THE GENETICS OF THE *INSULARIA* FORMS OF THE PEPPERED MOTH, *BISTON BETULARIA*

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SUMMARY

Insularia is a complex of forms in *B. betularia* ranging continuously between the paler, typical form and the black *carbonaria*. It appears that at least three alleles at the same locus as *carbonaria* control the *insularia* forms. *Carbonaria* is dominant to all *insularia* and to typical. The *insularia* forms show incomplete dominance; individuals carrying two *insularia* alleles are usually darker than corresponding *insularia*/typical heterozygotes.

1. INTRODUCTION

DESPITE the attention paid to industrial melanism generally, and to *Biston betularia* in particular, the range of forms known as *insularia* in this species has been little studied. In appearance *insularia* is intermediate between the pale, typical form and the extreme melanic form, *carbonaria*. It reaches frequencies as high as 50 per cent in parts of South Wales (Lees and Creed, 1975), and Holland (B. Lempke *in litt.* and in Kettlewell, 1973): even higher frequencies are found in parts of Scandinavia (Douwes *et al.*, 1973).

Previous work on the genetics of *insularia* has shown that both a form from the middle of the range from Oxford (Clarke and Sheppard, 1964) and a slightly darker, though not extreme, form from Herefordshire (Lees, 1968) are controlled by alleles at the *carbonaria* locus with *carbonaria* dominant to *insularia* which is, in turn, dominant to the typical form. Earlier, Lemche (1931), working with Danish material, had shown that the most probable interpretation of segregations in families reared by him was that a light form of *insularia* was allelic with *doubledayaria* (= *carbonaria*). Close examination of his plate VI, however, reveals that what he scored as *doubledayaria* were in fact very dark *insularia*. This inadvertent discovery is the only previous work into the genetic relationship of different *insularia* forms. The investigation reported here deals, in the main, with the genetics of *insularia* at the light and the dark extremes of what Kettlewell (1973) has termed the "*insularia* complex", both in relation to each other and to *carbonaria*, although the middle of the range of forms is also discussed.

2. SCORING

The "*insularia* complex" includes individuals almost as pale as typical at one extreme and as dark as *carbonaria* at the other (see plate I). Among lighter insects the presence of a predominantly black abdomen has been found to be characteristic of *insularia* whereas typical, with few exceptions, have a distinctly white abdomen with some black markings. The patterning of the wings in light *insularia* presents a more even speckling of black and

white scales than in typical, where the scales are so arranged as to present the appearance of black lines and patches on a white background. This scoring conflicts with Kettlewell (1973), whose two palest *insularia* categories would be scored by us, and also by Steward (1977) as typical; in Douwes *et al.* (1973) we would regard their fig. 1C to be typical and 1D to be I¹.

At the other extreme, dark *insularia* have been distinguished from *carbonaria* by the presence of a fine speckling of white scales on an otherwise black forewing. Those moths with a distinct line of white scales running transversely across both fore- and hind-wings, but lacking a general speckling of white scales, have been scored as *carbonaria*, as they have in Steward's (1977) analysis. This form, which is phenotypically identical with Kettlewell's (1958) "ancient heterozygotes", occurs, especially among males, in some families involving *carbonaria* and typical where *insularia* is not present.

Within South Wales populations, it is possible to classify the *insularia* phenotype "by eye" into three, rather arbitrary, categories, each representing an approximately equal proportion of the total variation. In increasing order of darkness these categories are referred to here as I¹, I² and I³; we are mainly concerned with the genetic relationship of I¹ and I³. In families involving segregation of I¹ and I³, no difficulty has been encountered in attributing any insect to either of the categories I¹ or I³ or to T and C. There is, however, some difficulty in distinguishing I² from I¹ and I² from I³ when the moths are scored subjectively. However, Steward (1977), using a more objective Discriminant Function Analysis confirms that there is justification for separating *insularia* into three categories, although his P, M and D *insularia* categories do not closely correspond with I¹, I² and I³ here. As Steward emphasises, his PI category contains over half of the total variation in *insularia*; PI therefore contains some of I² as well as all of I¹. MI would then correspond with the remainder of I² and some I³ while DI and the rest of I³ appear to be more or less equivalent (see fig.).

