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DEVELOPMENT AND THE GENETICS OF EVOLUTIONARY CHANGE WITHIN INSECT SPECIES

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■ **Abstract** Changes in genes and in developmental processes generate the phenotypic variation that is sorted by natural selection in adaptive evolution. We review several case studies in which artificial selection experiments in insects have led to divergent morphologies, and where further work has revealed information about the underlying changes at both the genetic and developmental levels. In addition, we examine several studies of phenotypic plasticity where multidisciplinary approaches are also beginning to reveal more about how developmental processes are modulated. Such integrated research will lead to a richer understanding of the changes in development that occur during evolutionary responses to natural selection, and it will also more rigorously examine how developmental processes can influence the tempo and direction of evolutionary change.

INTRODUCTION

Development is central to evolution because the processes of development translate genotypes into phenotypes; thus, developmental changes generate the variation on which natural selection can act—no variation, no evolution. Efforts to integrate evolutionary and developmental biology have gained much impetus in the past decade with the burgeoning understanding of developmental mechanisms. Much of this initiative has involved comparison of the development of major body features (e.g., segments, limbs, eyes) in a small number of model organisms that are widely disparate, both morphologically and taxonomically (Carroll et al. 2001, Davidson 2001, Raff 1996). These studies have discovered differences and also many surprising similarities in the developmental mechanisms employed by different phyla, but they do not address directly the developmental basis of variation in natural populations. Specific genes have been identified as central to the development of a trait through laboratory study of the consequences of major mutations, but does variation in these same genes also underpin variation in the trait arising within natural populations? This review seeks to demonstrate that understanding

development of the phenotype is useful for those ecologists and evolutionary biologists interested in the process of adaptation because insights about development provide the potential for improved predictions about evolutionary change.

Some researchers have recently started to examine the development of subtle differences in morphology among more closely related organisms, including species of flies (Gormpel & Carroll 2003, Kopp & True 2002, Kopp et al. 2000, Simpson 2002, Skaer et al. 2002, Stern 1998, Sucena & Stern 2000, Sucena et al. 2003, True et al. 1999), nematodes (Felix et al. 2000), centipedes (Arthur & Kettle 2001), fish (Peichel et al. 2001), and salamanders (Parichy 1996). An important issue, however, is whether genes underlying the differences between even closely related species also contribute to the phenotypic variance for the same traits within a species. Few studies have attempted to understand directly how developmental mechanisms are modulated in an evolutionary response to selection within a particular species. Evolutionary geneticists study the genetic changes that underlie the differences in phenotype that result from such responses.

Relating genetic variation to modification of the developmental processes that generate the phenotype is becoming one major goal of evolutionary developmental biology, or "evo-devo" (Arthur 2002, Beldade & Brakefield 2002, Stern 2000), and the field will increasingly focus on the origins of ecologically and evolutionarily relevant phenotypic variation within species. The emphasis in current work is on morphological traits but this will grow to include the whole functional phenotype, which also encompasses life history and behavioral and physiological traits. Furthermore, there is likely to be an increasing interest in a multidisciplinary study of the evolution of phenotypic plasticity, which will help to understand the modulation of development (Frankino & Raff 2003, Pigliucci 2001, Schlichting & Pigliucci 1998, West-Eberhard 2003).

Artificial selection is a valuable tool of evolutionary geneticists. It can efficiently screen populations for the allelic variation and combinations of genes (genotypes) that underlie the generation of phenotypes of evolutionary relevance (Barton & Partridge 2000). These selected phenotypes can then be examined, both with respect to their fitness consequences and to the ways in which development has been modified. Scharloo (1983, 1987) was an early practitioner of the latter approach although he was unable to go beyond models of how the underlying developmental processes might have been modulated through genetic change to yield the selected phenotypes. The subsequent rapid expansion in knowledge of developmental mechanisms now allows explicit developmental analyses for at least certain examples of morphological evolution. One potential advantage of artificial selection from the evolutionary perspective is that it focuses attention on standing genetic variation within outbred stocks, which is probably more relevant to understanding microevolutionary processes in nature than is developmental change resulting from novel genetic variation induced by mutagenesis (Haag & True 2001).

The emphasis in this review is on the use of artificial selection, or comparisons between different locally adapted populations, in order to examine the genetic

changes that underlie evolutionarily relevant phenotypic variation. We have limited the scope to work on insect species (which provide some of the clearest examples) and to studies that have at least started to explore how genetic change operates through the modulation of developmental processes. First, we introduce relevant aspects of the process of development and then we focus in some depth on particular case studies of microevolutionary change.

DEVELOPMENT: PRODUCING THE PHENOTYPE

In the development of a multicellular animal, typically from a fertilized egg, dramatic differences gradually appear between the cells. Specific cells will divide rapidly or slowly, equally or unequally, in random or highly predictable orientation. Some cells will enlarge, elongate, move, or die. The cells become spectacularly diverse in structure and function as they differentiate into epidermal, muscle, cartilage, nerve cells, and so on. These cellular differences appear appropriately in time and space, so that coherent and functional tissues and organs are formed, and they all result from selective gene expression (see Carroll et al. 2001, Davidson 2001). All cells of an individual contain all its genes (to a first approximation), but only a characteristic subset of them is active in each cell type, such that the cells will differ in the proteins produced and consequently in their properties. The central feature of development, then, is the coordinated control of gene expression, and this occurs primarily at the level of transcription, by the action of transcription factors (see below). A large number of gene products function as components of the array of developmental mechanisms, often called the toolkit (Carroll et al. 2001), that ensure that gene expression is appropriately controlled in the cells as development proceeds. Any genetic change that alters one of these developmental mechanisms may change the final organism—the phenotype.

In all embryos, differences in gene expression between the cells originate through some combination of two factors: initial heterogeneity within the fertilized egg and local interactions among the growing population of cells (Figure 1*A*). Cell interaction in a developing embryo is mediated by the operation of signaling pathways (as illustrated in Figure 1*B* by the Notch pathway). Typically, a pathway consists of a transmembrane receptor that is activated by binding a specific extracellular ligand, initiating a cascade of protein modifications (e.g., phosphorylation, cleavage) that constitute a signal transduction pathway and end in the activation of a transcription factor. The ligand may be a transmembrane protein on an adjacent cell, or it may be secreted by cells that are nearby (as in the Hedgehog or Wingless pathways) or remote (as in the Insulin or Steroid Receptor pathways). In some cases, the ligand (e.g., a steroid) may directly penetrate the cell to interact with its receptor. The signal transduction pathways may be very complex and they may share components or interact in other ways to integrate the different signals that may be impinging on a cell.

