

Allelic Melanism in American and British Peppered Moths

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Abstract

Parallel evolutionary changes in the incidence of melanism are well documented in widely geographically separated subspecies of the peppered moth (*Biston betularia*). The British melanic phenotype (f. *carbonaria*) and the American melanic phenotype (f. *swettaria*) are indistinguishable in appearance, and previous genetic analysis has established that both are inherited as autosomal dominants. This report demonstrates through hybridizations of the subspecies and Mendelian testcrosses of melanic progeny that *carbonaria* and *swettaria* are phenotypes produced by alleles (isoalleles) at a single locus. The possibility of close linkage at two loci remains, but the simpler one-locus model cannot be rejected in the absence of contrary evidence.

Industrial melanism in peppered moths has been a worldwide textbook example of natural selection [for reviews see Grant (1999), Grant and Clarke (2000), and Majerus (1998)]. The melanic phenotype, unknown in Britain prior to 1848, rose in frequency and spread throughout industrialized regions, nearly replacing the “peppered” or “typical” phenotype by the end of the 19th century. Then, in the latter half of the 20th century, the melanic phenotype declined and is now becoming rare (Grant et al. 1998). Changes in moth populations are broadly correlated with environmental modifications brought about by human activity, and substantial experimental work points to differential predation on the moths by birds as the proximal mechanism of selection (Cook 2000).

The darkest melanic phenotype, named *carbonaria*, is nearly solid black and is easily distinguished from the much paler typical form (wild type) of the moth. Phenotypes that are intermediate between typicals and *carbonaria* are called *insularia*. The phenotypic differences result from multiple alleles at a single locus that exhibit an approximate dominance hierarchy: the *carbonaria* allele shows complete dominance over the *insularia* and typical alleles, and the several *insularia* alleles show incomplete dominance over the typical allele (Lees and Creed 1977). For analyses of *insularia* in natural populations, see Cook and Grant (2000).

Parallel evolutionary changes in the incidence of melanism have occurred in populations of the American subspecies of the “pepper-and-salt” geometer, *Biston betularia*

cognataria (Grant and Wiseman 2002). Owen (1962), who pioneered these studies, described American and British typicals as “quite distinct in wing pattern.” American typicals are “generally darker and browner, the postmedial and antemedial lines are usually clear, whereas the wings of [British typicals] are much whiter, with the postmedial and antemedial lines broken up into spots and blotches.” American melanics (named *swettaria*), however, are indistinguishable from British *carbonaria*. Intermediates also occur in the American subspecies, and the degree of pigmentation is also influenced by multiple alleles at a single locus (West 1977). Representative phenotypes are shown in Figure 1.

Although it has been assumed that the parallel evolution of melanism in American and British subspecies of peppered moths involved orthologous genes, specific tests for allelism have not been performed until now. This report demonstrates by Mendelian crosses that *carbonaria* and *swettaria* are products of alleles at the same gene locus. Additional evidence supports that dominance is a property of the melanic allele, unaffected by modifiers.

Materials and Methods

Tests for Allelism

Male British peppered moths attracted to an assembling (pheromone) trap containing caged American female *B.*



Figure 1. Phenotypes of the American “pepper-and-salt” geometer, *Biston betularia cognataria*: typical (wild type) (top); intermediate (middle); melanic (f. *swettaria*) (bottom).

betularia cognataria as lures were hybridized to produce an F₁ generation from male *carbonaria* (melanic) and female *swettaria* (melanic). The *swettaria* were known heterozygotes from bred stocks derived from a Pennsylvania population. The wild-caught *carbonaria* were presumed with 98% confidence to be heterozygotes, as the frequency of melanism in their local population near Liverpool was approximately 7% (Grant et al. 1998). Heterozygosity was confirmed by progeny test.

The F₁ hybrid melanics were test crossed by mating them to siblings of the recessive (typical) phenotype. Seventeen testcrosses were attempted, of which 14 produced broods that survived hand-rearing to the pupal stage. Of these, scorable adult progeny emerged from only 10 broods, 2 years after the initial hybridization between the subspecies (one generation per year). The testcrosses were reciprocal: females served as the melanic parent in four crosses, and males served as the melanic parent in six.

Effect of Genetic Background on Dominance

A breeding stock of the Japanese subspecies *B. betularia parva* was generously provided by Dr. Takahiro Asami. With the exception of a single melanic specimen captured by Dr. Soichiro Kinoshita (Figure 2), Japanese peppered moth populations are monomorphic for the typical phenotype (Asami and Grant 1995). Japanese typicals (hereafter referred to as *parva*), though pale, resemble the wing pattern of American typicals more closely than British typicals.