FIG.—Diagrammatic representation of the range of variation in *Biston betularia*, indicating a comparison of three scoring methods: (i) Kettlewell (1973); (ii) this study; (iii) Steward (1977). The variable should not be taken as darkness, since some typicals may be considerably darker than pale I¹.

3. MATERIALS AND METHODS

Apart from female parents of the 1972 families, which were from Oxford stock, all of the material used to investigate I³ and I¹ is of South Wales origin. In thus restricting the origin of parents it was hoped to avoid any scoring complications due to breakdown of dominance in an alien gene complex, such as that shown with *carbonaria* by Kettlewell (1965). The

Plate I

Biston betularia from families used in this work. Top left: *insularia*, 2 male I^s and 2 male I¹ ex B/8/72. Top right: *insularia*, 2 female I^s and 2 female I¹ ex B/9/72. Middle: *insularia*, 3 male I¹, a male I^s and a male I^s ex B/8/73. Bottom: left, female typical ex B/9/73; right, female *carbonaria* ex B/1/75. (All life size.)

material used to examine the I^1/I^2 relationship is more varied, involving crosses of Oxford $\times$ Oxford and Oxford $\times$ South Wales material.

The majority of the families were reared in the laboratory in cages constructed of wood and nylon gauze using willow (*Salix* spp.) as the larval food plant. Care was taken to prevent larval mortality from disease or starvation which, if selective, would affect the segregation ratios. The few remaining families were reared, with similar precautions, out of doors in polyester gauze sleeves over willow bushes.

4. RESULTS

(i) Genetic relationship of I^3 and I^1

Table 1 contains details of the parentage and progeny of the crosses used to establish the relationship between I^3 and I^1 .

TABLE 1

Families of Biston betularia used to examine the relationship of I^3 and I^1

Family	Parents		Progeny					Total
	Male	Female	T	1	2	3	C	
B/1/72	T <i>ex</i> Cardiff	C <i>ex</i> B/3/71	134				123	257
B/8/72	I^3 <i>ex</i> Beddau	T <i>ex</i> Oxford		21		27		48
B/9/72	I^3 <i>ex</i> Beddau	T <i>ex</i> Oxford		34		25		59
B/1/73	I^3 <i>ex</i> B/9/72	T <i>ex</i> B/1/72	59			48		107
B/2/73	I^3 <i>ex</i> B/8/72	T <i>ex</i> B/1/72	92			72		164
B/4/73	I^3 <i>ex</i> B/8/72	T <i>ex</i> B/1/72	70			62		132
B/7/73	I^1 <i>ex</i> Beddau	I^3 <i>ex</i> B/8/72	66	55		135		256
B/9/74	I^1 <i>ex</i> Beddau	T <i>ex</i> B/4/73	88	56	25			169

Both B/8/72 and B/9/72 are families whose male parents were phenotypically I^3 and which came from Beddau, which lies about 16 km NW of Cardiff. Both were crossed to typical females from Oxford stock and among the progeny of both families a 1 : 1 segregation of I^3 : I^1 resulted.

This segregation is explicable in either of two simple ways: either (a) I^3 and I^1 are controlled by alleles at the same locus with I^3 dominant to I^1 , or (b) I^3 and I^1 are controlled at separate loci with I^3 epistatic to I^1 . In this case the males must have been homozygous at the I^1 locus; if they were not, typical would appear among the progeny.

To test which of these two alternatives applies, I^3 males from B/8/72 and B/9/72 were crossed to typical females (B/1, 2 and 4/73). In all three crosses, I^3 and typical segregate in approximate equality, which indicates that I^3 and I^1 must be allelomorphs; the only segregation possible if the genes controlling I^3 and I^1 were not alleles is 2 (I^3) : 1 (I^1) : 1 (T).

Confirmation of the allelomorph relationship of I^3 and I^1 is provided by the overall 2 (I^3) : 1 (I^1) : 1 (T) segregation in B/7/73 whose parents were I^1 male $\times$ I^3 female. This in itself, however, is not conclusive in the absence of the information provided by B/1, 2 and 4/73.

