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DEVELOPMENT, FUNCTION, AND THE QUANTITATIVE GENETICS OF WING MELANIN PATTERN IN *PIERIS* BUTTERFLIES

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Abstract.—Do genetic correlations among phenotypic characters reflect developmental organization or functional coadaptation of the characters? We test these hypotheses for the wing melanin pattern of *Pieris occidentalis* butterflies, by comparing estimated genetic correlations among wing melanin characters with a priori predictions of the developmental organization and the functional (thermoregulatory) organization of melanin pattern. There were significant broad-sense heritabilities and significant genetic correlations for most melanin characters. Matrix correlation tests revealed significant agreement between the observed genetic correlations and both developmental and functional predictions in most cases; this occurred even when the overlap between developmental and functional predictions was eliminated. These results suggest that both developmental organization and functional coadaptation among melanin characters influence the genetic correlation structure of melanin pattern in this species. These results have two important implications for the evolution of melanin pattern in *P. occidentalis* and other butterflies: 1) most phenotypic variation in pattern may reflect variation among, rather than within, sets of developmentally homologous wing melanin characters; and 2) in a changing selective environment, genetic correlations may retard the disruption of functionally coupled melanin characters, thus affecting the evolutionary response to selection.

Key words.—Coadaptation, development, genetic correlation, melanin, phenotypic characters, *Pieris occidentalis*.

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Many complex phenotypic traits involve sets of interrelated, quantitative characters that are polygenically inherited. The genetic variance-covariance matrix (**G**) provides a convenient description of the genetic structure of such complex traits. **G** determines the evolutionary response to phenotypic selection, thus affecting the rates and trajectories of phenotypic evolution (Lande, 1979; Lande and Arnold, 1983). It is of interest, then, to identify factors determining the structure of **G**.

Both developmental and functional interrelationships among characters have been suggested to influence the structure of phenotypic and genetic covariation. For example, pleiotropic effects of genes on phenotypic characters that share common developmental pathways can lead to genetic covariation among the characters. As a result, the structure of genetic covariation for a set of interrelated characters may reflect the pattern of developmental organization or homology (Lande, 1980, 1984; Cheverud, 1982; Atchley, 1984). In addition, the structure of **G** may reflect functional (selective) relationships among phenotypic characters. Thus, Cheverud (1984) has argued that, near adaptive peaks, genetic correlations among characters will evolve to coincide with the curvature of the fitness surface—that is, with the selective correlations among characters. Theoretical analyses (Lande, 1980, 1984; Turelli, 1988) show that at evolutionary equilibria, **G** is a function of both developmental relationships (given by the matrix of mutational effects on variance and covariance) and functional relationships (given by the matrix representing multidimensional stabilizing selection).

Evaluation of these hypotheses about determinants of **G** has been incomplete, because of the difficulties of distinguishing developmental from functional effects. In their classic studies of morphological integration, Olson and Miller (1958) recognized the need to separate functional from developmental correlations, but provided no criteria nor case studies that do so. Several studies have shown that sets of developmentally related characters are strongly correlated genetically (Atchley et al., 1981, 1985; Cheverud et

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al., 1983). Cowley and Atchley (1990) recently concluded that phenotypic and genetic correlations among body parts in *Drosophila* reflect the developmental organization of the imaginal discs. Cheverud (1982) demonstrated that functionally and developmentally related characters in the macaque skull are strongly correlated genetically, but did not separate functional from developmental effects. Similarly, Venable and Burquez (1990) showed that the phenotypic and genetic correlation structures for a set of morphological and life-history traits in the composite *Heterosperma pinnatum* agreed significantly with developmental and functional predictions, but did not distinquish between the two effects. Zelditch and Carmichael (1989) developed independent hypotheses about functional and developmental integration in rat skulls, and tested these hypotheses using ontogenetic data on phenotypic covariation. The problem is compounded by the fact that functional and developmental effects are not necessarily mutually exclusive: The functional relations and developmental relations among characters may be similar. Indeed, under multivariate stabilizing selection, developmental, and functional relations may evolve to coincide (Cheverud, 1984; Wagner, 1986). To identify functional and developmental effects will require independent evidence about developmental organization and functional coadaptation (Zelditch and Carmichael, 1989) that can be compared to estimates of genetic covariation.

In this paper we consider wing melanin pattern in the Western White Butterfly, *Pieris* (*Pontia*) *occidentalis,* as a complex phenotypic trait that consists of sets of separate but interrelated wing melanin characters (Kingsolver and Wiernasz, 1987, 1990). We use empirical evidence about developmental and functional organization of melanin pattern to generate independent predictions about developmental correlations and functional correlations among wing melanin characters. We then estimate the genetic correlations among wing melanin characters, and compare these to the predicted developmental and functional correlations. Our results suggest that both developmental organization and functional coadaptation are important in structuring genetic covariation in this complex trait.