Transcription of a eukaryotic gene is regulated by the binding of transcription factors to short sequences (binding sites) in the associated *cis*-regulatory DNA (Figure 1*B*). A transcription factor will typically have binding sites associated with many target genes and, reciprocally, a given gene will typically have binding sites for many different transcription factors, which will be both activators and repressors and will interact in characteristic ways upon binding to determine whether transcription occurs (Carroll et al. 2001, Davidson 2001). Hence, the *cis*-regulatory DNA acts to integrate the presence of different transcription factors within the cell. Furthermore, a gene's regulatory DNA may be very extensive and is typically organized into discrete and functionally independent modules, enhancers, each of which can initiate transcription in response to the appropriate activators (Davidson 2001). Hence, a gene, which may itself code for a transcription factor or a signaling pathway component, will be expressed in different contexts under different regulatory control, and will be performing different functions as development progresses. Clearly, a mutation in the coding region may alter the gene product, possibly affecting all functions, whereas a mutation in one of its *cis*-regulatory regions may change only one aspect of the expression pattern and affect only a single function (Stern 1998).

From an evolutionary perspective, it is significant that the signaling pathways used by animal cells are ancient and highly conserved, with the complete pathway being homologous across the metazoa, from arthropods to vertebrates (Carroll et al. 2001, Davidson 2001). Also, there are relatively few basic pathways (approximately 20—see Raff 2000) and they are used repeatedly, at different stages and in different parts of the developing embryo. Numerous genes code for transcription factors, but again, many of the major gene families are highly conserved and are recognizable across the animal kingdom. The same genes are, however, typically expressed in many different contexts during development (Carroll et al. 2001, Davidson 2001). Consideration of development suggests that developmentally important genes are likely to show both epistasis and pleiotropy, as the gene products interact in mediating cell interaction and controlling gene expression, repeatedly in the formation of different parts of the organism. Below we examine how changes in these genetic networks can produce evolutionary change in morphology, in particular, by describing three case studies.

EVOLUTION OF BRISTLES IN FLIES

An important issue for evolutionary developmental biology is to understand the origins of evolutionarily relevant variation in the phenotype: What is the genetic and developmental basis of the variation in a quantitative trait, and do the same genes also underlie trait differences between related species? Artificial selection on the phenotypic variation from natural populations can be used to generate large phenotypic differences between the selected lines, facilitating powerful genetic analysis to identify the relevant genes or gene regions. In *Drosophila*, development

is understood in considerable detail and it can be determined whether the identified quantitative trait loci (QTL) do correspond to genes with known functions in development of that trait. A particularly well worked out example of this approach is the analysis of variation in bristle number in *D. melanogaster*.

The bristles of *D. melanogaster* are external sensory organs of the peripheral nervous system that have been studied for many years, from perspectives ranging from development to quantitative genetics. The number of sternopleural (ST) and abdominal (AB) bristles has been advocated as an ideal system for study of the nature of genetic variation (Mackay 1995). It is a classical quantitative genetic character for which ample genetic variation is described (Mackay 1995) and for which major mutations have been documented (Lindsley & Zimm 1992).

Development of Bristles

In *Drosophila*, many genes have known functions in development of the peripheral nervous system and these are plausible candidates for contributing to the standing genetic variation in bristle number. Bristle formation starts with the expression of genes of the *achaete-scute* complex (ASC) in a cluster of cells at a particular location in the epidermis. Detailed analysis has shown that specific enhancer sites in the ASC function to regulate expression for each of the cell clusters and some of the controlling transcription factors have been identified (Ghysen & Dambly-Chaudiere 1988, Gomez-Skarmeta et al. 1995). Expression of the ASC is required for neural development, and this expression gradually becomes restricted to one cell within the cluster through a lateral inhibition process mediated by the Notch signaling pathway (Figure 1). Briefly, binding of the ligand Delta to the Notch receptor results in the activation of transcription factors that upregulate *Notch*, but inhibits expression of the *Delta* and ASC genes. Only one cell of the initial cluster remains uninhibited, producing the ligand and expressing ASC genes, and this cell undergoes subsequent divisions to produce the cuticular bristle and associated sensory neuron. There are thus numerous potential places in the regulatory pathway that could be altered to generate phenotypic change in the bristle pattern.

Quantitative Genetics of Variation in Bristles

Several routes have been taken to finding and identifying the QTL relevant to variation in bristle number (Mackay 2001). For instance, after *P*-element mutagenesis, some of the insertions with large effect on bristle number were mapped to genes known to control either the ASC or the Notch pathway (Lyman et al. 1996). This result is suggestive but does not shed light on the issue of whether these genes are relevant to natural variation (but see Long et al. 2000).

Artificial selection for AB and ST bristle number has been conducted on strains of *D. melanogaster* recently established from the wild (Gurganus et al. 1999, Long et al. 1995). Crosses were then performed between the divergent selected populations, and marker genotypes were analyzed for their phenotype using QTL mapping techniques (Lynch & Walsh 1998). Many QTL were found, with effects

that were variable but which, together, accounted for most of the difference in bristle number between the parental lines (Gurganus et al. 1999, Long et al. 1995). In both studies, most of the QTL mapped to the approximate positions of candidate genes that are components or controlling factors of either the ASC or the Notch pathway in the development of the peripheral nervous system (Gurganus et al. 1999, Long et al. 1995). The two sets of selected lines were further intercrossed to increase recombination in the QTL intervals, and thus improve the precision of mapping (Mackay 2001). In doing so, 26 QTL for bristle number were identified, 20 of which mapped to candidate gene positions (Nuzhdin et al. 1999; see also Dilda & Mackay 2002). In addition, by use of molecular markers and multiple backcrossing, putative QTL from the selected lines have been introgressed singly into homogeneous nonselected genetic backgrounds to attempt to confirm the existence of QTL mapping to specific candidate genes (Mackay 2001).

Another approach has been complementation testing using available mutants for the candidate genes. The rationale is that if the phenotypic effect of the mutant differs between crosses to the high and low selected lines, this failure to complement indicates that the candidate gene is indeed involved in causing the selection response (Mackay 2001). For both the AB- (Long et al. 1996) and the ST-selected lines (Gurganus et al. 1999), mutant alleles at some of the candidate loci failed to complement. Interpretation of these results is complex, however, as the noncomplementing locus may actually be allelic to the QTL in the selected lines, or it may be involved through a genetic interaction (i.e., epistasis). Indeed, mutants of some genes lying outside the QTL intervals did show significant interactions with the selected lines (Gurganus et al. 1999). Overall, the above results can be taken as a first indication of evolutionarily relevant variation in natural populations, which maps to genes with developmental functions that have previously been determined using mutations of large effects. However, even after high-resolution mapping, QTL map to the intervals containing many genes and the one truly affecting the trait may well not be the candidate gene (Mackay 2001).