A further, more complicated and less likely, explanation of the segregation of I^3 and I^1 in B/8/72 and B/9/72 is that I^3 and I^1 are the result of a modifying locus affecting *insularia* expression and converting I^3 to I^1 and vice versa. In this context family B/9/74 is crucial since its parents were a

wild male I^1 and a typical female from B/4/73, all of the *insularia* in which were I^3 . Thus the progeny of B/4/73 should on this hypothesis carry the modifier for I^3 and, as a consequence, the *insularia* in B/9/74 should contain I^3 . The absence of this morph from the family suggests that I^3 and I^1 are indeed allelomorphic and not due to the influence of a modifier acting on the locus controlling *insularia*.

(ii) *Genetic relationship of I^3 and carbonaria*

Table 2 contains details of the crosses relevant to this section. In order to establish whether I^3 and I^1 are controlled by alleles at the *carbonaria* locus, two crosses were made (B/6/74 and B/10/74) of female I^3 from B/4/73 (table 1) to *carbonaria* males from backcross (C : T) families. The male parents were therefore CT heterozygotes.

TABLE 2

Families of *Biston betularia* used to examine the relationship of I^3 and *carbonaria*. * I^3I^3 homozygotes, see text

Family	Parents		Progeny					Total
	Male	Female	T	1	2	3	C	
B/4/72	C ex Beddau	T ex Oxford	10				13	23
B/9/73	T ex B/1/72	C ex B/4/72	103				98	201
B/10/73	T ex B/10/72	C ex B/5/72	125				122	247
B/5/74	I^3 ex B/2/73	I^3 ex B/4/73	66			149	77*	292
B/6/74	C ex B/9/73	I^3 ex B/4/73	14			20	31	65
B/10/74	C ex B/10/73	I^3 ex B/4/73	61			73	127	261
B/6/75	C ex B/6/74	T ex B/9/74				47	62	109
B/7/75	C ex B/10/74	T ex B/1/74			1	83	62	146
B/9/75	C ex B/10/74	T ex B/2/74			3	64	57	124
B/10/75	T ex Beddau	C ex B/6/74				60	87	147
B/11/75	T ex Beddau	C ex B/10/74	53				60	113
B/12/75	C ex B/6/74	T ex B/11/74				52	51	103

The progeny in both B/6/74 and B/10/74 segregated in the ratio 2C:1 I^3 :1T. This is possible whether I^3 and *carbonaria* are controlled by alleles or not. Therefore six crosses (B/6, 7, 9, 10, 11, 12/75) were established to test this; in each cross *carbonaria* from B/6/74 or B/10/74 as mated with typical. If *carbonaria* and I^3 are controlled by alleles at the same locus, two alternative segregations are possible:

C : T in equality,

C : I^3 in equality.

Similarly, if they are controlled by separate loci, with *carbonaria* epistatic to I^3 , two segregations are possible:

C : T in equality

C : I^3 : T in the ratio 2 : 1 : 1

Thus either a 1C : 1 I^3 or a 2C : 1 I^3 : 1T segregation would be diagnostic. From table 2 it is clear that both 1C : 1 I^3 and 1C : 1T segregations occur among the six 1975 families; in none of them do three phenotypes occur. The C : I^3 segregation is possible only if I^3 and C are allelomorphic, with *carbonaria* dominant to I^3 .

The relationship of I^3 to C is made more complicated by the segregation obtained in the progeny of B/5/74 which resulted from crossing male and female I^3T heterozygote parents. It will be seen from table 2 that slightly more than a quarter of the offspring were scored as C and half as I^3 . The segregation in both sexes is close to 1 : 2 : 1 (for both sexes combined, $\chi^2(2) = 0.952$; $0.8 > P > 0.7$). It is certain from the provenance of the family that the "*carbonaria*" in B/5/74 are genetically I^3 and from the segregation it seems likely that they represent the homozygous (I^3I^3) genotype.

(iii) Genetic relationship of I^1 and I^2

In contrast with the segregation in families containing I^1 and I^3 , those containing I^1 and I^2 (table 3) show a much less clear segregation when

TABLE 3

Families of *Biston betularia* used to examine the relationship of I^1 and I^2 . The progeny of B/10/73 fell within the range of I^1 and I^2 but were not categorised within it