Materials and Methods

Melanization Pattern in Pieris occidentalis

Wing color in *Pieris* is determined by two types of pigments: white and yellow pteridine pigments, and black melanin pigments. The background pteridine pigments are controlled by a single locus with multiple alleles (Watt and Bowden, 1966). The pattern of wing melanin is more complex, and does not have a simple single-locus basis (Shapiro, 1984*a*).

To quantify wing melanin pattern in *P. occidentalis,* we measured 25 wing melanin characters, each a linear measure of a specific element of the melanin pattern (Kingsolver and Wiernasz, 1987) (Fig. 1A). We made 35-mm color slides (Ektachrome 160) of the dorsal and ventral wing surfaces for each butterfly under standard lighting and exposure conditions. Each slide was projected onto a computer-interfaced (Compaq II) digitizing pad (Houston Instruments, True Grid 1011) at a magnification of 4×. The length or width of each melanin character (see Fig. 1A) and of the radius of the forewing were measured with the digitizer's stylus; all measurements were taken on the right forewing and hindwing. The error of repeat image measurements was ~5%. All melanin characters and forewing length (FWL) were log-transformed to achieve normality. For these transformed data, each melanin character was regressed on FWL for each data set (see below). The residual value for each character after removal of the effect of FWL was used in all subsequent analyses. For nearly all characters in each data set, FWL explained less than 10% of the variance, and the qualitative results of our tests are the same whether residuals or the original data were used.

Developmental and Functional Organization of Pattern

The determination and deposition of wing melanin in butterflies occurs during late larval and pupal life (e.g., Nijhout, 1980, 1985). Comparative and experimental studies with many Lepidoptera (Schwanwitsch, 1924;

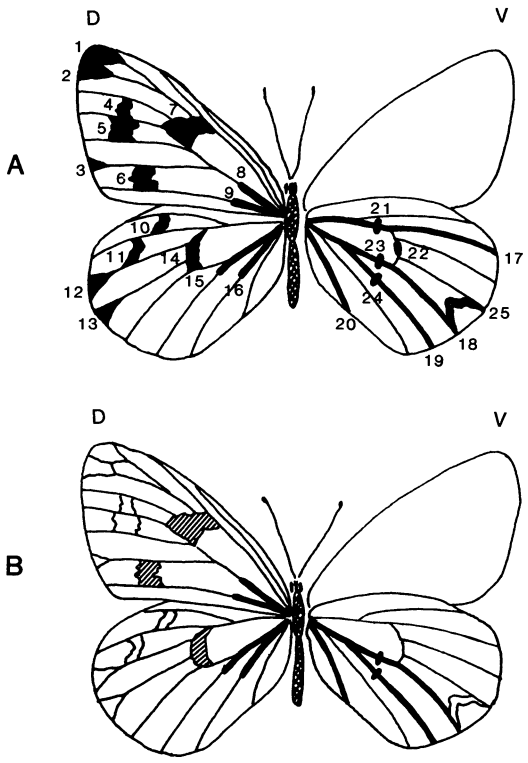

FIG. 1. Wing melanin pattern of pierine butterflies. Wings on the left are dorsal surfaces (D), on the right are ventral surfaces (V). A. The 25 melanin characters measured on *P. occidentalis* in this study. Each character is a length or width of a region of melanin, where each region is identified with respect to specific wing veins. See Kingsolver and Wiernasz (1987) for further details. See Table 1 for the names of the characters. B. Functional map relating effects of melanin characters in different wing regions on body temperature during thermoregulation for *P. occidentalis*. The map is given in terms of the effect of increased melanin for relevant melanin characters on body temperature: heavy solid lines indicate increased body temperature; striping indicates decreased body temperature. See text and Table 3.

Kuehn and Henke, 1936; Nijhout, 1984, 1985) indicate that melanin pattern is composed of several distinct types of pattern elements, that in general run from the anterior to the posterior margin of each wing surface. Within each of these elements, recurring pattern features in different wing cells (regions bounded by wing veins) are considered to be serially homologous (Nijhout, 1984, 1985).

Shapiro (1984*b*) (see also Schwanwitsch, 1956) has examined developmental organization in pierid butterflies, based on comparative studies and the order of appearance of different melanin characters during pupal development. In Shapiro's view, melanin pattern in pierids (including *P. occidentalis*) is composed of three elements (Fig. 1A and Table 1): a marginal band; a transverse band proximal to the marginal band; and melanin overlying the wing veins. These three features may occur on each wing surface, and melanin pattern on each wing surface is independent. We have used pupal cold-shock experiments (cf. Goldschmidt, 1938) with *P. occidentalis* to determine whether sets of developmentally related melanin characters respond similarly to cold-shocks during pattern development (Wiernasz and Kingsolver, submitted). Our results are generally consistent with the developmental organization of pattern proposed by Shapiro (1984*b*). These studies allow us to identify sets of serially homologous melanin characters on each wing surface (Table 1).

