

THE GENETICS OF MELANISM IN *MALACOSOMA DISSTRIA*
HÜBNER (LEPIDOPTERA: LASIOCAMPIDAE)

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ABSTRACT

The forest tent caterpillar is polymorphic for two melanic genes affecting wing color of moths. These are the first genetically determined morphological traits reported for the genus. Dark (*D*) is a sex-limited, autosomal dominant with a phenotype of dark brown males. Frequencies in population samples varied from 8 to 44%. Characteristics of Dark and nonmelanic males were compared. Larval development times, larval survival and pupal weights were not significantly different, but mean fecundity was higher for females with Dark progeny. Band (*b*), a darkened area across the forewings, occurred in low frequency in both sexes.

MOTHS of the genus *Malacosoma* are hardwood defoliators sharing several characteristics such as egg clumping, silk structures, communal feeding and wing color variability (STEHR and COOK 1968). Adult wing spans are 25 to 35 mm, coloring is light tan to dark brown. I studied the inheritance of melanic forms in *M. disstria*, the forest tent caterpillar, as part of a preliminary investigation of the population genetics of this species that also included quantitative characters (LORIMER 1979a) and isozymes (LORIMER 1979b). This information will ultimately be used to relate genetic organization and fluctuations in population size.

The evolutionary importance of melanism in the Lepidoptera is well illustrated by the phenomenon of industrial melanism. In the classic example, dark moths of *Biston betularia* L. (Selidosemidae) were protected from bird predation while resting on soot-blackened trees (reviewed by KETTLEWELL 1973). The frequency of melanic forms increased with proximity to industrial centers. But not all melanic polymorphisms are related to air pollution. Melanism appears to be a "basic and recurring necessity" that may come about for very different reasons (KETTLEWELL 1961). KETTLEWELL (1973) estimates that 10% of the 800 species of macrolepidoptera in Britain maintain melanic forms in other than industrial conditions. In-depth studies of melanic polymorphisms have revealed fascinating ecological, physiological and genetic interactions. Nevertheless, melanism has been examined in few American species of Lepidoptera, and records of genetic analyses are rare (GINEVAN 1971).

In this paper I report two polymorphic genes for melanism in *M. disstria*. One is sex-limited to males, the other autosomal and occurs in both sexes.

MATERIALS AND METHODS

M. disstria individuals were collected as eggs, larvae or pupae from Michigan, Minnesota, Indiana, and Alabama in two different years. Larvae were reared in the laboratory on prepared diet to the adult stage (LORIMER 1979). Individuals were measured for larval development time, pupal weight, and fecundity. Sex was scored at the pupal stage (MUGGLI 1974). Since each female lays her eggs in one clump, called an egg mass, individuals from one mass are siblings. During rearing, sibling groups were caged separately. Adult wing colors and patterns were scored soon after eclosion. Segregation of forms within sibling groups was noted. Frequencies of forms were also noted among adults that had been collected as larvae and pupae.

Pupae near eclosion were placed in small cages supplied with aspen twigs for oviposition

TABLE 1

Numbers of nonmelanic (+) and Dark moths in 27 sibling groups collected as egg masses from Michigan

Category	Sibling group	♀♀		♂♂	
		+	D	+	D
A	101	8	0	12	0
	104	7	0	18	0
	105	18	0	8	0
	106	7	0	9	0
	107	16	0	17	0
	108	7	0	20	0
	112	9	0	23	0
	115	18	0	16	0
	116	9	0	10	0
	117	5	0	16	0
	130	7	0	6	0
	Subtotal	111	0	155	0
	B	103	18	0	14
109		12	0	6	6
111		14	0	12	10
113		6	0	8	6
114		13	0	5	15
118		13	0	13	12
119		3	0	5	8
120		4	0	4	6
121		10	0	5	2
122		10	0	7	11
123		0	0	5	7
127		14	0	9	6
131		3	0	9	10
Subtotal	120	0	102	108	
C	102	10	0	0	11
	110	15	0	0	8
	126	4	0	0	7
	Subtotal	29	0	0	26
TOTAL	260	0	257	134	

See description of categories in text.

substrates. Pairs mated readily and females oviposited within 12 hr following eclosion. However, second generation masses failed to hatch after cold storage at -7° for five months.