Family	Parents		Progeny					Total
	Male	Female	T	1	2	3	C	
(a) B/9/74	I^1 ex Beddau	T ex B/4/73	88	56	29			173
B/10/74	I^1 ex Oxford	T ex Oxford	96	68	26			190
B/13/75	T ex Beddau	I^2 ex B/8/74	76	10	58			144
(b) B/13/73	I^1 ex Beddau	I^1 ex B/10/72	3	12	4			19
B/8/73	I^1 ex Beddau	I^2 ex B/10/72	97	125	152	38		412
B/11/73	I^1 ex Beddau	I^2 ex B/10/72	17	16	27	14		74
B/12/73	I^1 ex Beddau	I^2 ex B/10/72	4	6	6			16
B/11/74	I^2 ex Beddau	I^1 ex B/7/73	58	38	111	44		251
(c) B/10/72	I^2/I^3 ex Oxford	T ex Oxford		514				514
B/8/74	I^2 ex Beddau	T ex B/4/73		39	217	5		261

scored subjectively, as they have been here. Although the segregation of *insularia* to typical in these families is in accordance with Mendelian ratios, the segregation within the *insularia* category is not; this could in part be due to the mixed origin of most of the families (Oxford and South Wales). Nevertheless, some points do emerge which have a bearing on the genetics of moths falling within the range of variation encompassed by I^1 and I^2 .

The 10 families in table 3 have been arranged into three groups:

- (a) I^1 or $I^2 \times T$ where T appear in the progeny.
- (b) I^1 or $I^2 \times I^2$ where T appear in the progeny.
- (c) $I^2 \times T$ where no T appear in the progeny.

In both (a) and (b) the presence of T among the offspring indicates that all the *insularia* parents were *insularia*/typical heterozygotes, assuming that *insularia* is either dominant or epistatic to typical. On the same basis in (c) the male parents were *insularia* "homozygotes" in the sense that they had two, not necessarily identical, *insularia* alleles.

In the two families in which the *insularia* parent was I^1 (B/9/74 and B/10/74), there are more I^1 than I^2 among the offspring; the reverse is true in B/13/75, in which the *insularia* parent was I^2 . The difference in the relative proportion of I^1 and I^2 between the two types of family is highly significant ($\chi^2(1) = 59.12$).

In (b), those families where both parents were I^1T or I^2T heterozygotes, two families (B/13/73 and B/12/73) contain only a small number of offspring. However, in three families the number of offspring is large and insects scored as I^1 , I^2 and I^3 occur. The parentage of all three is similar, being $I^1 \times I^2$; no I^3 allele is present in any of them. Just as I^3I^3 homozygotes may be darker than I^3T heterozygotes (see above), it is tempting to suggest that some I^1I^2 individuals are being scored as I^3 ; that is to say, dominance within the *insularia* complex is not complete. However, if I^1 and I^2 were controlled by a pair of alleles then the expected segregation of genotypes in these families would be $1(TT): 1(I^1T): 1(I^2T): 1(I^1I^2)$. It is clear from table 3 that the offspring of B/8/73 ($\chi^2_{(3)} = 69.38$) and B/11/74 ($\chi^2_{(3)} = 52.83$) depart significantly from such a ratio though the offspring of B/11/73 do not ($\chi^2_{(3)} = 5.46$; $0.2 > P > 0.1$). This suggests that if distinct I^1 and I^2 alleles exist, then our scoring of *insularia* into the three categories does not correspond with the boundaries between genotypes.

Evidence supporting the above interpretation that *insularia* alleles may have an additive effect comes from one of the third group (c) in table 3, (B/10/72), the male parent of which was scored as being $I^{2/3}$ (i.e. at the light extreme of I^3 , or dark extreme of I^2). The large number of progeny were all *insularia* and distinctly lighter than the male parent. However, in the similar cross, B/8/74, the male parent is not darker than the progeny although it must have been carrying two *insularia* alleles.

5. DISCUSSION

From this study it is clear that insects at the light (I^1) and dark (I^3) extremes of the *insularia* complex are controlled by alleles at the *carbonaria* locus. Re-examination of material kept from a previous investigation by one of us (Lees, 1968) reveals that the *insularia* in families B/1 and 5/67 of that study would here be scored as coming from neither extreme of the *insularia* range (I^2). This is confirmed by Steward's (1977) scoring of the 1968 material; it falls within his M *insularia* category. It is probable that Clarke and Sheppard's (1964) material also represents this middle *insularia* class. If it does, then there is good evidence for three *insularia* alleles, all at the same locus as *carbonaria*.