Our knowledge of the developmental organization of melanin pattern in *P. occidentalis* (Table 1) allows us to make qualitative predictions about the structure of genetic correlations (Table 2): Melanin characters within a homologous set (Table 1) will have high, positive genetic correlations ($r_g = 1$), whereas nonhomologous characters will be relatively uncorrelated genetically ($r_g = 0$) (see also Cowley and Atchley, 1990). For convenience we shall call these predictions the developmental organization hypothesis. Because development on each wing surface is independent, we generate predictions for each wing surface separately. Table 2 (above diagonal) gives genetic correlations predicted by the developmental organization hypothesis for the dorsal forewing (DFW, Table 2A), dorsal hindwing (DHW, 2B), and ventral hindwing (VHW, 2C).

Our hypotheses about functional organization are based on analyses of the thermoregulatory functions of melanin pattern in *P. occidentalis*. *Pieris occidentalis,* like other pierine butterflies, require thoracic (body) temperatures of 28 to 40°C to take off and sustain flight (Kingsolver, 1985*a*), and they achieve these temperatures by behavioral posturing (orientation to solar radiation). *Pieris* use three main thermoregulatory postures: Reflectance basking, lateral

TABLE 1. Sets of homologous melanin characters for *P. occidentalis*. Numbers in parentheses indicate the character # denoted in Figure 1. See text for explanation.

Surface	Vein-associated	Transverse	Marginal
DFW:	FB3 (8)	FT3 (4)	FM1 (1)
	FB4 (9)	FT4 (5)	FM2 (2)
	FDS (7)	FT6 (6)	FM6 (3)
DHW:	HB4 (15)	HT1 (10)	HM3 (12)
	HB5 (16)	HT2 (11)	HM4 (13)
	HDS (14)		
VHW:	VL2 (17), VL5 (18)	VT6 (25)	
	VL6 (19), VL8 (20)		
	VX2 (21), VDS (22)		
	VX5 (23), VX6 (24)		

TABLE 2. Predicted genetic correlations, according to the developmental organization hypothesis (above diagonal), and the development-only hypothesis (below diagonal), among melanin characters on the dorsal forewing (2A), dorsal hindwing (2B), and ventral hindwing (2C). See Figure 1 and text.

2A. Dorsal forewing

	FB3	FB4	FDS	FT3	FT4	FT6	FM1	FM2	FM6
FB3	—	1	1	0	0	0	0	0	0
FB4	0	—	1	0	0	0	0	0	0
FDS	1	1	—	0	0	0	0	0	0
FT3	0	0	0	—	1	1	0	0	0
FT4	0	0	0	1	—	1	0	0	0
FT6	0	0	0	1	1	—	0	0	0
FM1	0	0	0	0	0	0	—	1	1
FM2	0	0	0	0	0	0	1	—	1
FM6	0	0	0	0	0	0	1	1	—

2B. Dorsal hindwing

	HB4	HB5	HDS	HT1	HT2	HM3	HM4
HB4	—	1	1	0	0	0	0
HB5	0	—	1	0	0	0	0
HDS	1	1	—	0	0	0	0
HT1	0	0	0	—	1	0	0
HT2	0	0	0	1	—	0	0
HM3	0	0	0	0	0	—	1
HM4	0	0	0	0	0	1	—

2C. Ventral hindwing

	VL2	VL5	VL6	VL8	VX2	VDS	VX5	VX6	VT6
VL2	—	1	1	1	1	1	1	1	0
VL5	1	—	1	1	1	1	1	1	0
VL6	1	0	—	1	1	1	1	1	0
VL8	1	0	0	—	1	1	1	1	0
VX2	1	1	1	1	—	1	1	1	0
VDS	1	1	1	1	1	—	1	1	0
VX5	1	0	0	0	1	1	—	1	0
VX6	1	0	0	0	1	1	0	—	0
VT6	0	0	0	0	0	0	0	0	—

TABLE 3. Predicted genetic correlations among melanin characters according to the functional coadaptation hypothesis (above diagonal) and the function-only hypothesis (below diagonal). See Figure 1 and text.

	FB3	FB4	FT6	FDS	HB4	HB5	HDS	VL5	VL6	VL8	VX5	VX6
FB3	—	1	−1	−1	1	1	−1	1	1	1	1	1
FB4	0	—	−1	−1	1	1	−1	1	1	1	1	1
FT6	−1	−1	—	1	−1	−1	1	−1	−1	−1	−1	−1
FDS	−1	−1	1	—	−1	−1	1	−1	−1	−1	−1	−1
HB4	1	1	−1	−1	—	1	1	1	1	1	1	1
HB5	1	1	−1	−1	0	—	−1	1	1	1	1	1
HDS	−1	−1	1	1	−1	−1	—	−1	−1	−1	−1	−1
VL5	1	1	−1	−1	1	1	−1	—	1	1	1	1
VL6	1	1	−1	−1	1	1	−1	0	—	1	1	1
VL8	1	1	−1	−1	1	1	−1	0	0	—	1	1
VX5	1	1	−1	−1	1	1	−1	0	0	0	—	1
VX6	1	1	−1	−1	1	1	−1	0	0	0	0	—

basking, and heat-avoidance posture (Kingsolver, 1985*a*; Kingsolver, 1987; Wiernasz, unpubl. data). Comparative and experimental studies (Kingsolver, 1985*b*, 1987; but see Heinrich, 1990) demonstrate how changes in melanin in specific wing regions can affect body temperature during thermoregulation (Fig. 1B). Heinrich (1990) has recently questioned the importance of reflectance basking in butterflies; but his paper fails to address the direct experimental manipulations of wing reflectivity in *Pieris* (Kingsolver, 1987) upon which the predictions in the current study are based.