Linkage disequilibrium mapping (Mackay 2001) was used to approach more closely to the quantitative trait nucleotide (QTN), in other words the polymorphism in the gene that is actually causing the phenotypic effect. Candidate gene regions of interest from wild-type flies were placed in a homozygous genetic background and polymorphisms within the candidate gene were associated with the bristle phenotype of carriers of these polymorphisms. This type of approach has proved successful for several genes [including ASC (Long et al. 2000, Mackay & Langley 1990), *Dl* (Long et al. 1998), *h* (Robin et al. 2002), and *sca* (Lyman et al. 1999)], with polymorphisms at these loci shown to be associated with divergence in bristle phenotypes. It is clear that very few systems allow such an elaborate genetic analysis as *D. melanogaster*. In the bristle example, the QTL mapping approach has drawn a direct connection between genes with a central role both in the development of the trait and in natural variation in that trait [including the genotype by environment interaction (see Gurganus et al. 1998)].

Considerable progress has been made in relating developmental mechanisms to naturally occurring variation in bristle patterns. However, an important disadvantage of bristle traits for evolutionary studies is that we have little understanding of the precise function of the bristles or of the relationship between the trait variation and fitness. In this respect, body size is a more promising trait.

EVOLUTION OF INSECT BODY SIZE

There is substantial (and rapidly increasing) information about the developmental control of growth and size, particularly in *Drosophila* and other insects (see below). Furthermore, body size is closely related to fitness (see Partridge & French 1996) and covaries with traits such as developmental time, growth rate, mating success, and progeny size.

That selection is acting on body size in natural populations is strongly indicated by the observation that genetic, latitudinal size clines have been described for several ectotherms from different taxa: Body size increases with increasing latitude (Endler 1977, Partridge & French 1996). The most convincing data come from studies on the cosmopolitan fruit fly *D. melanogaster*, in which latitudinal clines have been found on all major continents. The genetic and developmental basis of variation in body size has been much studied in *Drosophila* in the context of the geographical size clines and also of laboratory populations artificially selected for body size.

Developmental Control of Insect Body Size

Insects grow through a series of larval moult cycles that are controlled by changing titers of ecdysteroids and juvenile hormone (JH). The size of the adult depends on the dimensions of the cuticle secreted by the epidermis, and this is limited by the changes in hormone levels that occur through the last larval instar, to result in moulting—pupation in the case of holometabolous insects. When the larva reaches a critical size, the level of JH falls, allowing neurosecretory cells to release prothoracicotropic hormone (PTTH), which triggers ecdysteroid release, causing the larva to stop feeding and progress toward the moult (Nijhout 1994a).

The growth rate and final size of developing organs is controlled by organ-intrinsic as well as -extrinsic mechanisms (Bryant & Simpson 1984, Conlon & Raff 1999). In a holometabolous insect such as *D. melanogaster*, the adult epidermis grows as separate imaginal discs that fuse together and replace the larval epidermis during metamorphosis. Growth of the imaginal discs is intrinsically regulated (Bryant & Simpson 1984), as the discs will not grow beyond their normal size, even if pupation is delayed (Simpson et al. 1980). In addition, immature discs cultured in adult female hosts will terminate growth at a cell number close to that normally attained by the time of pupation (Bryant & Levinson 1985). The developing imaginal discs also communicate with the larval neurosecretory system because their damage and subsequent regenerative growth results in an extended

larval period and a delay in the timing of the ecdysone release (Simpson et al. 1980, Berreur et al. 1979).

The intrinsic control of growth (cell number and cell size) is closely related to establishing patterns of cell fate within the developing imaginal disc. Several intercellular signaling pathways have been implicated in these processes. For example, the signals encoded by *decapentaplegic* and *wingless* are produced in specific narrow regions and specify cell fate across different axes of the wing blade. If these signals cannot be transduced the cells cannot grow or divide (Edgar & Lehner 1996), whereas their ectopic expression provokes local cell proliferation and pattern duplication (e.g., Zecca et al. 1996). *Wingless* negatively regulates expression of the *Drosophila* homologue of *myc* and a loss of function mutation in *dMyc* retards the growth and division of disc cells, whereas its overexpression promotes these processes (Johnston et al. 1999).

Apart from extrinsic control by ecdysteroids and intrinsic control by local cell interactions, it has become clear that imaginal disc growth is controlled through the highly conserved insulin/IGF pathway (Leevers 2001, Oldham et al. 2000). As discussed below, this pathway responds to external conditions, such as nutrition availability, to modulate both cell growth and division.

Various manipulations of signal transduction from the insulin receptor (Dinr) can increase or decrease wing size, altering both cell size and number (Leevers et al. 1996, Weinkove et al. 1999). Reduction of receptor activity and its overexpression cause decreases and increases in wing size, respectively, involving both cell size and number (Brogiolo et al. 2001). Members of a family of insulin-like peptides are expressed in various tissues (e.g., the brain and imaginal discs), and the overexpression of one of these putative ligands enlarges the adult, increasing both the number and size of its cells (Brogiolo et al. 2001). Thus, the insulin/IGF pathway regulates cell growth as well as cell division (Leevers 2001), and only one associated gene, ribosomal *S6 kinase*, has been shown to influence only cell size (see Oldham et al. 2000). The gene *chico*, which encodes the receptor substrate component of the insulin pathway, is implicated in fat storage in *Drosophila* (e.g., Bohni et al. 1999). This, together with work in the nematode, *Caenorhabditis elegans* (Guarente & Kenyon 2000), is strongly suggestive that the insulin/IGF signaling pathway plays a significant role in coordinating growth with the nutritional status of the developing larva (but see Oldham et al. 2000, Stern 2003).

Evolution of Body Size in *Manduca Sexta*

Much of our understanding of the hormonal control of insect moulting and metamorphosis comes from detailed studies on the large moth *Manduca sexta* (see Nijhout 1994a). As outlined above, adult size depends on the following variables during the last larval instar: the initial weight, the critical weight (influenced by the initial weight), the growth rate, the delay while JH level falls after attaining critical weight, and a further delay associated with photoperiodicity in PTTH release (which completes feeding and growth). These were all measured in the early

1970s for laboratory stocks recently taken from the wild. Reexamination of size and growth control of the cultures 30 years later showed a dramatic 50% increase in body size (D'Amico et al. 2001). This could be fully explained by increases in the critical weight, the growth rate, and the delay before PTTH could be released, whereas there had been no change in the weight at the start of the final instar or in the photoperiodicity of PTTH release (D'Amico et al. 2001). The genetic changes in body size had presumably occurred in response to changes in factors such as density and parasitism, and perhaps also to inadvertent selection for large individuals for breeding. It is fascinating (but perplexing) why only particular aspects of growth control were affected—increasing growth rate in the final instar, for example, but apparently not in earlier instars (D'Amico et al. 2001).