Despite this evidence of three *insularia* alleles, the phenotypic gradation from typical to *carbonaria* appears subjectively to be more or less smooth. Although *carbonaria* shows complete dominance over both *insularia* and typical, dominance within the *insularia* complex is not complete and phenotypic boundaries are blurred. Kettlewell (1973) mentions that the typical form varies in its darkness, and that this variation is polygenically controlled. If some, or all, of the genetic and environmental factors affecting the darkness of typical also affect the expression of the *insularia* alleles, then this would have the effect of further blurring the distinction between separate *insularia* phenotypes.

Although there is considerable variation in the expression of typical between families used in this study, variation of typical within families is relatively small. There is no evidence of segregation of different typical phenotypes in any family; we therefore suggest that Kettlewell's (1973) I^1 and I^2 categories represent the genetic or environmental darkening of the

TT genotype, rather than the effect of different alleles at the *carbonaria* locus.

Both this study, using a subjective scoring technique, and Steward's (1977) more objective analysis (which uses some of the same material), are in agreement that at the pale end of the *insularia* range there is a tendency for homozygotes to be darker than the corresponding *insularia*/typical heterozygotes. These findings corroborate Dr C. J. Cadbury's suggestion (*in litt.* and reported in Kettlewell, 1973, p. 140).

At the dark end of the *insularia* range, I^3I^3 homozygotes would seem to be darker than I^3T heterozygotes and to be similar phenotypically to *carbonaria*. Although this is based on only one family (B/5/74) in this study, and needs replicating, it does lend confirmation to an indication in Bowater's (1914) stock. His family 10.2 had a wild male parent which was scored as *doubledayaria* (= *carbonaria*). On crossing with a typical female, all the resulting progeny were dark *insularia*. This indicates that the male was homozygous for *insularia* and certainly darker than his offspring. However, on intercrossing two of the *insularia* progeny of 10.2, Bowater reports that their offspring were all *insularia* and no mention is made of segregation within this class, into paler and darker individuals.

If I^3I^3 homozygotes are found consistently to be phenotypically *carbonaria*, this would complicate the estimation of *carbonaria* gene frequencies in wild samples of the species, especially where *insularia* forms are common and *carbonaria* relatively rare, e.g. South Wales (Lees and Creed, 1975) and Scandinavia (Douwes *et al.*, 1973).

The additive effect of *insularia* alleles is therefore supported by a variety of evidence, and this, coupled with the modifying effects of other loci increases the difficulty of further genetic analysis. However, it may be that, in future, scoring of all progeny for several "key" characters drawn from those used by Steward (1977) would increase the precision of this type of analysis.

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6. REFERENCES

- BOWATER, W. 1914. Heredity of melanism in Lepidoptera. *J. Genet.*, 3, 299-315.
- CLARKE, C. A., AND SHEPPARD, P. M. 1964. Genetic control of the melanic form *insularia* of the moth *Biston betularia* (L.). *Nature*, 202, 215-216.
- DOUWES, P., PETERSEN, B., AND VESTERGREN, A. 1973. Melanism hos *Biston betularius* L. i. Danmark och södra Sverige (Lepidoptera). *Entomologen*, 2, 15-20.
- KETTLEWELL, H. B. D. 1958. A survey of the frequencies of *Biston betularia* (L.) (Lcp.) and its melanic forms in Great Britain. *Heredity*, 12, 51-72.
- KETTLEWELL, H. B. D. 1965. Insect survival and selection for pattern. *Science*, 148, 1290-1296.
- KETTLEWELL, H. B. D. 1973. *The Evolution of Melanism*. Clarendon Press. Oxford.
- LEES, D. R. 1968. Genetic control of the melanic form *insularia* of the Peppered Moth *Biston betularia* (L.). *Nature*, 220, 1249-1250.
- LEES, D. R., AND CREED, E. R. 1975. Industrial melanism in *Biston betularia*: the rôle of selective predation. *J. Anim. Ecol.* 44, 67-83.
- LEMICHE, H. 1931. *Amphidasis betularia* (L.) and its melanic varieties. *J. Genet.*, 24, 235-241.
- STEWART, R. C. 1977. Multivariate analysis of variation within the *insularia* "complex" of the melanic moth *Biston betularia*. *Heredity*, 39, 97-109.