

Competition among body parts in the development and evolution of insect morphology

(relative growth/genetic constraint/developmental constraint/allocation tradeoff/growth compensation)

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ABSTRACT Changes in form during ontogeny and evolution depend in large measure on changes in the relative growth of the various parts of the body. The current consensus in developmental biology is that the final size of appendages and internal organs is regulated autonomously, within the structure itself. Size regulation of body parts typically requires no external control and is thought to be relatively insensitive to signals from the developmental environment. We show in two very different systems, butterfly wings and beetle horns, that experimentally induced changes in the allocation of developmental resources to one trait produces compensatory changes in the relative sizes of other traits. These findings illustrate that interaction among body parts in development is part of the mechanism of size regulation of those parts. Furthermore, in the case of beetle horns, we show that the tradeoff in size is manifest as a significant negative genetic correlation among the involved body parts and, therefore, constitutes a developmental source of genetic constraint on the evolution of body form.

. . . I see hardly any way of distinguishing between the effects, on the one hand, of a part being largely developed through natural selection and another and adjoining part being reduced by this same process . . . , and, on the other hand, the actual withdrawal of nutriment from one part owing to the excess of growth in another and adjoining part. [Darwin (ref. 1, p. 147)]

It has long been supposed that the dynamics of developmental processes restrict the range of morphological variation that can be produced (2, 3) and that, insofar as natural selection can only operate on available variation, this restriction constrains evolution to the paths permitted by the developmental mechanism (4–6). Yet despite a long history of interest in this problem, empirical studies that demonstrate how development biases the generation of morphological variation are rare (7–11), and empirical links between developmental phenomena and genetic constraints on evolution are all but nonexistent (12–14). Herein we demonstrate one way in which development may limit the generation of morphological variation, namely, through a common developmental environment within the organism that imposes resource allocation tradeoffs among growing body parts. When a limiting resource is shared among several body parts, the degree to which it is used by one part diminishes its availability to another (15). Such a resource could then constitute a signaling mechanism that controls the relative growth of body parts.

The concept that, during development, different parts of an organism could be in competition with each other is old (1, 3, 16), and intuitive, but has proven difficult to demonstrate in

practice. One reason for this difficulty may be that resource allocation tradeoffs are difficult to measure in organisms that grow and feed continuously, because changes in demands on developmental resources can be met by changes in resource acquisition (17–19). The peculiar growth of the adult structures of holometabolous insects, by contrast, provides us with an ideal system in which to examine whether morphological allocation tradeoffs occur during development.

Many of the structures that make up the body of the adult insect develop during larval life either as imaginal discs or as small undifferentiated patches of larval epidermis (20, 21). Cell number in these imaginal precursors increases exponentially throughout the larval period, and most of the increase in size of these traits is concentrated in a period of apparently explosive growth just prior to metamorphosis to the pupal stage (20–23). Most of the growth of the imaginal discs occurs after the larva stops feeding and, therefore, takes place within a closed system. Growth of the imaginal precursors occurs at the expense of reserves and tissues accumulated during larval life. Theoretical models of imaginal disc growth predict that under most circumstances there will be competition among imaginal structures for these reserves. When resources are limiting, diminished growth of one trait should be compensated for by excessive growth in another (24). Herein we test this prediction experimentally in two systems and by three techniques.

METHODS AND RESULTS

Butterfly Wings. In butterflies, the wings are by far the largest structures that develop from imaginal discs, the four wings accounting for nearly 20% of the dry weight in the adult (Fig. 1*a*). Because wings represent such a large fraction of the adult body mass, we predicted that developing wings would be especially likely to compete for resources with other traits. If resource allocation tradeoffs exist, then perturbation of the development of a wing (such as removal of one or more of the developing wing imaginal discs before any significant growth occurred) should result in shifts in the relative sizes of other traits, as resources that would have been used by the developing wings became available for use by other growing structures.

We removed one or two hind wing imaginal discs from larvae of *Precis coenia* (Lepidoptera: Nymphalidae) during the first day of the final larval instar. At this time the discs were still very small and were less than 0.1% of the weight of the larva (26). Larvae of *P. coenia* were reared on an artificial diet at a constant temperature of 26°C and a 16-h light/8-h dark photoperiod. Before surgery, larvae were anesthetized by submersion in water for 10 min. Imaginal discs of the meta-

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Abbreviation: JH, juvenile hormone.