The *Manduca* study has defined changes in the developmental/physiological control of growth and size, but has not investigated their genetic basis: For this, we need to move to the extensive studies of the evolutionary genetics of body size in *Drosophila*.

Genetic Variation and the Evolution of Body Size in *Drosophila Melanogaster*

In *D. melanogaster*, latitudinal clines in body size have been found across the Middle East to Africa (Tantawy & Mallah 1961), Japan (Watada et al. 1986), North America (Coyne & Beecham 1987), Eastern Europe to Central Asia (Imasheva et al. 1994), Australia (James et al. 1995, James et al. 1997), and South America (van't Land et al. 1999). In all cases, populations from the higher latitudes give the bigger flies, even when all are reared in standard conditions. The similar evolutionary responses to life at higher latitudes and at lower laboratory temperatures implicates temperature as a selective agent on body size (Atkinson & Sibly 1997, Partridge & French 1996). Many climatic factors vary with latitude, but regression analysis for both the Australian (James et al. 1995) and South American (van't Land 1997) clines in *D. melanogaster* has shown the closest correlations to be between temperature, latitude, and body size. Moreover, in two independent studies, laboratory populations of *D. melanogaster* kept at different temperatures show rapid evolution—again toward genetically larger flies at the lower temperatures (Cavicchi et al. 1985, 1989; Partridge et al. 1994). There is also an intriguing parallel with the developmental response to temperature: Across a wide range of ectotherms, including *D. melanogaster*, there is an inverse relationship between rearing temperature and adult body size (see Atkinson 1994, Partridge & French 1996).

Body size in an adult insect depends on the dimensions of the cuticle and thus on the number and size of the underlying epidermal cells. These parameters are most conveniently analyzed on adult wings, where each epidermal cell secretes one hair (or trichome). Trichome counts showed that populations maintained at different temperatures had evolved their different body sizes through changes to cell size, with little or no effects on cell number. Thus, thermal evolution at the lower

temperature produced flies with larger cells (Partridge et al. 1994). Intriguingly, the developmental response of size to rearing temperature was also shown to be mediated by changes in cell size, rather than cell number in the wings (Partridge et al. 1994), and also in the eyes and legs (Azevedo et al. 2002).

In contrast to the laboratory responses to temperature, size differences along the geographical clines involve both cellular parameters. In the Australian populations, the increase in size with latitude is mainly caused by an increase in the number of cells (James et al. 1995, Zwaan et al. 2000). In the South American cline, however, more than 40% of the size increase with latitude is caused by increased cell size, as compared with less than 20% in the Australian cline (Zwaan et al. 2000). Comparable differences have been found in the cellular basis of recently established clines for wing size in *D. subobscura* in North and South America (Calboli et al. 2003). These results indicate that increased body size (and wing size) is being selected for at lower temperatures, but the precise cellular basis is less important (Zwaan et al. 2000). Body size can be readily altered in the laboratory by artificial selection. Two independent selection experiments, targeting thorax length or wing area and using different base populations, both showed that an increase in fly size was achieved by increasing the number of cells, whereas the response to selection for a smaller adult was a reduction in cell size (Partridge et al. 1999). This interesting result suggests that, in establishing the Australian cline, selection from the founding population could have been mainly in the direction of an increase in body size, whereas in the expansion to form the South American cline, selection was for both smaller and larger body size (Zwaan et al. 2000) (Figure 2).

Why do animals evolve to larger sizes at lower temperatures? Much of this topic is beyond the scope of this review (see Partridge & French 1996), but some points are directly relevant here. For instance, fat content and starvation resistance showed no correlation with latitude for the South American cline (Robinson et al. 2000), suggesting that, although overall size varies, body composition of the flies does not. Moreover, in both the thermal evolution laboratory lines and the populations from the Australian and South American clines, growth efficiency was higher for the populations that had adapted to lower temperatures (Robinson & Partridge 2001). This increased growth efficiency may result from differences in the acquisition and allocation of resources, producing the larger body size and shorter development time in a trade-off against other phenotypic characteristics. Such trade-offs need to be pinned down to specific developmental and physiological mechanisms (see Leroi 2001). Because the *Drosophila* insulin/IGF pathway is now known to regulate growth in relation to food availability (see above), genes associated with this pathway are plausible candidates for variation between lines differing in body size.

QTL mapping approaches have been taken to identify genetic variation associated with the size differences that have evolved among different geographical populations. In the comparison between populations at the two ends of the Australian cline, QTL were found on the second and third chromosomes (Gockel et al. 2002), including a continuous stretch of the third chromosome that contains genes from the insulin/IGF signaling pathway. Previously, five microsatellite loci were

shown to vary with latitude (Gockel et al. 2001), but none of these loci was significantly associated with QTL [probably because of the low resolution (see also Merila & Crnokrak 2001)].

In analyzing the lines resulting from artificial selection on wing size, significant QTL were found on all major chromosomes and, again, some of these mapped to positions of genes in the insulin/IGF signaling pathway (B.J. Zwaan, B. Seifeid, G. Gedes & L. Partridge, unpublished results). The asymmetry in the cellular response in this selection experiment is highly relevant, as it might be anticipated that the large and small lines would identify genes with variation affecting cell number and cell size, respectively. Indeed, the QTL for large and small wing size do not map to the same positions. In selection experiments for fitness traits, asymmetrical responses are predicted (Roff 1997), and these may often be caused by variation in different genes contributing to the responses in different directions. It was also found that QTL from the lines selected for high or low bristle number did not correspond (Nuzhdin et al. 1999).

These studies of the evolution of body size in insects have thus explored genetical, developmental, and functional issues using variation among both natural and laboratory populations. They have detected examples of differences in the developmental mechanisms underlying examples of comparable changes in phenotype. Another variable morphological trait that has proved amenable to a multidisciplinary approach to understanding phenotypic diversity is the eyespot patterning on the wings of many species of butterfly.

EVOLUTION OF WING COLOR PATTERN IN BUTTERFLIES

The wings of butterflies and moths display striking, and often very intricate, patterns of colored scales that show great diversity across species and frequently also vary within a species (Nijhout 1991). The ultimate aim of studies on the evolutionary genetics and development of butterfly wing patterns is to understand the processes involved in generating the morphological diversity of color patterns observed in present-day species.