We can use our functional 'map' (Fig. 1B) relating different melanin characters to body temperature to generate predicted genetic correlations among functionally related melanin characters (Table 3, above diagonal). For convenience we shall call these predictions the functional coadaptation hypothesis. Melanin characters with the same effect on body temperature (e.g., FB3 and FB4; or FT3 and FT4) are predicted to be positively correlated genetically ($r_g = 1$); whereas characters with opposite effects on body temperature (e.g., FB3 and FT3; or FB4 and FT4) are predicted to be negatively correlated genetically ($r_g = -1$). We consider only those 12 melanin characters that affect thermoregulation in these predictions (see also results).

The predicted genetic correlations based on developmental organization (Table 2, above diagonal) and functional coadaptation (Table 3, above diagonal) are identical for some melanin characters. For example, both the functional and developmental hypotheses predict positive genetic correlations among characters VL5, VL6, VL8, VX5, and VX6 (see Tables 2 and 3). To reduce the confounding of developmental and functional effects in our tests, we can alter the above predictions in the following way: when the developmental organization and functional coadaptation hypotheses make identical (nonzero) predictions for the genetic correlation between two characters, we set the predicted correlation to zero. This generates two additional predicted matrices: first, a 'development-only' hypothesis (Table 2, below diagonal), in which only those genetic correlations predicted by developmental organization that are *not* predicted by functional coadaptation are given nonzero values; second, a 'function-only' hypothesis (Table 3, below diagonal), in which only those genetic correlations predicted by functional coadaptation that are *not* predicted by the developmental organization hypothesis are given nonzero values. As an example, both the developmental organization hypothesis (Table 2A, above diagonal) and the functional integration hypothesis (Table 3, above diagonal) predict a positive ($r_g = 1$) genetic correlation between characters FB3 and FB4; hence the predicted correlation between these characters is set to zero for the development-only hypothesis (Table 2A, below diagonal) and the function-only hypothesis (Table 3, below diagonal).

These considerations yield specific predictions about the structure of genetic cor-

relations among wing melanin characters that would result from developmental (Table 2) and functional effects (Table 3). These predictions can then be compared to estimates of genetic correlations in natural populations.

Quantitative Genetics of Pattern

Quantitative genetics of melanin pattern was studied for populations of *P. occidentalis* from western Colorado (Montrose and Mesa Counties, elevation 1,370–1,700 m), in a region where both thermoregulation and mate choice have been examined (Kingsolver, 1987; Wiernasz, 1989). For each breeding experiment, females were obtained from the field, fed twice daily on 20% sugar water, and allowed to oviposit in the lab on *Lepidium perfoliatum,* one of the common larval host plants at this site. Females were dissected after oviposition was completed to ensure that only singly mated females were used in the experiments. Families of full sibs (40–80 individuals/family) were reared in a single walk-in environmental chamber at 25°C with continuous light, on a diet of greenhouse-grown *L. perfoliatum.* First- and second-instar larvae were reared in groups of 5–10 full sibs in a Petri dish; later instar larvae and pupae were reared individually in the dishes. Wing melanin characters of each emerging adult were measured using the digitizing system described above. These studies enable us to estimate broad-sense heritabilities and genetic correlations, that include additive and nonadditive genetic components as well as common environmental effects. We do not expect wing melanin characters to have important maternal effects (recall that each character has the effect of forewing length, and hence body size, removed before analysis). Additional genetic estimates based on laboratory females derived from these lines showed qualitatively similar heritabilities and genetic correlations to these experiments using wild-caught females (Wiernasz and Kingsolver, unpubl. data). We report results from two independent breeding experiments: Experiment 1, with 14 familes (319 males, 252 females); and Experiment 2, with 18 families (480 males, 453 females). The females initiating both experiments represented the first brood of the season. Because the females initiating each experiment were collected in different months and years (March, 1987 for Experiment l; April, 1988 for Experiment 2), and because melanin pattern differed significantly between experiments for both males and females (MANOVAs, $P < 0.001$), results for the two experiments were analyzed separately. Because of the differences in melanin pattern between males and females, and the possibility of function differences between the sexes (Wiernasz, 1989), each sex was analyzed separately.