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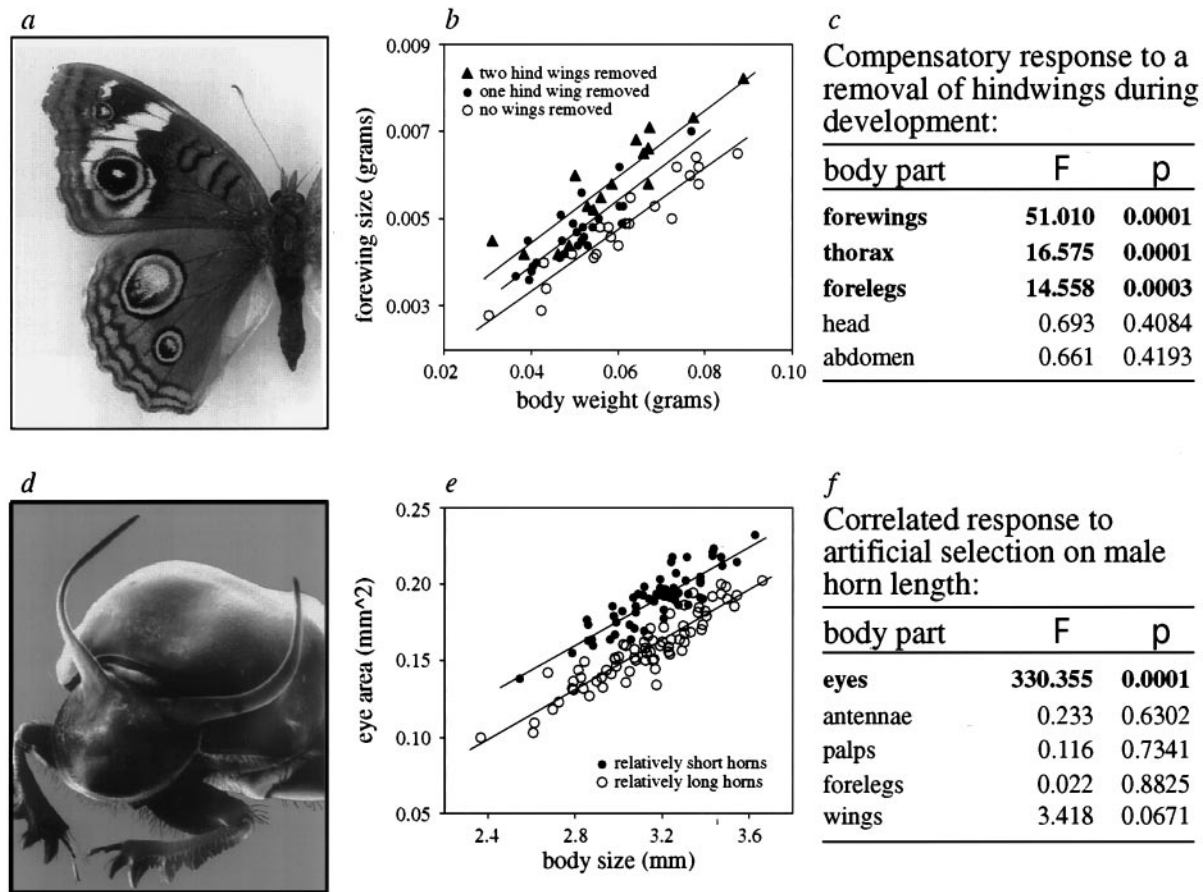


FIG. 1. Resource allocation tradeoffs in the development of butterfly wings and beetle horns. Species illustrated are *P. coenia* (a) and *O. taurus* (d). One or two hind wing imaginal discs were excised from caterpillars of *P. coenia* during the second day of the final larval instar and the compensatory responses of fore wings and other tissues was measured in the adults that developed from these larvae. (b) Animals with excised hind wings developed disproportionately large fore wings for their body sizes (one-way analysis of covariance, with body dry weight as covariate $F = 51.010$, $P = 0.0001$), and the magnitude of this effect depended on the number of hind wings removed. (c) Removal of hind wings also affected other traits. Males with reduced number of hind wings developed disproportionately large thoracic dry weight and foreleg femur length, but the relative dry weights of the head and abdomen were unaffected (F values shown are for the effect of hind wing removal in one-way analysis of covariance, with body dry weight as covariate). Developmental time was not affected by the surgery [duration of final larval instar (mean \pm SD): sham-operated controls, 6.50 ± 0.86 days; one wing removed, 6.42 ± 0.99 days; two wings removed, 6.94 ± 0.68 days]. In *O. acuminatus*, male horn size was altered through artificial selection. After seven generations of selection, males selected for relatively long horns had significantly longer horns than males selected for relatively short horns (25). (e) Heritable changes in the relative length of male horns resulted in negatively correlated changes in the sizes of male eyes (one-way analysis of covariance with body size, measured as width of prothorax, as the covariate, $F = 330.355$, $P = 0.0001$). (f) Compensation only involved the compound eyes; the relative sizes of other structures were unaffected by selection on horn size (F values for one-way analysis of covariance for the effect of artificial selection on horn length with prothorax width as the covariate). Results of these experiments show that butterfly wings and beetle horns compete with certain other traits during growth and that the relative sizes of these traits is significantly affected by resource allocation tradeoffs within the developing animal.