The lepidopteran wing originates as an internal imaginal disc within the larva, and as it grows by cell division and extension in the late larva and pupa, the cells acquire their different developmental fates with respect to subsequent scale formation and pigment synthesis (Nijhout 1991). Rows of specialized scale cells differentiate within the epidermal cell layer that forms each surface of the pupal wing, and each scale cell develops a large protrusion. A color pattern arises at late pupal stage, just before adult eclosion, as the cells at different locations on the wing surface synthesize and deposit different pigments in their scale cuticle. A long tradition of comparative analysis of wing color patterns, especially within the Nymphalid butterflies, has led to the concept of a groundplan (see Beldade & Brakefield 2002; Nijhout 1991, 2001) characterized by pattern elements such as

transverse bands, chevrons, and eyespots. There has been much brilliant research on the evolutionary genetics of several types of wing pattern elements, especially those involved in the evolution of mimicry in species of *Papilio* and *Heliconius* (see Joron & Mallet 1998, McMillan et al. 2002, Nijhout 1994b). Unfortunately, current knowledge of development of pattern elements is largely limited to the eyespots (Beldade & Brakefield 2002, Brakefield & French 1999). Each eyespot consists of concentric rings of color, usually surrounding a central pupil located midway between wing veins. Eyespots can function in startling predators (Blest 1957), in deflecting predator attacks away from the vulnerable body (Lyytinen et al. 2003, Wourms & Wasserman 1985), and also in mate choice (Breuker & Brakefield 2002).

Evolutionary research on butterfly eyespots has built on earlier studies in ecological genetics on *Maniola jurtina* (Brakefield 1984, Brakefield & Shreeve 1992, Ford 1964). Information was gathered about patterns of phenotypic variation and its genetic basis, and about consequences for fitness in natural populations. However, as for many comparable studies, this system eventually proved frustrating because the mechanisms by which the phenotypes mapped onto genotypes remained elusive. Study of eyespot development began with Nijhout's (1980) demonstration of the signaling role of the center of a developing eyespot in *Precis* (= *Junonia*) *coenia* (see below). This work has been extended by use of new systems and by combining surgical manipulations with studies of gene expression patterns and the application of the tools of quantitative genetics (Beldade & Brakefield 2002).

Development of Eyespots

Surgical experiments, studies of gene expression, and analyses of wing pattern mutants in *Bicyclus anynana* have suggested that development of the butterfly eyespot proceeds by the initial specification of a central focus, followed by signaling to the surrounding cells and their subsequent synthesis of specific pigments (Brakefield et al. 1996, Brunetti et al. 2001).

Many genes are known to regulate wing development in *Drosophila* and study of their homologues has suggested that several of them have evolved additional functions in eyespot formation in butterflies (Carroll et al. 1994). For example, *Distal-less* (*Dll*) is expressed along the margin and in each subdivision of the wing disc in mid last larval instar (as in *Drosophila*), but then strong expression persists only in groups of cells that correspond to the centers of the future eyespot patterns (Figure 3). Hence, focus formation correlates with *Dll* (and *engrailed*) expression and this appears to be established as a response to signals provided by *hedgehog* expression in flanking cells (Keys et al. 1999).

Ablation and transplantation of small regions of early pupal wing epidermis demonstrate that signals from the focus instruct the surrounding cells to form the eyespot pattern (Brakefield & French 1995, French & Brakefield 1995, Nijhout 1980). At this stage, several regulatory genes become expressed in nested rings around the focus, corresponding to the different fates of cells in forming the color

pattern (Brunetti et al. 2001). The genes (e.g., *engrailed* and *spalt*) encode transcription factors that may control pigment synthesis, as strikingly illustrated by comparison of the gene expression and eyespot phenotypes of the wild-type and *Goldeneye* mutant of *B. anynana* (Figure 3). In several different butterfly species, the same transcription factors are expressed in the developing eyespot fields, but in different relative spatial domains and different relationships to the eyespot color scheme (Brunetti et al. 2001).

These observations show that genes (e.g., *engrailed*) involved in early establishment of the eyespot foci may also play later roles following focal signaling, and they also indicate a remarkable flexibility in the regulatory interactions downstream of focal signaling. This may have facilitated the diversification in the color composition of eyespots, perhaps following the evolutionary novelty (in some group of basal Lepidoptera) of forming a focus and a simple response, resulting in an undifferentiated spot pattern (Brunetti et al. 2001).

None of the genes known to be expressed in the focus encode an intercellular signal, so there is no direct information on the mechanism of focal signaling. The results of surgical experiments are broadly compatible with the simple gradient model: The focus produces a diffusible morphogen, the declining levels of which specify rings of future pigment synthesis over the surrounding wing epidermis (see Beldade & Brakefield 2002, Nijhout 1990, French & Brakefield 1995). Focal effects can extend for at least 100 cells across the early pupal wing epidermis, however, and this is farther by an order of magnitude than the demonstrated range of any intercellular patterning signal (e.g., in *Drosophila*). Thus, the mechanism by which the focus patterns the entire eyespot is likely to prove more complex than a single, long-range signal.

Linking Genetic Variation and Eyespot Development

Although the study of several spontaneous mutants of *B. anynana* has given information on eyespot development (Brakefield 1998, Brunetti et al. 2001, Monteiro et al. 2003), evolutionary genetical work has focused on the application of artificial selection.

The wild-type dorsal forewing of *B. anynana* has a small anterior and a large posterior eyespot, each with a central white pupil, a broad black disc, and an outer narrow gold ring (Figure 4*B*). Directional selection has been applied in both upward and downward directions to several features of the large posterior eyespot. Eyespot size and color composition have both shown progressive responses to selection with heritabilities of approximately 50% (Monteiro et al. 1994, 1997a). The analysis of crosses between lines selected for ventral eyespot size suggest that 5–10 genes are involved in producing highly divergent phenotypes (Wijngaarden & Brakefield 2000). These observations all show that eyespot size and coloring behave as classic morphometric traits, but one other eyespot feature does behave differently in terms of genetic variation: Shape shows much lower heritability in lines selected for eyespots ellipsoidal in either of their axes (Monteiro et al.

1997b,c). This result may indicate some developmental constraint on producing asymmetry in the focal signal or the epidermal response.