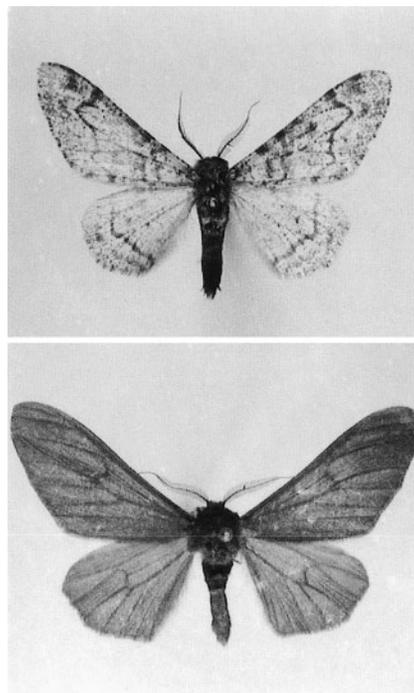


Figure 2. Phenotypes of the Japanese “frosted, branch-measuring” moth (*Oo-shimofuri-eda-shaku*), *Biston betularia parva*: typical (wild type) (top); melanic (bottom). These specimens were caught, pinned, and photographed by Dr. Soichiro Kinoshita.

A wild-caught American melanic (*swettaria*) male from Michigan and a *parva* female were hybridized. Melanic siblings from the F₁ were reciprocally backcrossed to *parva*. The backcrossing scheme introduced the *swettaria* allele to a genetic background that was approximately 75% Japanese in origin. Three female and two male backcrosses were successful in producing scorable adult progeny, but continued backcrossing over successive generations designed to enrich the Japanese genetic background beyond 75% failed following the loss of breeding stock.

In separate crosses, two wild-caught American intermediates (one darker than the other) were mated to *parva* females and the darkest among the F₁ progeny from each brood were reciprocally backcrossed to *parva*. Three female and two male backcrosses produced progeny in the “dark” line, and one female and two male backcrosses produced progeny in the “light” line.

Results

Tests for Allelism

Two of three matings observed between American and British melanics produced F₁ progeny. Both broods approximated 3:1 ratios of melanics to typicals (26:14, $\chi^2 = 2.20$, $P > .10$; and 14:3), confirming the heterozygosity of both sets of parents. The 3:1 phenotypic ratio is expected

Table 1. Single-locus and two-locus genotypic and phenotypic expectations from hybridizations between American and British moths heterozygous for their respective dominant genes for melanism

Single-locus model	
Heterozygous American melanistic × heterozygous British melanistic	
$Cc \times C'c'$	
Hybrids: $\frac{1}{4} CC' : \frac{1}{4} Cc' : \frac{1}{4} C'c : \frac{1}{4} cc'$	
Two-locus model	
Heterozygous American melanistic × heterozygous British melanistic	
$Aabb \times a'a'B'b'$	
Hybrids: $\frac{1}{4} Aa'B'b : \frac{1}{4} Aa'bb' : \frac{1}{4} aa'B'b : \frac{1}{4} aa'bb'$	

Although the genotypic distributions are different under the two models, both predict 3:1 phenotypic ratios in the first generation.

from this hybridization, whether or not the dominant melanistic genes are alleles at the same locus or nonallelic genes at independent loci. Table 1 illustrates that single-locus and two-locus predictions are identical for the first generation. However, the genotypic distributions are different from the two models and these predict different outcomes among broods in subsequent testcrosses.

The key outcome expected from the single-locus model is that one-third of the testcross broods should yield melanistic progeny only, indicating homozygosity of the melanistic hybrid parent. The remaining two-thirds of the testcross broods should show segregation of alleles by producing both phenotypes in equal frequency (1:1), indicating heterozygosity of the melanistic hybrid parent. Under the two-locus model, assuming independent assortment (no linkage), all of the testcross broods produced by melanistic hybrids should show segregation of alleles at one or both loci (either 1:1 or 3:1 ratios, respectively). No melanistic-only broods are predicted by the two-locus model for these testcrosses.

That any of the testcrosses of sufficient size should fail to produce nonmelanistic progeny is inconsistent with the independent two-locus model. Broods 13 and 14 (in Table 2) exclude that model, but are consistent with the predictions of the single-locus model. Brood 8 is too small, but assuming a 1:1 prediction, the binomial probability of all melanistic progeny in a family of five is $P = .03$.