thoracic wings were removed from larvae submerged in lepidopteran saline, through a small slit made in the lateral thoracic body wall. After surgery the larvae were allowed to recover for 1 h on saline-wetted filter paper and then transferred to individual rearing containers with fresh diet. Control larvae underwent a sham operation, consisting of anesthesia and wounding but without removal of the discs.

After metamorphosis, a compensatory response was observed in the relative size of the adult fore wings, and this response was proportional to the number of hind wing discs removed (Fig. 1b). Larvae whose hind wing discs were removed developed into adults with fore wings that were disproportionately large for their body size. We also examined other body parts for compensatory growth and found additional changes in the relative sizes of the thorax and forelegs but not in the head or abdomen (Fig. 1c).

These results reveal competition among growing traits in these butterflies and illustrate that the ontogeny of body form may be sensitive to resource allocation tradeoffs operating within a developing animal. Because in these butterflies the

fore wings, hind wings, and legs appear to compete for resources during development, enlargement of one trait should interfere with the simultaneous enlargement of the others. This competition among body parts may restrict the range of morphological variation that is possible, and variant phenotypes in which more than one of these structures are simultaneously enlarged may be produced less frequently than others, if at all.

Beetle Horns. To address the question whether morphological allocation tradeoffs can, in fact, affect character evolution, we tested whether competition among body parts was responsive to artificial selection in horned beetles. Beetle horns are exaggerated extensions of exoskeleton, expressed only in males (Fig. 1d). They are used in intrasexual combat over access to females and provide one of the classical examples of sexual selection (1, 27–29). Like butterfly wings, beetle horns constitute a substantial investment for a developing animal. And as in butterfly wings, horn development does not begin until the larva has stopped feeding and thus occurs in an essentially closed system in which resource allocation tradeoffs might be detectable.

In *Onthophagus taurus* and *Onthophagus acuminatus* (Coleoptera: Scarabaeidae), the possession of horns is a dimorphic character in males. Small-bodied males lack horns and large-bodied males possess large horns (25, 30). The developmental switch to horn production at intermediate body sizes can be manipulated experimentally, and this gives us the means to investigate whether horn development is associated with a compensatory response in other structures. We manipulated allocation to horns in two ways: (i) we altered the hormonal environment of developing males to induce changes in horn growth and (ii) we used artificial selection to generate populations that differ genetically for horn growth.

Juvenile Hormone Treatment. In *O. taurus* we have established that the body size above which horns will develop can be increased by the application of juvenile hormone (JH) during a critical period of development in the last larval instar (unpublished results). Upon JH application during this critical period, intermediate-sized animals with a body size that would normally be horned either failed to develop horns or produced horns substantially smaller than expected for their body size (Fig. 2a).

O. taurus larvae were reared individually in cavities made in plaster blocks, supplied with horse manure, and kept in darkness at 28°C. Larvae were observed and weighed daily to characterize growth and physiological age. Third-instar feeding-stage larvae were treated with a 5- μ l topical application of the JH analog methoprene (approximate dose of 400 μ g of methoprene per g of larval weight). Control larvae were treated with a similar volume of acetone alone. Larvae were then returned to their individual containers and monitored as above until adult eclosion.