There is very little genetic correlation between eyespot size and color composition, as pairs of lines obtained by artificial selection had diverged only for the target feature of the eyespot. Although these features show closely similar genetic properties, reciprocal transplantations demonstrate a clear difference in their developmental basis (Beldade & Brakefield 2002; Monteiro et al. 1994, 1997a). When a focus is grafted ectopically between pupae from different selected lines, the size of the resulting eyespot is largely dependent on the identity of the donor (i.e., of the grafted focus), whereas its color composition depends on the identity of the responding host animal. Thus differences in eyespot size are attributable mostly to changes in properties of the focal signal, whereas those in color depend entirely on the sensitivity thresholds of the responding cells.

Linking Development to Specific Allelic Variation

Studies of gene expression patterns in developing butterfly wings have implicated some genes (e.g., *Dll*) and some developmental pathways (e.g., Hedgehog signaling) in eyespot formation, although direct evidence of their function awaits the use of methods of manipulating gene expression. Studies of gene expression and function cannot, however, identify those genes contributing to phenotypic variation that could be the basis of evolutionary change in eyespot pattern. The crucial issue in evolutionary developmental biology of identifying such genes has now also been examined in *B. anynana*.

Dll expression in late larval and pupal wings is associated with eyespot foci and the expression pattern changes in parallel with shifts in adult eyespot morphology (Brakefield et al. 1996) (Figure 3). In lines of *B. anynana* selected for the size of both dorsal forewing eyespots (see Figure 4*B*), *Dll* expression patterns have also diverged (Beldade et al. 2002a). Such correlations could occur through upstream genetic and developmental changes, but the study by Beldade et al. (2002a) tested whether variation in the *Dll* gene contributes directly to the responses in these selected lines. Informative molecular polymorphisms were identified in this gene and then F2 individuals from crosses between the selected lines were scored for both the parental origin of their *Dll* alleles and their eyespot size. In several crosses there was a clear association between the *Dll* genotype and eyespot phenotype, providing strong evidence that variation mapping to this gene contributes to phenotypic variation of potential relevance to evolutionary change within *B. anynana*. It is probable, but not yet demonstrated, that this variation lies in the *cis*-regulatory regions of the *Dll* gene.

Eyespot Development and the Flexibility of Morphological Change

In *B. anynana*, all developing eyespots have shown the same patterns of gene expression (Brunetti et al. 2001), and both forewing eyespots behave similarly in

response to transplantation or ablation experiments (Brakefield & French 1995, French & Brakefield 1995). Furthermore, most single mutations affect all eyespots (Figure 3) (Brakefield 1998), and selection on one specific eyespot results in concerted changes in the other eyespots, especially those on the same wing surface (Beldade et al. 2002b, Beldade & Brakefield, 2003, Monteiro et al. 1994). The developmental coupling of the eyespots suggested that, although parallel changes could be readily accomplished by selection, it might be more difficult to uncouple eyespots or change them in different directions (Brakefield 1998). When this prediction was tested by selection on both the small anterior and large posterior forewing eyespots, however, both coupled and uncoupled changes were readily achieved (Beldade et al. 2002c). Final phenotypes after 25 generations were widely divergent in all directions of selection from the phenotypic range of the original base population (Figure 4*B*). This was especially noteworthy in one of the uncoupled directions (large anterior, small posterior eyespot) because none of the 80 or so extant species of *Bicyclus* show this phenotype (Figure 4*A*). Thus, although this phenotype might be suspected of being a "forbidden morphology," the experimental result indicates that its absence in nature is more likely to have resulted from lack of appropriate natural selection in any lineage.

Although eyespots share a common developmental mechanism, the pattern in relative size of the dorsal forewing eyespots can readily move through morphospace under appropriate selection regimes (Figure 4*B*). This result demonstrates variation in genes that differentially modify the causal mechanism that underlies eyespot formation, giving the eyespots individuality within the overall wing pattern. Analysis of eyespots on the other wing surfaces of the selected butterflies indicates a modular organization of the pattern (Beldade et al. 2002b, Beldade & Brakefield 2003). The ventral hindwing has a full series of seven eyespots, which show a conserved pattern of relative size in wild-type *B. anynana*. In response to the regimes of selection on the dorsal forewing, parallel changes occurred in the eyespot sizes on the ventral hindwing: The four most anterior changed in the direction of the anterior forewing eyespot, whereas the more posterior (especially the most anal two) shifted with the posterior forewing eyespot. Current work is exploring the genetic and molecular basis of this individuality and modularity in eyespot development (Monteiro et al. 2003). The flexibility apparent in the response to selection on eyespot size in *B. anynana* may indicate a long legacy of natural selection favoring the evolution of divergence among the eyespots, or subsets of eyespots, in butterflies (Beldade et al. 2002b).

Beyond the Eyespot

The examination of eyespot development has partly bridged the gap from genes to the eyespot phenotype and its functions in particular environments. We have restricted the discussion of lepidopteran wing patterns to studies concerned with the eyespot because, unfortunately, there is no comparable information about the development of other types of pattern element. From comparative analysis,

Nijhout has argued that signaling foci underlie formation of the colored bands and patches found in the much-studied, polymorphic and mimetic species of *Papilio* and *Heliconius (*Nijhout 1991, 1994b). However, these foci have yet to be demonstrated. In these species, allelic differences at a few major pattern loci give dramatic shifts in wing pattern (for references, see Nijhout 1994b, McMillan et al. 2002). When more is known about the mechanisms by which the *Papilio* and *Heliconius* patterns develop, and when the major loci become amenable to molecular study, we can hope for an exciting and fuller understanding of the adaptive evolution of mimicry. Furthermore, it will be fascinating to be able to compare the genetic and developmental mechanisms of evolutionary change in butterfly wing patterns with those of other morphologies, including bristle patterns in Diptera (Gompel & Carroll 2003, Simpson 2002, Stern 1998, Sucena & Stern 2000, Sucena et al. 2003) and the melanic wing patterns of species of *Drosophila* (True et al. 1999).

PHENOTYPIC PLASTICITY

Phenotypic plasticity is variation across environments in the phenotype developed from a given genotype. Its interest for ecologists and evolutionary biologists lies in being a means of adaptation to divergent environments, and it provides insight into understanding control of the stability of developmental mechanisms (Frankino & Raff 2003, Pigliucci 2001, Schlichting & Pigliucci 1998). From a genetic perspective, there is interest in the possibility of specific regulatory genes that mediate plasticity (genes for plasticity) and in how they might function. Few analyses of phenotypic plasticity in insects have included any examination of how developmental pathways are modulated. Here we discuss studies that illustrate the potential for a multidisciplinary approach, from genetic variation through hormonal regulation of development, to phenotype and function.