Estimation and significance testing of heritabilities (h^2) were performed using ANOVA with the SYSTAT statistical package. Estimation and significance testing of genetic correlations (r_g) were performed with the FREESTAT statistical software package (see Mitchell-Olds and Shaw, 1987, Appendix D); in this package significance testing is done using a permutations test. The "table-wide" significance of each genetic correlation matrix was estimated using a sequential Bonferroni test (Rice, 1989). Recall that our primary goal is to test qualitative predictions about the matrices of genetic correlations, rather than determining the value or significance of particular genetic correlations.

To examine the correspondence between predicted (developmental organization, development-only, functional coadaptation, or function-only) and estimated genetic correlation matrices (**G**), the matrix correlation coefficient Z (Pearson product-moment correlation) was computed for each predicted-estimated matrix pair. Significance of each matrix correlation Z was determined using matrix permutations, in which rows and columns in the data (estimated) matrix were randomly permuted to obtain a distribution of expected matrix correlation values (Dietz, 1983; Cowley and Atchley, 1990). The p-value is defined as the probability that a Z value at least as large as the observed Z value was drawn at random from the empirically derived distribution of Z values, based on the matrix permutations (Kohn and Atchley, 1988). All 720 unique permutations were used for the DHW matrices; for all other matrices 10,000 permutations were used (Jackson and Somers, 1989).

TABLE 4. Broad-sense heritabilities of wing melanin characters for males and females for Experiments 1 and 2. Underlined values are significantly different from 0.00 at a table-wide P-value of 0.05 (sequential Bonferroni test).

	Experiment 1		Experiment 2	
Characters	Males	Females	Males	Females
FB3	0.52	0.57	0.80	0.83
FB4	0.46	0.71	0.39	0.60
FDS	0.24	0.41	1.15	0.87
FT3	0.80	0.49	0.68	0.53
FT4	0.45	0.46	0.71	0.64
FT6	0.40	0.46	0.85	0.79
FM1	0.10	0.13	0.09	0.77
FM2	0.44	0.27	0.10	0.60
FM6	0.55	0.58	0.67	0.81
HB4	0.29	0.61	0.63	0.54
HB5	0.64	0.42	0.56	0.34
HDS	0.62	0.76	0.23	0.42
HT1	0.60	0.16	0.48	0.54
HT2	0.26	0.06	0.53	0.59
HM3	0.57	0.22	0.64	0.65
HM4	0.65	0.23	0.67	0.41
VL2	0.10	0.28	0.34	0.69
VL5	0.37	0.30	0.52	0.60
VL6	0.41	0.34	0.31	0.54
VL8	0.71	0.22	0.18	0.23
VX2	0.29	0.50	0.39	0.24
VDS	0.36	0.60	0.88	0.73
VX5	0.29	0.43	0.48	0.47
VX6	0.30	0.53	0.18	0.27
VT6	0.19	0.29	0.64	0.47

RESULTS

Broad-sense heritability (h^2) estimates suggest that there was significant genetic variation in most (Experiment 1) or all (Experiment 2) melanin characters in males and females (Table 4). Heritabilities of some characters differed considerably between the two experiments, probably due to the small numbers of families used in the estimates. Heritabilities also differed substantially between the sexes for some characters. These significant levels of genetic variance justify the estimation of genetic correlations among these characters.

As an example we present genetic correlation matrices for Experiment 2 for the dorsal forewing (DFW, Table 5A), dorsal hindwing (DHW, 5B), and ventral hindwing (VHW, 5C). There were significant genetic correlations (r_g) among many melanin characters within each wing surface. Note that many of the large, positive values in the matrix represent genetic correlations between serially homologous characters (e.g., in Table 5A, r_g among FB3 and FB4; FT3, FT4, and FT6; FM1, FM2, and FM6).

We can compare the observed genetic correlation matrix (e.g., Table 5) with the matrix predicted by developmental organization (Table 2, above diagonal), for each wing surface. The results (Table 6A) showed significant ($P < 0.05$), positive correlations between the observed and predicted matrices for the dorsal forewing (DFW) and dorsal hindwing (DHW) for males and females in each experiment. For the ventral hindwing (VHW), there was no significant correlation between observed and predicted matrices for males or females in either experiment. Matrix correlations for the development-only hypothesis (Table 2, below diagonal), in which only those developmental predictions not predicted by functional coadaptation are given nonzero values, were lower than for the developmental organization hypothesis; but the results of the significance testing were generally similar (compare Tables 6A and 6B). There were significant positive correlations between observed and predicted matrices for the DHW for males and females in both experiments, and no significant matrix correlations for the VHW in any case. For the DFW, there were significant matrix correlations for males in both experiments and for females in Experiment 2; for females in Experiment 1, the matrix correlation was marginally significant ($P = 0.054$). These results suggest that, in agreement with the developmental organization hypothesis, genetic correlations reflect patterns of developmental homology among melanin characters for the dorsal wing surfaces, but not for the ventral hindwings (Table 6A). This result holds even if we remove the confounding effects of functional associations among melanin characters (Table 6B).