RESULTS

The inheritance of Dark: Moths were light tan or dark brown. In the darker moths, all areas of the wings and the body were chocolate brown. The two oblique lines across the forewings were still distinguishable because they were delineated by thin white lines. This form was designated Dark (*D*). Segregation of Dark and the more common light, nonmelanic (+) form was recorded for 27 sibling groups from Michigan (Table 1). Only males were Dark. Sibling groups could be divided into three categories: (A) no Dark males, (B) Dark males and + males, and (C) all Dark males. The numbers of groups in each category were 11, 13, and 3, respectively.

The ratio of nonmelanic to melanic individuals for the same group of data from the Michigan sibling groups (Table 1) was approximately 2 + females: 0 *D* females: 2 + males: 1 *D* males. The missing class of *D* females suggests two hypotheses: *D* is lethal in females, or the expression of *D* is sex-limited to males. The strongest argument against the first possibility is the presence of females in category C sibling groups. Groups from this category, where all males are Dark, would have had one *D/D* parent, and all females would also carry the gene. The sex ratio, skewed toward males in category B, may suggest that homozygous *D* is lethal for females. However, the sex ratio in category A (*D* individuals absent) was also significantly skewed toward males. The simplest explanation of the data is that the expression of *D* is sex-limited to males.

Dominance and linkage of the *D* melanic trait can be hypothesized from the expected and observed frequencies of the three categories of sib groups in the 1976 Michigan collection (Table 1). Among males, 65.7% were light. If Dark is taken as dominant to light, then, according to the Hardy-Weinberg relationship, $q^2 = 0.657$, and $q = 0.811$. The expected frequency of *D* in the sample will be $p = 0.189$. *D/D* will be 0.036 and *D/+* ($2pq$) 0.307. Expected (and observed) frequencies for sib group categories based on segregation of males if *D* is dominant are 0.432 (0.407) A, 0.498 (0.481) B, and 0.071 (0.111) C. Comparable figures obtained from assumptions of recessiveness and sex linkage do not agree as closely with the observed frequencies (Table 2).

Further evidence supports the hypothesis that Dark is dominant. The expected ratios of Dark to light males in the sib groups with both forms (and their frequencies) are 1:1 (81%) and 3:1 (19%), if *D* is dominant. Actual figures (Table 1) for the frequencies of Dark males reflect the following: seven out of 13 groups had ratios differing from 1:1 by no more than sampling variation, three were in the range of 3:1, three were in the range of either ratio. If *D* is recessive, the expected ratios are 1:1 (59%) and 1:3 (41%). However, only three groups were compatible with either a 1:1 or 1:3 ratio, seven were in the range of 1:1, and three were incompatible with either. The high incidence of 1:1 groups and the relatively few 1:3 groups support the supposition that *D* is dominant to +.

The inheritance of band: A second melanic form identified in this study was

TABLE 2

Probabilities of sibling group types based on the frequency of Dark males in the 1976 Michigan egg collection, in relation to hypothetical modes of inheritance, all sex-limited

Linkage	Dominance	All + (A)		+ and D (B)		All D (C)	
		Frequency	Deviation from observed	Frequency	Deviation from observed	Frequency	Deviation from observed
Autosomal	Dominant	0.432	(6.1%)	0.498	(3.5%)	0.071	(36.0%)
	Recessive	0.313	(23.1%)	0.567	(17.9%)	0.118	(6.3%)
Sex-linked	Dominant	0.532	(30.7%)	0.249	(48.2%)	0.219	(97.3%)
	Recessive	0.935	(129.7%)	0.058	(87.9%)	0.007	(93.7%)
	Observed	0.407		0.481		0.111	

designated band (*b*). The wings and the body retained the tan coloration of the common form, but the area between the two oblique lines on each wing were darkened, giving the appearance of a dark band across each forewing. The segregation of band in two sib groups reared from the 1976 Indiana egg mass collection was as follows:

Sib group	Females		Males	
	+	<i>b</i>	+	<i>b</i>
203	15	10	8	6
208	7	2	8	6

In contrast to Dark, band is not sex-limited. There are insufficient data to hypothesize whether *b* is dominant or recessive.

Phenotype frequencies: The frequency of Dark males varied from 8 to 44% in nine insect collections (Table 3). Appropriate tests indicated significant differences in the frequency of *D* only between Michigan moths collected as larvae in 1976 and 1977 ($0.01 < P < 0.001$). Small sample sizes may have affected the statistical significance of other comparisons that were border line ($0.10 < P < 0.05$).

Frequencies of band moths were much lower than *D* moths in most collections.

