Natural Selection and Developmental Constraints in the Evolution of Allometries

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In animals, scaling relationships between appendages and body size exhibit high interspecific variation but low intraspecific variation. This pattern could result from natural selection for specific allometries or from developmental constraints on patterns of differential growth. We performed artificial selection on the allometry between forewing area and body size in a butterfly to test for developmental constraints, and then used the resultant increased range of phenotypic variation to quantify natural selection on the scaling relationship. Our results show that the short-term evolution of allometries is not limited by developmental constraints. Instead, scaling relationships are shaped by strong natural selection.

Among species, populations, and even sexes, morphological traits exhibit an impressive diversity of scaling relationships with body size; most traits scale positively with body size, although the rate at which trait size changes with overall size often differs from isometry and can even be nonlinear (1). Changes with overall size are common (2–7). This is particularly true of insects, which exhibit extremes in trait-body size allometries (3). This extreme variation among groups is in marked contrast to the extent of variation among species; typically, individuals within these groups exhibit little variation around some average allometry, reflecting a tight scaling between body parts and overall size [e.g., (4–7)].

Although these patterns have long been recognized (2, 8), surprisingly little is known about the evolution of scaling relationships (3, 9); in particular, the relative importance of processes shaping their evolution is largely uninvestigated (10). Presumably, tight adherence to particular allometries results from external selection against traits with atypical or nonfunctional relative sizes. Such selection is predicted to favor the evolution of genetic and developmental systems that properly scale the growth of traits across body sizes, maintaining functional size relationships in the face of environmental and genetic variation (11, 12). However, this scenario presents a paradox: The proximate mechanisms that evolve to maintain the relative size of traits will then produce developmental constraints [as defined in (10)] that must be overcome if allometries are to evolve. Here, we present empirical data addressing the relative roles of natural selection and developmental constraints in the evolution of the allometry between forewing area and body size in the butterfly Bicyclus anynana.

In the context of the evolution of allometries among morphological traits, the scaling relationship between wing area and body size (i.e., the ratio of body size to wing area, or “wing loading”) is of interest, because it is ecologically important and taxonomically diverse (13, 14). The size of the wings and flight musculature relative to body mass affects flight performance directly (14), as well as indirectly through thermoregulatory effects while basking or during ectotherm flight (13). Lepidoptera have the lowest average wing loading among flying insects (15) and exhibit lineage-specific, seasonal morph–specific, or sex-specific scaling relationships associated with life historical or behavioral correlates [e.g., (6, 16–20)]. As with most insects, adult body size in B. anynana is a highly plastic trait (21), and forewing area (FW) exhibits a strong, positive phenotypic correlation with total body mass (BS) across the natural range of body size (Pearson correlation coefficient = 0.86, N = 691 stock population females, P = 0.0001). Moreover, artificial selection for changes in FW and pupal mass revealed a genetic correlation between these traits (r = 0.75) (Fig. 1) (20).

The strong genetic correlation between FW and body size should constrain their independent evolution (22), inhibiting phenotype evolution in a direction perpendicular to that of the wild-type allometry (23). To determine whether such internal constraints limit the short-term evolution of the scaling relationship, we performed artificial selection on the FW/BS allometry (20). The FW/BS allometry evolved rapidly, diverging ~2 SD in each direction relative to that of the control lineage to produce distinct, novel phenotypes [Fig. 2; discriminant function analysis correctly classified 94.8% of females from generation 13 (~2 log likelihood = 107.4; N = 766, replicates pooled)]. The response to selection resulted almost entirely from changes in FW (Fig. 3); BS changed in the appropriate direction in only one lineage (~FW/BS, lineage E; $F_{1,11} = 5.55$, $P = 0.038$ (20). This extreme asymmetry in the contribution of each trait to the evolution of the allometry was unexpected, as both individual FW and body size exhibited very similar realized heritabilities (Fig. 1), indicating adequate and equivalent genetic variation in both traits. Moreover, the observed pattern of response is not due to differences in the phenotypic variance between the traits, because they were subject to similar indirect selection pressures in all but one case (Fig. 3). A low frequency of alleles in our starting population that affect BS independently of FW or a sieving out of key alleles that affect FW but not BS could account for the pattern. In any case, the rapid evolution of the allometry demonstrates a surprising absence of developmental constraints restricting change in this scaling relationship. However, the pattern of response exhibited by FW and BS indicates a strong
bias [or developmental constraint (10)] in how these traits respond indirectly to direct selection on their scaling relationship. Hence, the allometry itself is not developmentally constrained; what does appear to be constrained is the way in which the individual components contribute to the evolution of this complex phenotype.