A particularly striking mode of phenotypic plasticity is polyphenism, where development can produce discrete, alternative phenotypes. In seasonal polyphenism, changing environmental cues lead to alternative adult phenotypes being produced by generations developing at different times of the year. There are several dramatic examples in butterflies (Shapiro 1976), including *B. anynana* (Brakefield 1997, Brakefield & French 1999, Beldade & Brakefield 2002).

Physiological Control of Polyphenic Development

In *B. anynana*, adults of the wet season form (WSF) have ventral wing surfaces with a pale band and conspicuous eyespots that may function to deflect bird attacks away from the vulnerable body (see Lyytinen et al. 2003). In contrast, dry season form (DSF) butterflies are uniformly brown in color, almost lacking ventral eyespots, and they rely on camouflage among brown leaves for survival. The results of field studies demonstrate that seasonal polyphenism in *Bicyclus* butterflies is adaptive (see Brakefield & French 1999). Field surveys (Brakefield & Mazzotta

1995, Brakefield & Reitsma 1991, Windig et al. 1994), together with controlled rearing experiments (Kooi & Brakefield 1999), reveal that temperature provides the predictable cue for the adult environment. Larvae experiencing high temperature develop as WSF, whereas those in cooler conditions form DSF adults.

Ecdysteroid hormones mediate the development of the seasonal forms in *B. anynana* (Koch et al. 1996), as well as those of some other species of butterfly (Koch 1992). The increase in ecdysteroid titer after pupation occurs at a later stage in pupae of the DSF of *B. anynana* than in those of the WSF. When animals are reared to produce the DSF and then microinjected as young pupae with ecdysteroid, the adult wing pattern is shifted toward the larger eyespots characteristic of the WSF (Koch et al. 1996). Understanding precisely how larval rearing temperature influences the secretion of ecdysteroids, and how the ecdysteroid titer in the early pupa then regulates eyespot development [and only on the ventral wing surface (see Brakefield et al. 1998)], are exciting challenges for the future.

The butterfly, *Precis* (= *Junonia*) *coenia*, also shows seasonal polyphenism, producing two forms differing in dorsal wing color. Rountree & Nijhout (1995a) found that response to photoperiod in the larval stage leads to differences in ecdysteroid titer in early pupae, and then to the divergent adult phenotypes. They also isolated a mutant that constitutively expresses only one phenotype (Rountree & Nijhout 1995b) and showed that the gene does not affect the endocrine system but alters the developmental response to the hormone in early pupae. Variation in the mode of control of such patterns of polyphenism is characteristic of perhaps the most intensively examined system, that involving variation in wing development in crickets (for general review, see Zera & Harshman 2001).

In the sand cricket, *Gryllus firmus*, there are two distinct phenotypes, a long-winged morph capable of flight (LWM) and a short-winged, flightless morph (SWM). The LWM has well-developed flight muscles, high lipid reserves, and underdeveloped ovaries, whereas in the SWM wing muscles are underdeveloped and there are low fat reserves but large ovaries. This polyphenism is thought to be a functional response to conflicting life history demands: reproduction or dispersal (Zera & Denno 1997). In different species and sometimes within species, the alternative phenotypes can be caused directly by genetic polymorphism, induced exclusively by environmental cues (such as crowding, temperature, photoperiod) or involve both mechanisms (Zera & Denno 1997). Although the morphs of *G. firmus* are discrete in phenotype, expression of wing morph is under polygenic control (Roff & Fairbairn 1999). Juvenile hormone (JH) is a key gonadotropic hormone in insects (Nijhout 1994a), and the differences in ovarian (and wing muscle) development between morphs may be caused by differences in JH titer (Zera & Cisper 2001, Zera & Huang 1999, Zera et al. 1998). In addition, ecdysteroid titers in the first week of adulthood are significantly higher in the SWM than in the LWM (Zera & Bottsford 2001). The dynamics and interaction between the hormones is complex, and more experimental work is needed to fully understand morph expression in this system (Fairbairn & Roff 1999, Zera 1999).

Within adults of both morphs, ovary and muscle size respond to feeding conditions (Roff & Gelinas 2003), and some LWM females change their phenotype through histolysis of their flight muscles and increase their ovarian growth and fecundity. Such histolyzed phenotypes can also be induced by topical application of a JH analogue (Zera & Cisper 2001), suggesting that there may be a common hormonal control of these suites of traits and that perhaps the same genes are responding both in juvenile development and in adult change.

Hormonal regulation of polyphenic variation has also been investigated in numerous other insects including species of aphid and hymenoptera (see Nijhout 1994a, 1999; Abouheif & Wray 2002). There have, however, been few studies in which geographical variation in polyphenism has also been examined. One such system is the development of horns in dung beetles, including the species *Onthophagus taurus*. Male beetles vary continuously in body size as a function of larval feeding conditions (Moczek 1998), and only males that exceed a critical size eventually form a pair of long, curved horns on their adult heads (Moczek & Emlen 1999). Smaller beetles, including females, remain almost hornless. Horns are used to prevent access to females, and the hornless males adopt different tactics to acquire matings (Emlen 1997, Moczek & Emlen 2000). Experiments involving the application of JH indicate that the horn dimorphism is controlled by threshold responses to JH titer at sensitive periods during the last larval instar (Emlen & Nijhout 1999, 2001).

O. taurus is endemic to the Mediterranean basin but was introduced in the 1960s to the eastern United States and, as part of a program to control cow dung, to Australia (Moczek & Nijhout 2002, Moczek et al. 2002). These introduced populations have now diverged in the critical body size above which horns are developed in the male beetles, probably in response to differences in ecology and levels of competition (Hunt & Simmons 1998). Evolution of this difference has been shown to involve both a change in sensitivity of the head tissue to JH and a change in the timing of the sensitive period during which the hormone titer is monitored (Moczek & Nijhout 2002, Moczek et al. 2002). It will indeed be exciting if the relevant genetic differences between the populations can be identified and to determine whether they are the same as those that underlie similar changes in critical size produced under artificial selection in a related beetle (Emlen 1996). Furthermore, there is an exciting challenge to be met in determining whether the hormone response can be linked directly to the developmental mechanisms of horn formation.

Genetics and Development of Polyphenism

Artificial selection has been used in *B. anynana* to survey genetic variation available for the evolution of phenotypic plasticity in wing pattern. Although field populations show classical seasonal polyphenism with rather discrete phenotypes (Windig et al. 1994), laboratory experiments demonstrate that the underlying reaction norms are continuous in form (Brakefield & Mazzotta 1995). Quantitative variation in ventral eyespot size at a single rearing temperature has provided the basis for artificial selection on plasticity. In general, the response is rapid and

heritabilities are high, and there are positive genetic covariances between the target and the other ventral eyespots, and for the same eyespot across rearing temperatures (Holloway & Brakefield 1995, Holloway et al. 1993). In selection experiments that progressively increased (low line) or decreased (high line) rearing temperatures over the generations, the high line eventually developed the WSF phenotype across all temperatures, although plasticity remained (with high temperatures giving larger adult eyespots). In sharp contrast, the low line produced only butterflies lacking eyespots (DSF) at all temperatures (Brakefield et al. 1996).

