					
B/MT/25	?	?	0	48	0
B/1/67L	Typical	*carbonaria*	4	13	0
B/5/67L	Typical	*carbonaria*	1	20	4
B/3/73	Typical	Dinsul	0	8	40
B/6/73	Typical	Pinsul	52	3	0
B/7/73	*carbonaria*	Pinsul	68	1	0
B/13/74	Pinsul	Typical	62	3	0
B/18/74	Pinsul	Typical	69	9	0
B/19/74	Pinsul	Typical	34	11	0
B/21/74	*carbonaria*	Pinsul	55	14	0
B/23/74	Dinsul	Typical	1	14	47
B/29/74	*carbonaria*	Dinsul	1	1	13
2. *Families in which both parents were heterozygous* insularia					
B/8/73L	Pinsul	Pinsul	60	31	0
B/14/74	Minsul	Pinsul	53	62	0
B/16/74	Pinsul	Pinsul	56	24	0
B/26/74	Pinsul	Pinsul	114	8	0
B/8/73	Dinsul	Minsul	18	7	37
3. *Families in which " homozygous "* insularia *was crossed with typical*					
B/8/72L	Dinsul	Typical	10	0	12
B/9/72L	Dinsul	Typical	20	1	10
B/10/72L	Minsul	Typical	53	7	0

6. ORDER OF DOMINANCE OF THE *insularia* PHENOTYPES

The segregations in the crosses between " homozygous " *insularia* and typical (Group 3, table 3) can be explained if the male parents of B/8/72L and B/9/72L carried both the P *insularia* and D *insularia* alleles, while the male parent of B/10/72L was homozygous for P *insularia*. The progeny in B/10/72L were therefore all genotypically P *insularia*/typical heterozygotes and this is consistent with the scoring of families in which an *insularia* from B/10/72L was used as one of the parents (table 1). However, the scoring of the parent of B/10/72L as M *insularia* suggests that homozygous P *insularia* may be darker than the P *insularia*/typical heterozygotes. Cadbury (Kettlewell, 1973) has suggested that homozygous *insularia* may be darker than heterozygotes. In discussion of the appearance of the " *insularia* heterozygotes " in this paper it is the *insularia*/typical heterozygote which is being referred to, while those *insularia* which were not carrying either a *carbonaria* or a typical allele are referred to as " *insularia* homozygotes ".

In this paper the three *insularia* alleles which are considered to be involved are distinguished according to the phenotype which they produce in the heterozygous state with typical. None of the families examined involved homozygotes of the M *insularia* and D *insularia* alleles.

The phenotypes of the male parents of B/8/72L and B/9/72L indicate that the D *insularia* allele is dominant in expression to the P *insularia* allele.

This is also indicated by the segregation in B/8/73 of approximately twice as many D *insularia* as P *insularia* phenotypes. The female parent of B/8/73 originated from B/10/72L and so must have been genotypically a P *insularia*/typical heterozygote, although it was one of the darkest of the progeny of B/10/72L.

Kettlewell (1973) has suggested that the order of dominance among the *insularia* phenotypes is likely to be in order of increasing darkness. This is supported by the results of a cross between a M *insularia* and a P *insularia* (B/14/74). If M *insularia* were dominant to P *insularia*, then among the *insularia* progeny of B/14 there should be a ratio of 2 M *insularia* : 1 P *insularia*. Table 3 shows that there is some indication of this ratio, with more M *insularia* than P *insularia* among the progeny of this family. In both B/8/73 and B/14/74 the overall segregation ratio of *insularia* : typical was not significantly different ($P > 0.05$) from the expected 3 : 1 ratio.

It is possible to examine the suggestion that " homozygous P *insularia* " may be phenotypically M *insularia* by considering three families which were crosses between two P *insularia*/typical heterozygotes, namely B/8/73L, B/16/74 and B/26/74. In these families, assuming that there is no differential viability of the genotypes, one-third of the *insularia* offspring should be genotypically homozygous P *insularia* and two-thirds should be P *insularia*/typical heterozygotes. Therefore, from the suggestion that the P *insularia* phenotype is the result of incomplete dominance of an allele, which in the homozygous state produces M *insularia* phenotypes, the *insularia* progeny of these three families would be expected to show a ratio of 2 P *insularia* : 1 M *insularia*. For B/16 and B/26, only 9 and 11 respectively, of the *insularia* offspring were too damaged to be scored for all 13 variables. All the other *insularia* offspring were scored on emergence. For B/8/73L, a random sample ($n = 91$) of the *insularia* progeny was scored. In B/16/74 and B/8/73L, the overall segregations of *insularia* to typical were 89 I : 17 T and 315 I : 97 T respectively. For both families, approximately one-third of the *insularia* offspring were classified as M *insularia* (B/16, 24 out of 80; B/8, 31 out of 91) while the rest were classified as P *insularia*.