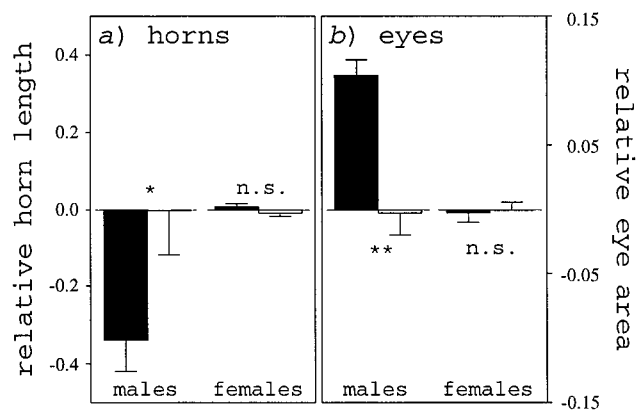


FIG. 2. Resource allocation tradeoffs in developing *O. taurus* revealed by treatment with the JH analog methoprene. Solid bars, animals receiving methoprene in acetone; open bars, acetone-treated controls. (a) Topical application of JH during the second and third days of the final larval instar induced males to reduce allocation to horns. Males just above the critical size for horn production developed significantly shorter horns relative to their body size than control males (Mann-Whitney U test on relative horn length: $U_{7,7} = 9$, $Z = -1.981$, $P = 0.0476$). Bars indicate the residual horn length (mean \pm SEM), calculated as the difference between actual horn length and that expected for a male of the same body size. Expected values were generated from the best-fit curve relating horn size to body size in unmanipulated males (25). Females never develop horns and were not affected by the JH treatment (Mann-Whitney U test: $U_{13,13} = 61$, $Z = -1.205$, $P = 0.228$). (b) JH-induced diminution of male horns was accompanied by a significant increase in the size of male compound eyes (Mann-Whitney U test: $U_{7,7} = 1$, $Z = -3.003$, $P = .0027$). Bars indicate the mean \pm SEM of residual eye size, calculated from the mean relationship between eye surface area and body size of unmanipulated males. Female eye size was unaffected by JH treatment (Mann-Whitney U test: $U_{13,13} = 75$, $Z = -0.487$, $P = 0.626$), indicating that increased eye size in males resulted from reduced allocation to horns rather than as a direct response to JH.

In normal animals there is a negative phenotypic correlation between horn size and the size of the compound eyes: large animals with large horns have relatively small compound eyes, and vice versa (D.J.E., unpublished data). In our JH-treated animals, whose horns were experimentally reduced, we found a compensatory increase in the size of the compound eyes. The relative sizes of the eyes of males whose horns were experimentally reduced by JH treatment were larger than those of control males (Fig. 2b). That the eyes did not respond directly and independently to JH is suggested by the fact that the relative sizes of the eyes of females (which never develop horns) was not affected by JH treatment (Fig. 2b). These results suggest that resource allocation tradeoffs operate between horns and eyes within developing beetles in much the same way as between hind wings and fore wings in *P. coenia*.

Artificial Selection. To address the question whether resource allocation tradeoffs during development can, in fact, affect morphological evolution, we tested whether competition among body parts was responsive to artificial selection in horned beetles. In *O. acuminatus*, allocation to horns was altered through artificial selection on the relative length of male horns (for methods used, see ref. 30). Two selection lines were established. In one line, selection was imposed for increased horn size relative to body size, and in the second line selection was imposed for decreased horn size relative to body size. After seven generations of artificial selection, males selected for long horns had significantly longer horns for a given body size than males selected for short horns (30). As with *O. taurus*, changes in horns of males were accompanied by inverse changes in the sizes of eyes of the same animals. Males selected for relatively short horns developed eyes that were large relative to body size, whereas in males selected for relatively long horns, the size of the eyes decreased (Fig. 1e). The shift in the relative size of *O. acuminatus* eyes produced through artificial selection on horns reveals a negative genetic correlation among these traits (31–33). Because these correlated shifts in male eyes are identical in magnitude and direction to the compensatory shifts induced by JH treatment, we suggest that this negative genetic correlation results from the competition between horns and eyes for a shared limiting resource. If true, this would provide a direct link between the developmental environment of growing traits and the genetic correlation between those traits (34) and establish resource allocation tradeoffs as a mechanism by which developmental processes can generate genetic constraints on evolutionary change.

The compensatory responses for the loss of hind wings or the diminution of horn size were evidently not homogeneously distributed across the body but seemed to be preferentially focused on one or a few structures (Fig. 1c and f). In butterflies, we had expected that at least some of the resources that were not used in the manufacture of hind wings would be retained in the abdominal fat body and not used for growth, but the abdominal weight was not affected by removal of the hind wings. Instead, affected traits were all located within the vicinity of the developing wings. It is interesting in this regard to note that in species of scarab beetles that have thoracic horns instead of head horns, a negative phenotypic correlation between horn size and wing size has been found—males with disproportionately large horns have disproportionately small wings (35). We did not find a compensatory response in the wings of the two species of *Onthophagus* we studied, but in these species, horns develop adjacent to eyes not wings. In each of these cases, as well as in the butterflies that we studied, the structures exhibiting the strongest compensatory response were ones that developed in relatively close proximity to the manipulated trait.