However, the prediction from a two-locus model with complete linkage is indistinguishable from a single-locus model. As crossing over is presumed to be limited to males, based on other geometrid species studied (Suomalainen 1965), testcrosses of melanistic males are more informative than the reciprocal. Unfortunately, even the combined results of broods 8, 13, and 14 are too small to reject the possibility of tight linkage.

The testcross broods listed in Table 2 fall into two categories: those that show no segregation (all melanistic progeny) and those that show melanistic and nonmelanistic progeny. In no case were melanistics missing from broods. Among broods with melanistic and nonmelanistic progeny, three phenotypic categories are evident: full melanistics, typicals, and unexpected intermediates. The intermediates cannot have

Table 2. Testcross results of melanistic hybrids from British *Biston betularia* f. *carbonaria* × American *B. betularia cognataria* f. *swettaria*

Brood ID	Melanistic	Intermediate	Typical	N
2	11	6	9	26
4	5	2	1	8
5	17	13	8	38
7	31	10	15	56
9	3	0	2	5
10	1	4	5	10
12	4	2	1	7
8	5	0	0	5
13	11	0	0	11
14	64 + 1 ^a	0	0	65

Broods 2, 4, 9, and 12 were produced by melanistic females mated to typical males, and broods 5, 7, 8, 10, 13, and 14 were reciprocal (typical females × melanistic males).

^a Flecked.

resulted from the presence of a hidden *insularia* allele because had the melanistic parent been heterozygous for *insularia*, typicals would not be expected among the progeny. Compared to the number of melanistic progeny in these broods, the typicals are underrepresented. If the intermediates and typicals are grouped as a single phenotypic category called nonmelanistic, then the overall ratio of melanistics to nonmelanistics among broods fits the predicted 1:1 ratio very well ($\chi^2 = 0.24$, $P > .5$). From this it would appear that the intermediates are not modified melanistics, but instead are modified typicals. This point will be expanded in the discussion.

The sex of each moth in all 10 broods, including those unscorable for melanism because of malformed wings, was recorded. Females outnumbered males (154:111, $\chi^2 = 6.98$, $P < .01$). Among broods showing variance for melanism, there was no interaction between sex and melanistic phenotype (melanistic versus nonmelanistic) (contingency $\chi^2 = 0.823$, $P > .3$), nor was there an interaction between sex and phenotype within the nonmelanistic categories (intermediate versus typical) (contingency $\chi^2 = 2.647$, $P > .1$).

Genetic Background

The results of the hybrid backcrosses to *parva* are summarized in Table 3. Within lines the progeny of F₁ siblings of the same sex have been pooled for convenience. Reciprocal backcrosses of siblings do not differ with respect to melanistic phenotypes within broods (*swettaria* versus

Table 3. Progeny from F₁ *cog/par* melanistic and intermediate hybrids separately backcrossed to *parva*

F ₁ hybrid ID	Melanistic	Intermediate	Typical	N
Melanistic male	33	3	45	81
Melanistic female	12	1	16	29
Intermediate male	0	19	21	40
Intermediate female	0	19	26	45