In contrast, for B/26, only eight out of the 122 *insularia* which were scored, were classified as M *insularia*; all the other progeny were scored as P *insularia* (Group 2, table 3). The overall ratio of *insularia* : typical in this family was 133 I : 69 T, which is significantly different from the expected 3 : 1 ratio ($\chi_1^2 = 8.56$, $0.01 > P > 0.001$) but almost exactly a 2 : 1 ratio. This, even in the absence of any additional information, would strongly suggest that few homozygous *insularia* had survived to emerge in this family. This provides an explanation of why nearly all of the offspring were phenotypically P *insularia*. The segregation in B/26, therefore, both supports the suggestion of incomplete dominance of the P *insularia* allele and gives direct evidence of a low fitness of the *insularia* homozygotes. There is some reason to expect a deficiency of *insularia* homozygotes in B/26 as it was the only one of the *insularia* × *insularia* families which was fed on polluted foliage. However, as B/26 was not partitioned into two batches, one fed on polluted foliage and the other fed on Cardiff foliage, it is not certain that the apparent deficiency of homozygous *insularia* was due to the experimental treatment. Two other families, involving crosses between *insularia*/typical heterozygotes and typicals, were partitioned into " experimental " and " control " halves and the results showed that the different diets had not produced any

difference in the range of darkness of either the *insularia* or typical pheno-
types in these families (unpublished).

The analysis of the variation of the *insularia* phenotypes within families
indicates the existence of three distinct *insularia* alleles which in the hetero-
zygous state (with typical) produce three different phenotypes. The evidence
from families such as B/MT/25, B/1/67L and B/5/67L, where heterozygous
insularia show a range of variation limited to the M *insularia* phenotype,
demonstrates that two different alleles are likely to be involved in producing
the M *insularia* phenotype; one of these alleles is incompletely dominant and
in the heterozygous state produces the P *insularia* phenotype.

7. CLASSIFICATION OF THE *insularia* IN RANDOM SAMPLES

The conclusion about the number of distinct *insularia* phenotypes
involved comes from a relatively small number of families. However,
similar analyses of the *insularia* in random samples from over 30 localities in
England and Wales also indicate that three distinct phenotypes are involved.
Table 4 shows the result of using the discriminant functions that were

TABLE 4

Classification of the insularia *in random samples into dark, medium and pale phenotypes*

Locality	Year	Phenotype numbers			Phenotype per cent			Sample	Summer smoke
		Pinsul	Minsul	Dinsul	Pinsul	Minsul	Dinsul		
Clyne	1974	5	1	0	13·1	2·6	0	38	12
Swansea	1973-4	6	2	11	10·9	3·6	20·0	55	23
Tonna	1973	3	1	7	9·7	3·2	22·6	31	12
Neath	1974	24	9	43	15·0	5·6	26·9	160	21
Margam	1974	11	9	15	13·6	11·1	18·5	81	18
Tythegston	1974	10	4	7	19·2	7·7	13·5	52	6
Ogmore	1974	21	10	15	15·2	7·2	10·9	138	7
Ystradowen	1973	29	14	11	23·4	11·3	8·9	124	—
Ystradowen	1974	40	24	35	14·6	8·7	12·7	275	—
Ystradowen	1973-4	69	38	46	17·3	9·5	11·5	399	9
Radyr	1973	9	4	8	17·3	7·7	15·4	52	12
Cardiff	1973	9	4	7	26·5	11·8	20·6	34	26
Thornhill	1973	6	2	5	25·0	8·3	20·8	24	12
Tongwynlais	1973	11	10	15	13·4	12·2	18·3	82	26
Beddau	1972-3	64	28	56	21·6	9·5	19·0	296	13
Cefn Pennar	1973-4	11	1	8	31·4	2·9	22·9	35	40
Abercanaid	1974	15	11	11	16·5	12·1	12·1	91	14
Clydach	1974	10	5	15	18·2	9·1	27·3	55	17
Bwlch	1972	3	4	3	4·5	6·0	4·5	67	12
Sennybridge	1972	0	3	2	0	6·0	4·0	50	3
Risca	1974	12	7	10	17·9	10·4	14·9	67	21
Llanbeder	1973	6	3	11	11·8	5·9	21·6	51	17
Glascoed	1973	21	15	20	18·1	12·9	17·2	116	16
Staunton	1974	34	14	18	17·3	7·1	9·2	196	9
Much Birch	1974	8	4	4	19·5	9·8	9·8	41	9
Marlborough	1974	19	1	3	—	—	—	—	—
Wytham	1974	23	4	2	—	—	—	—	—

The samples from Bwlch, Sennybridge, Marlborough and Wytham were collected by Dr D. R. Lees,
and the sample from Beddau was collected by Dr E. R. Creed. The figures for summer smoke concentra-
tions ($\mu g/m^3$) at the sites in south Wales are shown.

calculated from the *insularia* families, to classify all of the *insularia* in 23 random samples from south Wales. In comparison, the classification of *insularia* in samples from Marlborough and Wytham in southern England is shown.