Table 7 gives estimated genetic correlations $(r)_g$ between functionally related melanin characters for males and females from Experiment 2. There were significant r_g between many melanin characters both within and across wing surfaces. By comparing observed correlation matrices with those pre-

TABLE 5. Estimated genetic correlations among melanin characters on the dorsal forewing (DFW, 5A), dorsal hindwing (DHW, 5B), and ventral hindwing (VHW, 5C) for females (above diagonal) and males (below diagonal) from Experiment 2. See Table 2 for comparison with developmental predictions. Correlations that are underlined are significantly different from 0.00 at a matrix-wide *P*-value of 0.05 (sequential Bonferroni test).

5A. Dorsal forewing

	FB3	FB4	FDS	FT3	FT4	FT6	FM1	FM2	FM6
FB3	—	0.71	0.19	−0.33	0.09	0.13	0.02	0.04	0.11
FB4	0.78	—	0.48	−0.42	0.17	0.28	0.38	0.43	0.42
FDS	−0.12	0.03	—	0.30	0.50	0.45	0.27	0.44	0.57
FT3	−0.69	−0.54	0.35	—	0.67	0.61	0.12	0.27	0.09
FT4	−0.45	−0.58	0.13	0.82	—	0.93	0.10	0.36	0.59
FT6	−0.19	−0.09	0.42	0.62	0.76	—	0.03	0.27	0.48
FM1	−0.32	−0.08	0.19	0.34	0.15	0.21	—	0.88	0.78
FM2	−0.24	−0.32	−0.08	0.33	0.28	0.12	0.79	—	0.88
FM6	−0.23	−0.03	0.36	0.60	0.38	0.45	0.86	0.73	—

5B. Dorsal hindwing

	HB4	HB5	HDS	HT1	HT2	HM3	HM4
HB4	—	0.95	0.60	0.46	0.29	0.66	0.59
HB5	0.85	—	0.66	0.52	0.39	0.62	0.54
HDS	0.69	0.61	—	0.30	0.23	0.09	0.05
HT1	0.32	0.52	0.70	—	0.96	0.51	0.56
HT2	0.48	0.58	0.82	0.85	—	0.51	0.55
HM3	0.40	0.54	0.23	0.06	0.22	—	1.01
HM4	0.45	0.63	0.22	0.03	0.15	1.00	—

5C. Ventral hindwing

	VL2	VL5	VL6	VL8	VX2	VDS	VX5	VX6	VT6
VL2	—	0.93	0.82	0.36	0.78	0.55	0.88	0.78	0.34
VL5	0.93	—	0.94	0.61	0.68	0.69	0.79	0.68	0.55
VL6	0.92	1.00	—	0.46	0.70	0.59	0.80	0.68	0.65
VL8	0.77	0.84	0.87	—	0.17	0.78	0.34	0.49	0.70
VX2	0.88	0.75	0.70	0.50	—	0.52	0.96	0.90	0.39
VDS	0.14	0.17	0.08	0.17	0.24	—	0.55	0.73	0.43
VX5	0.95	0.90	0.89	0.72	0.93	0.12	—	0.96	0.48
VX6	0.81	0.71	0.69	0.53	0.80	−0.08	0.92	—	0.50
VT6	0.46	0.65	0.67	0.74	0.19	0.34	0.45	0.33	—

dicted by the functional coadaptation hypothesis (Table 3, above diagonal), we found significant ($P < 0.05$), positive correlations between predicted and observed matrices for males in Experiment 1, and for females in both experiments (Table 8A). Matrix correlations for the function-only hypothesis (Table 3, below diagonal), in which only those functional predictions not predicted by developmental organization are given nonzero values, were lower than for the functional coadaptation hypothesis (Table 8B). There were significant positive correlations between the observed and predicted function-only matrices for both males and females in Experiment 1, and a marginally significant ($P = 0.079$) matrix correlation for females in Experiment 2.

These results provide partial support for the hypothesis that the structure of genetic correlations reflects the functional (thermoregulatory) relations among wing melanin characters in *P. occidentalis*, as predicted by the functional coadaptation hypothesis (Table 8A). This result holds even if we remove the confounding effects of developmental

TABLE 6. Matrix correlation coefficients (Z) between predicted and estimated genetic correlation matrices for the developmental hypotheses: (A) developmental organization; (B) development-only. Probability values (P) are based on 10,000 random matrix permutations for the DFW and VHW, and all 720 unique permutations for the DHW: † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.005$. See text for further explanation.

	Males			Females		
	DFW	DHW	VHW	DFW	DHW	VHW
A. Developmental organization						
Expt. 1.	0.485*	0.620**	0.104	0.444*	0.583*	0.573
Expt. 2	0.642***	0.687**	0.374	0.577***	0.635*	0.219
B. Development-only						
Expt. 1	0.399*	0.485*	−0.214	0.331†	0.521*	0.007
Expt. 2	0.586**	0.545*	0.222	0.503**	0.530*	−0.194

associations among melanin characters (Table 8B).