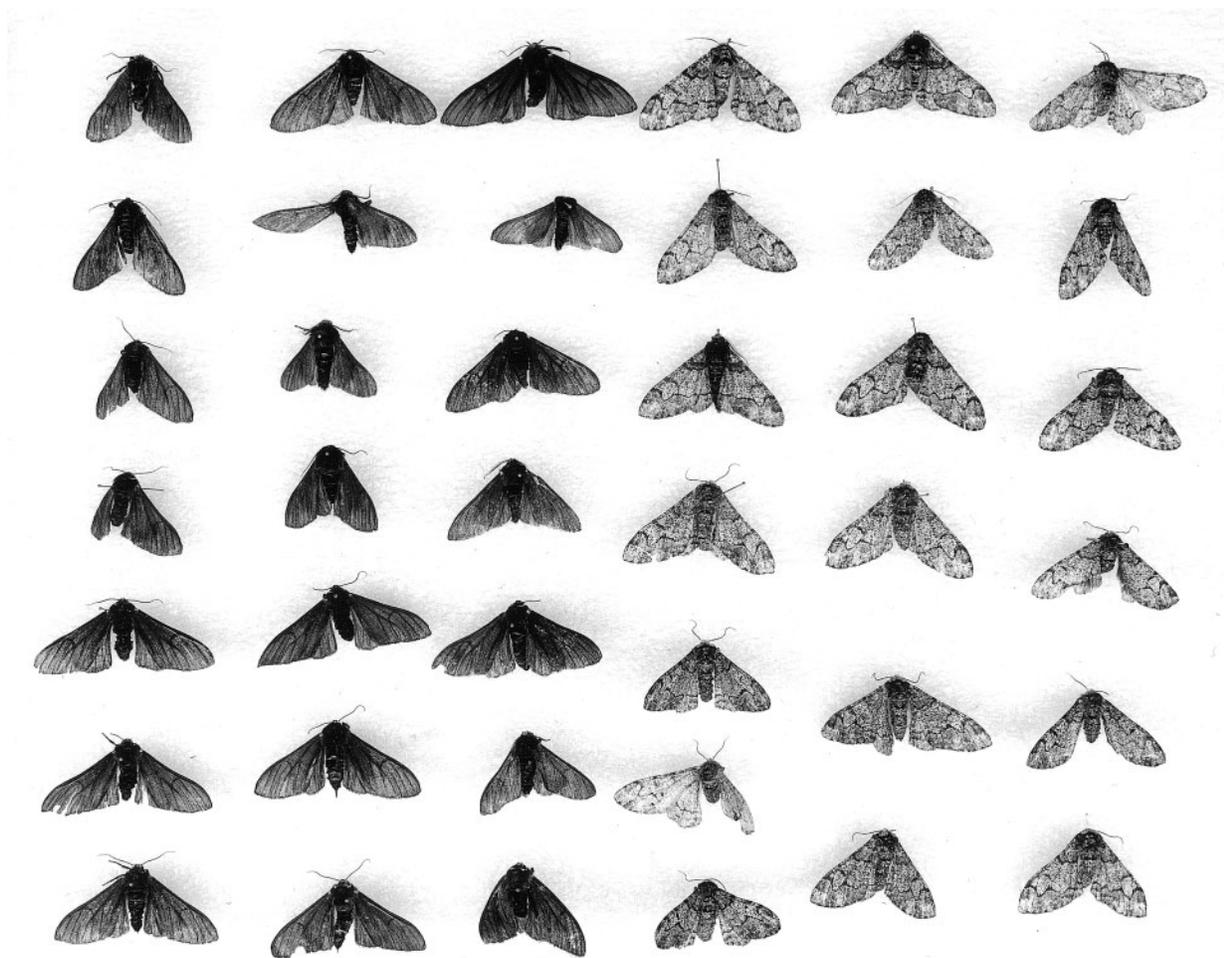


Figure 3. The phenotypes of the progeny from one brood (pbc10a) of the hybrid between an American melanic (f. *swettaria*) × *parva* backcrossed to *parva*. The left three columns are melanic, the right three columns are nonmelanic.

typical: contingency $\chi^2 = 0.03, P > .7$; *insularia* versus typical: contingency $\chi^2 = 0.07, P > .7$).

Excluding the occurrence of intermediates, the backcrosses of *swettaria* heterozygotes to *parva* produced melanic and typical progeny in a ratio of 1:1 ($\chi^2 = 2.415, P > .1$). Figure 3 shows one of the broods. The 4 intermediate progeny among the 110 scored are not more than usually occur among testcrosses within single subspecies. Table 4 compares the pooled results from three separate testcrosses (from Grant and Clarke 1999) involving only the American

Table 4. Progeny from F₁^{cog/par} hybrid melanics backcrossed to *parva* compared to pure *cognataria* melanic testcross progeny

Cross	Melanic	Intermediate	Typical	N
F ₁ ^{cog/par} × <i>parva</i>	45	4	61	110
<i>cog</i> × <i>cog</i>	198	7	191	396

$G_2 = 3.637; .10 < P < .25$.

subspecies (pure *cognataria*) to the results of the hybrid backcrosses to *parva*: the number of intermediates produced do not differ significantly ($G_2 = 3.637, P > .1$).

The backcross of F₁ hybrids from the darker-intermediate line produced intermediate and typical progeny in a ratio of 1:1 ($\chi^2 = 0.953, P > .3$). No fully melanic progeny (*swettaria*-like) occurred in any of these broods (Table 3). The wild-caught intermediate male parent initially hybridized to *parva* clearly was expressing an *insularia* allele.

The backcross of F₁ hybrids from the lighter-intermediate line produced 53 progeny, some darker than others, but no discontinuity separated phenotypes. The original male parent hybridized to *parva* was not likely carrying an *insularia* allele, or at least not one of strong penetrance, thus the heritability of its “smokiness” was not apparent from this cross.