TABLE 3

Phenotype frequencies of Dark (males) and band

Generation*	Location	Stage collected	Dark	(N)	Band
1976	Michigan	Eggs	0.34	(391)	0
		Larvae	0.19	(66)	0.03
		Pupae	0.23	(140)	0.03
	Indiana	Eggs	0.08	(275)	0.05
	Alabama	Pupae	0.17	(29)	0
1977	Michigan	Eggs	0.22	(95)	0.02
		Larvae	0.44	(41)	0
	Indiana	Eggs	0.19	(119)	0.02
	Minnesota	Pupae	0.33	(104)	0

* Year of defoliation.

The highest frequency was 5% band in the 1976 Indiana egg collection (Table 3).

Characteristics of the polymorphic forms: Individuals reared from egg masses were measured for several quantitative characters. Data from the 1976 egg collection from Michigan were analyzed to compare values for *D* and + moths. Mean pupal weights of *D* and + males from sib groups with both forms (category B) were 215 mg and 204 mg, respectively. Log pupal weights were not significantly different by a two-way analysis of variance (ANOVA) by wing color and sib group ($P = 0.13$). Differences in development time by wing color for the same individuals were also not significant ($P = 0.225$). In a second type of ANOVA, categories A and B were compared (Table 1). For both log pupal weight and development time, there were no significant differences between categories.

Differences among sib group types were further explored by comparing fecundities. Fecundities of mothers of sib groups were determined by counting the numbers of eggs in the original masses from which the insects were hatched and reared. Means were 73 eggs for masses producing sib groups in category A (no *D* males), 111 eggs for category B (both kinds of males), and 84 eggs for category C (all *D* males). By an ANOVA, sib group categories were significantly different ($P < 0.05$) for numbers of eggs per mass. The fecundities of females in the field may be affected by their own genotypes for Dark, since egg masses producing all nonmelanic males (mothers were probably +/+) had significantly fewer eggs than those in which *D* segregated (mothers may have carried *D*). The data for females reared in the lab did not repeat this pattern. Females from each of the three categories were dissected and their eggs counted. Mean fecundities were 104 eggs (A), 110 eggs (B), and 108 eggs (C), not significantly different among categories for the laboratory-reared generation.

Sibling group categories were compared for significant differences in survival. Mean survivorships from first-instar larvae to adults were 64%, 59%, and 64% for A, B, and C, respectively, not significantly different. However, survival from pupae to adults was significantly different ($P < 0.005$), 84% for A, 78% for B, and 63% for C.

DISCUSSION

KETTLEWELL (1961) defined melanism in insects as any darkening of the wings or other parts. I use the term in that broad sense since I have not investigated the biochemical characteristics of Dark and band in relation to the light form. Few studies on melanin pigment production have been undertaken in the Lepidoptera (ONSLow 1916).

The sex-limited nature of Dark in *M. disstria* implies that pigment formation may be hormonally mediated. Either the environment afforded by the male sex hormones enhances pigment production when the proper gene is present, or else female sex hormones inhibit this biochemical process. In birds, fish, and other animals, coloration is affected by hormones (KECK 1934; FOX and VEVERS 1960).

Sex-limited inheritance is a common feature in butterflies, particularly in mimetic species complexes. Males are almost always monomorphic, apparently

because females respond to males by sight during courtship (BROWER 1963). Moth morphism is not as constrained since scent, rather than sight, is a large component of the mating process. Even so, few cases of male sex-limited heredity are known in moths. In *Parasemia plantaginis* (Arctiidae) females are yellow, and males are yellow or white (COCKAYNE 1932).

Besides *M. disstria*, *Cynonia mendica* (Arctiidae) is the one other known case of sex-limited melanism in moths. In this species, females are white and males are white or brown (ONSLow 1921). Unlike *M. disstria*, the melanic form is apparently recessive (ONSLow 1921, ADKIN 1928a, 1928b). Most melanisms investigated genetically in Lepidoptera are inherited as simple autosomal dominants, and recessive polymorphisms are rare (reviewed by KETTLEWELL 1973).

WHITE (1877) was one of the first to suggest that melanic forms were maintained in populations by natural selection. Various physiological, ecological and behavioral factors have been invoked to account for melanism in Lepidoptera (reviewed by KETTLEWELL 1973). Too little is known about adult behavior in *M. disstria* to surmise whether or not cryptic advantage maintains the *D* and *b* polymorphisms. The adult portion of the life cycle is very short, adults in the laboratory seldom live longer than a week, and insemination and oviposition occur immediately after eclosion. If the same pattern holds in the field a cryptic form would be an advantage only for males. Further studies on adult behavior and ecology would clarify this point.

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