DISCUSSION

The notion that organisms are functionally and developmentally integrated systems dates at least to the beginnings of structuralist approaches to morphology (for discussion see Riedl, 1978). The more explicit hypothesis that developmentally and functionally related phenotypic characters will be correlated phenotypically and/or genetically has been documented for both fossil (e.g., Olson and Miller, 1958) and living (e.g., Cheverud, 1982) animals. Distinguishing between functional and developmental effects on the correlation structure has proven more elusive, however, because of the difficulties of generating independent, testable predictions about development and function (Zelditch and Carmichael, 1989; Venable and Burquez, 1990) that can be compared to estimates of genetic covariation.

The predictions of developmental and functional effects on genetic correlation structure of melanin pattern in *Pieris* that are tested in this study have several limitations. First, they yield qualitative, not quantitative, predictions about the structure of genetic covariation. As a result, the predictions involve genetic correlations, not genetic covariances. In contrast, theoretical models are framed in terms of genetic covariances, which are more directly related to the evolutionary response to selection (Lande, 1979, 1980; Turelli, 1988). Second, our estimates of genetic correlations include both additive and nonadditive genetic as well as common-environmental components. However, it is the additive component that is relevant to the long-term cor-

TABLE 7. Estimated genetic correlations among melanin characters involved in the functional coadaptation hypothesis, for females (above diagonal) and males (below diagonal) in Experiment 2. See Table 3 for predicted correlations.

	FB3	FB4	FT6	FDS	HB4	HB5	HDS	VL5	VL6	VL8	VX5	VX6
FB3	—	0.71	0.13	0.19	0.30	0.32	0.29	0.34	0.53	0.16	0.48	0.49
FB4	0.78	—	0.28	0.48	0.72	0.62	0.59	0.61	0.71	0.41	0.60	0.74
FT6	−0.19	0.09	—	0.45	0.24	0.15	0.36	0.62	0.62	0.25	0.48	0.31
FDS	−0.11	0.03	0.42	—	0.44	0.57	0.52	0.73	0.74	0.79	0.54	0.60
HB4	0.73	0.96	0.05	0.21	—	0.95	0.60	0.42	0.36	0.49	0.45	0.67
HB5	0.46	0.58	0.19	0.48	0.85	—	0.66	0.45	0.45	0.66	0.43	0.63
HDS	0.50	0.55	0.43	0.54	0.69	0.61	—	0.59	0.58	0.89	0.44	0.72
VL5	0.12	0.32	0.28	0.17	0.26	0.20	0.05	—	0.94	0.61	0.79	0.68
VL6	0.13	0.33	0.26	0.07	0.24	0.16	−0.03	0.97	—	0.46	0.80	0.68
VL8	0.12	0.43	0.36	0.11	0.27	0.21	−0.08	0.84	0.87	—	0.34	0.49
VX5	0.28	0.30	0.40	0.07	0.26	0.20	0.05	0.90	0.89	0.72	—	0.96
VX6	0.15	0.06	0.50	−0.16	0.10	0.16	−0.16	0.70	0.69	0.53	0.92	—

related response to selection, although nonadditive effects can contribute to the short-term response. Third, the precision of our genetic estimates is limited by the small number of families in our data sets. This likely contributes to the quantitative differences in heritabilities and genetic correlations between the two experiments. Fourth, our functional predictions concern performance 'surfaces' for thermoregulation, not fitness surfaces. If thermoregulatory performance and fitness are not positively correlated, our predictions do not follow from the expectations of the theoretical models (Lande, 1980, 1984; Cheverud, 1984).

Despite these limitations, our studies reveal some consistent results about genetic variation and covariation in melanin pattern in *Pieris*. There is significant heritable variation in most aspects of melanin pattern, and significant genetic correlations among many melanin characters. Thus, we would expect both direct and indirect evolutionary responses to selection on melanin pattern. The developmental tests (Table 6A) strongly support the hypothesis that serially homologous melanin characters are more highly correlated genetically than are nonhomologous characters for the dorsal wing surfaces. This is true even when the overlap between functional and developmental hypotheses is eliminated (Table 6B). These results suggest that the genetic correlation structure reflects the developmental organization of dorsal melanin pattern in this species.

The tests of the ventral hindwing (VHW) did not generally support the developmental organization hypothesis (Table 6). This may be an artifact of measuring only a single, nonvein-associated character (see Table 1). In fact, most melanin characters on the VHW were highly correlated genetically (Table 5C), as suggested by Shapiro (1984*a*, 1984*b*). Similarly, our pupal cold-shock experiments and factor analyses with *P. occidentalis* showed that all VHW melanin characters respond similarly to perturbations of development (Wiernasz and Kingsolver, submitted), suggesting that the entire VHW melanin pattern may form a single developmental unit.

Our results also provide partial support for the functional coadaptation hypothesis:

TABLE 8. Matrix correlation coefficients (Z) between predicted and estimated genetic correlation matrices for the functional hypotheses: (A) functional coadaptation; (B) function-only. Probability values (P) are based on 10,000 random matrix permutations: † $P < 0.10$, * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.005$. See text for further explanation.