The sex of each moth in all backcross broods to *parva* was recorded. Overall there was no significant difference in the number of females to males (133:115, $\chi^2 = 1.31, P > .20$). There was, however, a striking distortion of progeny sex ratio

from reciprocal backcrosses of F_1 hybrids. F_1 males produced female-biased broods (129:33), whereas in broods produced by F_1 females, female progeny were nearly absent (4:82). A strong interaction between progeny sex ratio and the sex of the hybrid parent is indicated (contingency $\chi^2 = 123.0$, $P < .001$). Nevertheless, there was no interaction between the sex of the progeny and melanic phenotype for either *swettaria* (contingency $\chi^2 = 0.876$, $P > .3$) or *insularia* (contingency $\chi^2 = 1.361$, $P > .2$).

Discussion

The Mendelian inheritance of melanism in peppered moths has been firmly established. Creed et al. (1980) list 12,569 progeny from 83 broods gathered from published reports. As numerous independent workers have been involved, subjectivity in scoring phenotypes is recognized, especially so for ranking the intermediates (*insularia*), but the volume of data demonstrating the heritability of *carbonaria* is beyond dispute.

Far less attention has focused on melanism in American *Biston*. At the time of Owen's (1962) original work on industrial melanism, no genetic data had yet been published confirming the inheritance of melanism in the American subspecies. West's (1977) was the first. Since that time we have assumed without direct evidence that the American melanic phenotype, *swettaria*, and the British melanic phenotype, *carbonaria*, were products of alleles at the same locus. The results of the crosses reported in Table 2 now allow us to change that assumption to a conclusion. Because *swettaria* and *carbonaria* are indistinguishable melanic phenotypes, the genes producing them can be regarded as *isoalleles*. The unresolved assumption is that they arose by independent mutation as the two subspecies are geographically widely separated, and have remained so presumably since the loss of the Bering land bridge (Rindge 1975). That assumption awaits molecular analysis.

The possibility remains that the genes producing *carbonaria* and *swettaria* are members of two tightly linked loci that failed to recombine in those crosses in which segregation was not observed. The probability of synteny of two genes drawn at random is the inverse of the haploid chromosome number; therefore parsimony requires that linkage of two loci must be demonstrated before a simpler model of one locus is rejected.

The backcrosses of hybrids to *parva* listed in Table 3 were attempts to test the dominance of American melanic alleles on a "naive" genetic background derived from populations where melanism was absent. The experiments were inspired by those of Kettlewell (1965), from which he concluded that dominance of the *carbonaria* allele had evolved by selection for modifiers at other loci. The "breakdown" in dominance that Kettlewell reported did not appear until the third backcross of melanic hybrids to Canadian stock from a region where melanism was unknown. Attempts by others to repeat that work failed to show any breakdown in dominance (Mikkola 1984; West 1977). The results in Table 3 are not comparable to their

experiments because backcrossing was terminated after only one generation. Nevertheless, the results are instructive in interpreting the unexpected appearance of intermediate phenotypes in diallelic crosses.

The backcross of melanic hybrids to *parva* (Table 3) produced conspicuously fewer intermediates than the testcross of American \times British melanic hybrids (Table 2). Indeed, the number of intermediates produced in the *parva* backcross did not differ significantly from what is observed occasionally among pure American broods (Table 4). In addition, the backcross of American *insularia* to *parva* produced no melanic (*swettaria*) progeny. The *swettaria* phenotype occurs only in broods where at least one parent shows that phenotype, consistent with the expectations of a fully penetrant, dominant gene.

The surplus of full melanics compared to the deficiency of typicals among the segregating broods produced by American \times British hybrids (Table 2) suggests that these particular intermediates are modified typicals rather than modified melanics, and may reflect segregation at other loci that distinguish American and British typicals. The intermediates are not a homogeneous group, but without clear criteria to categorize them, and the limited number of progeny in these experiments, further analysis at this point is hampered.

Perhaps the reason such a "breakdown" in the typical phenotype did not occur in Japanese backcrosses is because American and Japanese typicals bear a much closer resemblance in wing patterning than either does to the British typical. From a hybridization of a British melanic (*carbonaria*) to *parva*, the late Sir Cyril Clarke (unpublished data) scored 11 of 94 in the F_1 generation as "*insularia*-like" in an otherwise clear segregation (1:1) of melanics to typicals. Unfortunately the loss of breeding stock prevented Clarke from producing any backcrosses. Clearly more work is needed to analyze the genetic differences producing the divergent typical phenotypes of these subspecies.

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