All three phenotypes are present in the majority of the samples, although the M *insularia* morph is usually the least common phenotype and shows a smaller range in frequency. The total *insularia* frequency at some sites reaches almost 60 per cent, but the frequencies of the individual *insularia* morphs never exceed 32 per cent. The *carbonaria* frequencies in the samples from Margam and Tongwynlais were 17·3 per cent and 18·3 per cent and in all the other samples from south Wales the *carbonaria* frequency was less than 14 per cent. This means that the phenotype frequencies of the *insularia* morphs will give a good indication of their actual frequency, although because of the order of dominance, the dark *insularia* phenotype will tend to obscure the frequencies of the paler *insularia* forms. Lees and Creed (1977) have found evidence that individuals homozygous for the dark *insularia* allele may be as dark as *carbonaria* but the low frequency of the D *insularia* morph (table 4) indicates that this genotype will be rare.

8. DISCUSSION

Analysis of the phenotypic variation of *B. betularia*, in families and over 30 samples, confirms the view that the morphs can be readily allocated to one of three main categories: *carbonaria*, *insularia* and typical. Twenty of the families contained *insularia* progeny and analyses of these families show that at least three distinct morphs are included within *insularia*.

The analyses, in which *carbonaria*, *insularia* and typical were included, confirmed that the qualitative criteria used to distinguish these morphs resulted in a natural distinction between the morphs. The distinction between *carbonaria* and dark *insularia* follows that made by Kettlewell (1973). In *carbonaria*, the fore-wings are either completely black or if white scales are present these are arranged to form a thin broken band across the fore-wing, near the outer margin. Dark and light *carbonaria* occurred together in samples and in all the families of *carbonaria* examined; male moths tended to show the white scaling more often than females. The normal range in variation of the *carbonaria* phenotype is well illustrated in Kettlewell (1973, plate 7.1); the left-hand column shows examples of " dark " *carbonaria*, while the right-hand column, labelled " ancient heterozygotes ", shows the range of variation in light *carbonaria*. Examination of samples from southern England and Wales (including London and Birmingham) indicates that the light *carbonaria* phenotype is widespread.

In contrast to *carbonaria*, dark *insularia* always have some white scales on the fore-wing and these are never grouped to form a transverse band. As in *carbonaria*, females tend to be darker and in extreme examples there are fewer white scales present on the fore-wings than there are on some light *carbonaria*. Another important characteristic is, that while the dorsal surface of the thorax and abdomen of *carbonaria* is almost always completely black, dark *insularia* usually have a small white spot on areas 2 and 5 (fig. 1).

The criteria used to distinguish between the palest *insularia* forms and typical differ somewhat from those of Kettlewell (1973) but are supported

by the results of the analyses. A " natural " distinction between *insularia* and typical seems to result if the term *insularia* is restricted to those individuals in which the abdomens are black with variable amounts of white patterning and in which the fore-wings have at least as many black as white scales. There is considerable variation in the darkness of the individuals which are consequently classified as typical but only rarely do these forms have both dark wings and dark abdomens. In the typical phenotype, darkening of the fore-wings is due to a spreading of the normal black patterning. This gives the " dark typical " individuals a characteristic blotched appearance, very different from that of the palest *insularia* forms, in which black and white scales are much more uniformly distributed, so that the fore-wings have a pale greyish appearance.

It is likely that there may be several distinct forms included within the typical category and Kettlewell (1973, plate 9.1) distinguishes darker forms as " *insularia* 1 " and " *insularia* 2 ". However, close examination of the typical progeny in families suggested that, although the typical offspring within different families showed different ranges of variation in the darkness of the wings and body, there was no evidence of segregation into distinct phenotypes among the typical offspring within a single family. If subsequent rearing investigations showed that there was a dark phenotype within the typical category, which was inherited as a multiple allelomorph of *carbonaria*, this form could be regarded as a fourth *insularia* phenotype. The present evidence, however, suggests that the different typical phenotypes may be under polygenic control and affected by environmental influences. The variation in the typical phenotype is likely to be of considerable interest and recording the variation in the typical phenotype in different samples, by distinguishing between the paler and darker " typical " morphs, may prove to be of great value.