	Males	Females
A. Functional coadaptation		
Expt. 1	0.694**	0.667***
Expt. 2	0.143	0.454***
B. Function-only		
Expt. 1	0.532**	0.504**
Expt. 2	−0.016	0.200†

the observed genetic correlations show significant agreement with predictions based on thermoregulatory analyses of melanin pattern (Tables 3, 7, and 8A) for females in both experiments, and for males in Experiment 1. When the overlap between developmental and functional predictions is eliminated (Table 8B), only males and females of Experiment 1 showed significant agreement. Thus, the structure of genetic covariation may reflect directly the functional relations among wing melanin characters, as suggested by Cheverud (1984) and Wagner (1986), but the evidence is more ambiguous than for the developmental tests.

The predictions about functional coadaptation tested here are based on the consideration of a population at or near an evolutionary equilibrium (Lande, 1980, 1984; Turelli, 1988; Cheverud, 1984). An alternative hypothesis is that if directional selection is operating on two characters simultaneously, the genetic correlation between them will become negative in the short-term (cf. Falconer, 1981 p. 300). This would be expected if selection rapidly exhausts the existing positive genetic covariance between the two characters. Applied to the present case, this argument predicts genetic correlation opposite in sign to those from the functional coadaptation hypothesis considered here (Table 3). Because our functional coadaptation matrix (Table 3) is positively or not correlated with the estimated genetic correlation matrices in all cases (Table 8), our results are not consistent with this alternative hypothesis.

We note that the qualitative nature of the

predictions about genetic correlations here limits the interpretation of our results about functional coadaptation. For example, setting all −1.0 values to 0.0 in the predicted function matrix in Table 3, corresponding to the functional hypothesis that characters FT6, FDS, and HDS do not contribute to thermoregulatory function via reflectance, does not affect the matrix correlations or probability values in the results; this change amounts to a simple change of scale. This limits our ability to distinguish between possible alternative predicted matrices.

Our results are generally consistent with the hypothesis that both developmental organization and functional coadaptation influence the structure of genetic correlations in *P. occidentalis*. It is noteworthy that there are only two characters (FDS and HDS) for which developmental predictions and functional predictions are in direct opposition for this system (compare Tables 2 and 3). For all other correlations, the predictions from development and from function are either in agreement or are independent. Whether this lack of 'conflict' between developmental and functional effects holds in other systems is not known, but may contribute to the difficulties of distinguishing between the two (Olson and Miller, 1958; Cheverud, 1982; Wagner, 1986).

The genetic variance-covariance matrix influences directly the evolutionary response of a population to selection. Our results on the biological determinants of genetic correlation structure have two important implications for the evolution of melanin pattern in *P. occidentalis* and other butterflies. First, each developmentally homologous set of melanin characters will tend to evolve as a unit, relatively independent of other such homologous units. Factor analyses of one *P. occidentalis* population (Wiernasz and Kingsolver, submitted) and comparative analyses of pierid butterflies (Shapiro, 1984*a*, 1984*b*) suggest that most variation in melanin pattern is the result of variation among homologous sets of characters, rather than within homologous sets. Indeed, because many aspects of melanin pattern formation are common to all butterflies, the consequences of development organization for genetic correlation structure described here may be relevant to phenotypic pattern variation of butterflies in general. Along these lines, Nijhout (1991) has recently examined the relationship of developmental pattern formation to wing color pattern variation in all Lepidoptera. Cowley and Atchley (1990) document an analogous situation in which genetic and phenotypic correlation structure resulting from the organization of imaginal discs may influence patterns of morphological variation in many Diptera.

The second implication of our results concerns the evolution of functional coadaptation. Because genetic correlation structure reflects functional (thermoregulatory) relations among melanin characters in *P. occidentalis,* we would expect this to influence the variation among individuals in thermoregulatory function generated by melanin pattern variation. Principal components and discriminant analyses of biogeographic (Kingsolver and Wiernasz, 1987) and seasonal (Kingsolver and Wiernasz, 1991) variation in melanin pattern in *P. occidentalis* show that the loadings on the principal components and/or discriminant function match the thermoregulatory relations among characters (Fig. 1B). This will tend to increase the rate of evolutionary response to selection for populations in temporally and spatially variable thermal environments. On the other hand, if the shape of the phenotypic fitness surface—i.e., the functional relations among characters—changes, the genetic correlation structure will slow the rate of evolutionary response to selection: in this sense, the genetic correlation structure acts as an evolutionary constraint to the disruption of the functionally coupled characters (Lande, 1979; Burger, 1986; Wagner, 1986). Thus, the issue of whether genetic correlations act as evolutionary constraints on selection response must be discussed with respect to a specific fitness surface and to the location of the population on that surface. This emphasizes the importance of studying the shape of performance and fitness surfaces, for which there are few empirical data (Arnold, 1988; Kingsolver, 1988; Schluter, 1988).

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