Our results, together with the few other studies that have used artificial selection to alter scaling relationships between morphological traits in insects (24–26), indicate that even strong genetic correlations do not constrain phenotype evolution in the short term. It seems that the developmental basis of these genetic correlations is more important than their strength in determining the response to selection (27). In particular, under novel selection regimes such as the artificial one we imposed, the developmental program coordinating the growth of the individual traits may influence how these traits and the relationship between them evolves (28).

The lack of developmental constraints on the evolution of the allometry motivated us to determine the pattern of natural selection on wing loading. To examine the fitness consequences of deviating from the wild-type FW/BS scaling relationship, we measured the mating success of competing wild-type control and novel-phenotype males (two treatment male classes, +FW/−BS and −FW/+BS) in a spacious, naturally planted, tropical greenhouse. Treatment and control males taken from reciprocal crosses of the replicated lineages of each selected direction were selected for inclusion in the experiment on the basis of their static allometries (20). Hence, all males came from similarly outcrossed populations, and treatment and control males were drawn from the same genetic background. Mating success was determined by the transfer of phenotype class–specific colored powder from males to females (20, 29). In both trials, males with the wild-type phenotype acquired three times as many matings as did males from both phenotype classes with novel wing loadings (Fig. 4; trial 1, \( G = 30.2, P < 0.001; \) trial 2, \( G = 18.381, P < 0.001 \)). These results demonstrate strong stabilizing selection favoring the natural scaling relationship between forewing and body size in *B. anynana*.

Survival among male phenotype classes (recapture rates) did not differ (trial 1, \( G = 0.110, P = 0.947; \) trial 2, \( G = 0.641, P = 0.726 \)), a finding consistent with results from manipulative studies of wing loading in free-flying butterflies [e.g., (15, 30, 31)]. Because survival was the same among male phenotypes, the higher fitness of wild-type males must be due to other, nonexclusive, selective factors. In the greenhouse, males engage in prolonged bouts of chasing both other males and females, as they do in nature (29), which suggests that the lower fitness of treatment males may result from decreased locomotor performance (20). Intrasexual competition among male phenotypes may also play a role; +FW/−BS and −FW/+BS males may be excluded from females in the presence of superiorly flying, wild-type males. Alternatively, wild-type males may be selected by females because of favored signals produced during courtship or flight (29) or because treatment males are less appealing visually.

In any case, because treatment and wild-type males were drawn from the same outbred populations, any female preference must be largely unrelated to male genetic background in our experiment. Regardless of the cause of the higher fitness of wild-type males, we...
have documented strong stabilizing selection on male wing loading. Our findings indicate that it is not internal developmental constraints, but rather external natural selection, that is the primary basis of how the allometry evolved suggests that development may strongly influence how individual traits respond to selection on their scaling relationships.

References and Notes

Mechanisms of Hair Graying: Incomplete Melanocyte Stem Cell Maintenance in the Niche

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Hair graying is the most obvious sign of aging in humans, yet its mechanism is largely unknown. Here, we used melanocyte-tagged transgenic mice and aging human hair follicles to demonstrate that hair graying is caused by defective self-maintenance of melanocyte stem cells. This process is accelerated dramatically with Bcl2 deficiency, which causes selective apoptosis of melanocyte stem cells, but not of differentiated melanocytes, within the niche at their entry into the dormant state. Furthermore, physiologic aging of melanocyte stem cells was associated with ectopic pigmentation or differentiation within the niche, a process accelerated by mutation of the melanocyte master transcriptional regulator Mitf.

Qualitative and quantitative changes in stem and progenitor cells have been implicated in physiological (chronological) aging (1, 2), although the changes are poorly understood and the process of stem-cell aging has not been visually observed. Involvement of stem and progenitor cells in aging of multiple organ systems has been suggested in mice defective in DNA damage repair and telomere maintenance (3), but melanocytes may be unique in that the oxidative chemistry of melanin biosynthesis can be cytotoxic (4). This led to the suggestion that differentiated, pigmented melanocytes (rather than their unpigmented progenitors) are specifically targeted in hair graying (5, 6). The recent discovery of unpigmented melanocyte stem cells, distinctly located within the hair follicle (7), creates an opportunity to determine whether the process of hair graying arises specifically from changes in differentiated melanocytes or the stem-cell pool that provides them.

Stem cells are maintained in the niche microenvironment (8). Hair follicles contain a well-demarcated structure for the stem-cell niche (within the lower permanent portion), whereas differentiated melanocytes reside in the hair bulb (at the base of the transient portion of the hair follicle) (Fig. 1A) (7, 9).
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