DISCUSSION

The findings we describe bear on an old and still unanswered question in biology, namely, how the absolute and relative sizes of traits are regulated. Numerous experiments on regeneration and transplantation have shown that the final size of appendages and internal organs is regulated autonomously, within the structure itself; size regulation of body parts typically requires no external control (23, 36–40). For instance, when sections are cut from a *Drosophila* imaginal disc, adjoining cells divide to produce a normal-sized trait, even when the injured disc is placed outside of the environment of the developing larva (41, 42). When a wing imaginal disc of *Drosophila* is transplanted to the abdomen of an adult fly, it grows to the same size that it would have if it had been left in place in its normal larval environment (36–40). Likewise, when the limb of a salamander, or the liver of a mouse, is partially amputated, the remainder regenerates to its normal size and shape even though the context in which this regeneration occurs is very different from that in which the structure developed initially (43).

Yet it is clear that these trait-autonomous processes are also regulated at a higher level. In insects, developmental hormones such as the ecdysteroids and JHs and external factors such as starvation and extreme temperatures are known to affect whole-animal growth and thus also the final sizes of traits (44–46). Herein we illustrate a third and intermediate level of size regulation, manifest as what appears to be competition among growing traits within late-stage larvae immediately prior to pupation. Superficially, at least, the mechanism of trait competition described herein is similar to the process of cell competition and compensatory growth that operates within compartments of individual imaginal discs. Increased growth of one trait occurs at the expense of growth in other traits, without overall changes in the size of the animal, much as faster-growing cells (e.g., minute⁺ cells expressed in a minute⁻ background) out-compete neighboring cells for space within a growing imaginal disc without affecting the final size of the disc (36, 41, 47, 48).

It will be interesting to see whether mutations in *Drosophila* that affect the sizes of imaginal discs also reveal competition with other traits. At least some of the mutant phenotypes are suggestive. For example, the hyperplastic disc overgrowth mutations (e.g., *fat* or *dco*) lead to the failure of some discs to terminate growth and produce larvae with discs that retain their overall morphology but grow disproportionately large (36, 41, 49). These mutations are almost always lethal, but occasionally pharate adults are produced and these have characteristic morphological abnormalities that include enlarged wings and halteres but also reduced or absent eyes (37–40, 49). Similarly, overexpression of decapentaplegic (*dpp*) causes partial wing duplication but also severe reduction in scutellar size (50). Optic morphology (*Om*) mutations [particularly *Om(1E)*] produce flies with enlarged but structurally normal eyes and appear also to lead to reduction in scutellar size (figure 8 in ref. 51).

Our findings of allocation tradeoff raise two important questions. (i) Why has this phenomenon not been observed in the best-studied holometabolous insect *Drosophila melanogaster*? One reason trait competition may have been overlooked in *Drosophila* is that individual variation in body size is rarely accounted for, making changes in the relative sizes of specific body parts difficult to detect except in extreme cases. For example, recent studies of genetic correlations among body parts in *Drosophila* found large positive correlations among most traits (52, 53). However, these correlations were estimated from measures of the absolute sizes of traits, without taking variation of overall body size into account. Thus the correlation of traits with overall body size (wings and legs of large individuals are larger than wings and legs of small

individuals) may have masked subtler relations among the individual traits. (ii) What is the mechanism by which growth of one trait influences the growth of another? We have been deliberately vague as to the nature of the limiting resource—although our experiments were inspired by theoretical models of nutrient limitation, this is not the only possible mechanism; limitations of any factor whose value varies inversely with the size of a disc could produce the results we observed.

It is significant that the allocation tradeoff is not diffuse or generalized but is concentrated on only one or a few traits. There are several possible developmental mechanisms that could explain the preferential allocation of excess resources to certain tissues and not to others. It is possible that allocation is compartmentalized so that tissues that are physically close to the site of loss receive a disproportionate amount of the excess resource. Alternatively, it is possible that tissues whose growth periods coincide in time share certain resources that are not available to other tissues and, therefore, compete with each other but not with other tissues. Finally, it is possible that fast-growing tissues are resource-limited but slow-growing ones are not, so that a small excess in resources freed up by diminution or removal of one body part only affects the growth of the fastest-growing tissues. Each of these alternatives involves a different developmental mechanism, and experiments can be devised that can differentiate between them. The developmental mechanism of preferential allocation has implication for the evolution of suites of traits. Depending on the mechanism, it may be possible not only to change the strength of the allocation but also to change the allocation partners during evolution. Thus evolution of the allocation tradeoff itself may play a role in the evolution of morphological integration of traits.

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