In contrast to the evidence that qualitative characters give an efficient separation of *carbonaria*, *insularia* and typical, the examination of the variation within *insularia* suggests that a more quantitative scoring method is required to classify these individuals. This seems to be because of the wide range in the variation of the P *insularia* phenotype. The results of canonical analyses, in which the *insularia* progeny of different families, in each of which only one *insularia* allele could be segregating, were entered as separate groups, indicated that in different families the P *insularia* morph showed the same range in variation and had very similar mean values for the variables. Examination of the range in the *insularia* phenotype by eye suggests that the variation in the P *insularia* morph accounts for more than half of the variation in darkness of *insularia*. Thus, although the results of classifying *insularia* " by eye " into three categories agree closely with the phenotypes " *insularia* 5, 4 and 3 " distinguished by Kettlewell (1973, plate 9.1), " *insularia* 3 and 4 " both come within the range of P *insularia*. D *insularia* corresponds with *insularia* 5 but M *insularia* would come somewhere between categories 4 and 5. Similarly, of the three forms shown by Lees and Creed (1975) to represent the range in variation of *insularia*, the two paler forms are P *insularia*, while the darkest form would probably be scored as M *insularia*. Douwes *et al.* (1973) use a similar scoring method to that of Kettlewell (1973).

Examination of *insularia* individuals classified by the computer programme into the three morphs suggests that:

P *insularia* are those *insularia* in which the fore-wing areas 10 and 13

(fig. 1) have white markings larger than 1 mm in diameter, while areas 11 and 12 have a continuous network of white scales.

M *insularia* are specimens in which areas 10 and 13 are black with a few scattered white spots (less than 1 mm in diameter) and areas 11 and 12 are predominantly black with numerous white spots which tend, however, to remain distinct and not to merge as in P *insularia*. The thorax, abdomen and hind-wings are also darker for M *insularia* than for P *insularia*.

D *insularia* superficially resemble M *insularia* in general darkness of the body and wings but, whereas in M *insularia* the white spotting on the fore-wings causes the central part of areas 11 and 12 to be markedly paler than the rest of the wing, in D *insularia* the fore-wings are uniformly dark, with a light and more or less even scattering of white specks, each less than 1 mm in diameter.

The evidence from this study, together with the results of previous work by Lemche (1931), Clarke and Sheppard (1964) and Lees (1968), suggests that there are at least three distinct *insularia* morphs, each of which is produced in the heterozygous state by a multiple allelomorph of *carbonaria*. Lees (1968) has demonstrated that the M *insularia* of B/1/67L and B/5/67L are allelic to *carbonaria*, while Clarke and Sheppard (1964) demonstrated this for a paler *insularia* form (P *insularia* ?). The evidence obtained by Lees and Creed (1977) of allelism between P *insularia*, D *insularia* and *carbonaria* indicates that all three *insularia* morphs are inherited as multiple alleles. The segregations of *carbonaria*, *insularia* and typical in crosses between P *insularia* and *carbonaria* (B/7, B/21), and between D *insularia* and *carbonaria* (B/29), were consistent with this suggestion.

The evidence of incomplete dominance of an *insularia* allele seems to be of particular importance, as the great majority of melanic forms which have been studied have been found to be controlled by single genes, completely dominant in expression (Lees, 1974). There is the possibility that for an established laboratory stock of *insularia* it may be possible to distinguish between heterozygotes and homozygotes with a reasonable degree of accuracy, using a multivariate scoring system. This may prove to be of considerable advantage in genetic investigations.

The parents of the families reared in this study originated from Oxford, Cardiff or Beddau (16 km north-west of Cardiff). However, the incomplete dominance of the " P *insularia* " allele cannot be explained as being simply a result of crossing individuals from different populations, as it was observed in B/16/74, in which both parents were of south Wales origin, and in B/10/72L, where both parents originated from Oxford.

If the phenotype frequencies of the *insularia* morphs in the 23 samples from south Wales are compared with measurements of summer smoke levels ($\mu g/m^3$) at the nearest available recording site (Anon., 1965-73) there is evidence that both the dark and the pale *insularia* morphs reach their highest frequencies in urban areas. The correlation coefficient of D *insularia* frequency with square root summer smoke concentration is 0·615 (0·01 > P > 0·001), while for P *insularia* frequency the correlation with square root summer smoke is 0·461 (0·05 > P > 0·02). In contrast the correlation of M *insularia* frequency with square root summer smoke is only −0·046. Estimates of the relative crypsis of the *insularia* morphs and the *carbonaria* and typical forms, using a similar method to that of Lees and Creed (1975) indicate that the crypsis of the D *insularia* morph is greatest in polluted areas,

while that of P *insularia* is greatest in rural areas. Therefore, the variation in D *insularia* frequency in south Wales might be partially determined by selective predation but the positive relationship between P *insularia* frequency and pollution is the opposite to that which would be expected if selective predation were responsible for the variation in the frequency of this morph.

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