Surveys of hormone titers indicate that these selected lines show a difference in the timing of the pupal ecdysteroid peak, similar to that seen between the unselected stock when reared at high and low temperature (Koch et al. 1996, Brakefield et al. 1998). The different seasonal phenotypes, whether genetically or environmentally determined, are associated with a divergent pattern of *Dll* gene expression in the wings of one-day-old pupae (Brakefield et al. 1996). This follows the time when surgical experiments indicate that the focus signals to specify the eyespot pattern.

Following selection on the ventral eyespot pattern in *B. anynana*, pairs of lines diverged (sometimes dramatically) in the elevation of their reaction norms. A response in the degree of phenotypic plasticity will, however, necessitate a change in reaction norm shape, which is only possible where there is genotype by environment interaction. Recent experiments targeting shape per se failed to yield either substantially steeper or shallower reaction norms, or ones of divergent shape (Wijngaarden & Brakefield 2001, Wijngaarden et al. 2002). Extreme changes in reaction norm elevation can evolve rapidly but, presumably owing to positive genetic covariances across environments, the same is not true for shape. It is not clear why this should be (see Wijngaarden et al. 2002), but interestingly, a recent genetic analysis of plasticity of wing and ovary size in relation to food supply in the cricket, *Gryllus assimilus*, has also indicated genetic variation mainly in the elevation and not in the slope of the relationship (Roff & Gelinas 2003, see also Emlen 1996).

For many of these examples of plasticity in insects, we need a deeper understanding of the developmental mechanisms—of the ways in which environmental stimuli and/or genetic variation influence the changing hormone concentrations and tissue sensitivities, and how these then regulate formation of various aspects of the phenotype (Brakefield et al. 1998, Nijhout 1999). It may then become clearer how particular instances of polyphenism and phenotypic plasticity have arisen and why they may be able to evolve more readily in some directions than in others.

DISCUSSION

Here, we discuss three areas of interest that follow on from the case studies we have reviewed and that represent future aims of, and challenges for, evolutionary developmental biology.

First, mapping of quantitative trait loci (QTL) has already provided a rich source of data on the genetics of complex traits (Mackay 1995, 2001). We review several of

the studies of morphological traits that have begun to associate QTL variation with genes of known function in the development of the trait. Such an integration of data from gene mapping with known developmental pathways should gather pace as more systems become accessible to developmental investigation, gene expression studies, and genome-wide screens. This will give a more detailed mechanistic basis for properties of genes and genetic pathways, including epistasis and pleiotropy. Looking further ahead, a fusion of evolutionary developmental biology, quantitative genetics, and evolutionary functional genomics should provide a much deeper understanding of how functional phenotypes map onto genotypes in a variety of organisms. As this knowledge grows, mathematical modeling will become increasingly attractive as an approach to explore further the evolutionary dynamics of known gene networks and developmental processes (Rice 2002). Nijhout & Paulsen (1997) provided an early example based loosely on eyespot formation in butterflies, involving a one-dimensional diffusion gradient and threshold model and incorporating genetic variation for six developmental parameters. Although the biology was necessarily oversimplified, observations of emergent properties of the model illustrate the potential power of such approaches.

Second, complex morphological traits have numerous phenotypic dimensions. For such traits one can ask whether the generation of evolutionarily relevant variation in different features (e.g., size, pattern, or shape) of the trait is under the control of the same set of genes or whether different genes are involved. Similarly, are essentially independent developmental pathways involved, or is a single pathway regulated in a modular manner? Such ideas are implicit in thinking about developmental flexibility and its relationship to evolvability, or the capacity of a lineage to evolve (Kirschner & Gerhardt 1998, Leroi 2000, Wilkins 2002). Again, an increasingly detailed understanding of the genes and developmental processes involved in generating specific morphological and other phenotypic traits will enable increasingly robust predictions about the evolutionary dynamics of such modular structures (Wagner 1996, Wagner & Altenberg 1996). This goal is perhaps closest for certain morphological patterns comprising iterative repeats of a unit element such as the butterfly eyespot or *Drosophila* bristle. Two other examples from case studies we have covered in this review illustrate how developmental insights are likely to uncover important properties of evolvability. The two features of a butterfly eyespot, size and color, behave similarly with respect to the short-term responses to artificial selection, but their underlying development is very different (see Beldade & Brakefield 2002). Thus, although the estimates of genetic variances alone would lead to similar predictions about evolvability, the developmental changes may suggest different dynamics, at least for long-term responses to selection. Similarly, the asymmetry evident in the developmental mechanisms underlying selection responses for increased or decreased body size in *Drosophila* is also likely to have consequences for patterns of evolutionary change in this trait in natural populations.

Third, we have focused on case studies that reveal both genetic and developmental mechanisms of microevolutionary change, rather than on those underlying

divergence across species or among phylogenetically distant lineages. Indeed, researchers who compare convergent patterns of morphological evolution (both loss and gain of structures) across related species or groups of species are also beginning to explore the predictability of the underlying changes in development (e.g., Abouheif & Wray 2002, Gompel & Carroll 2003, Kopp et al. 2000, Stern 1998, Sucerna et al. 2003, True et al. 1999). There is, however, an additional issue: Do the genetic changes that occur in such microevolutionary responses to selection involve essentially the same sets of genes and developmental pathways as those that underlie more macroevolutionary patterns of divergence? The examples we have discussed begin to suggest that some, perhaps most or even all, of the evolutionary change in morphology observed within species also maps to genes with central developmental functions, and perhaps to their regulatory elements (see also Stern 2000).

CONCLUDING REMARKS

Do ecologists and evolutionary biologists interested in adaptive evolution need to address developmental mechanisms at all, and if so, why? A more explicit way of formulating this question is to ask whether an understanding of the role of development in generating evolutionarily relevant variation in the phenotype can provide the potential for improving predictions of evolutionary change. We believe that further multidisciplinary analysis of examples of phenotypic variation of the type we have discussed will demonstrate that developmental, as well as genetic, insights are indeed important for predicting the paths of adaptive evolution and for understanding properties of evolvability. In particular, phenotypes map onto genotypes through the mechanisms of development, and study of the properties of development will yield additional information that is not available from estimates of genetic variances and covariances alone.

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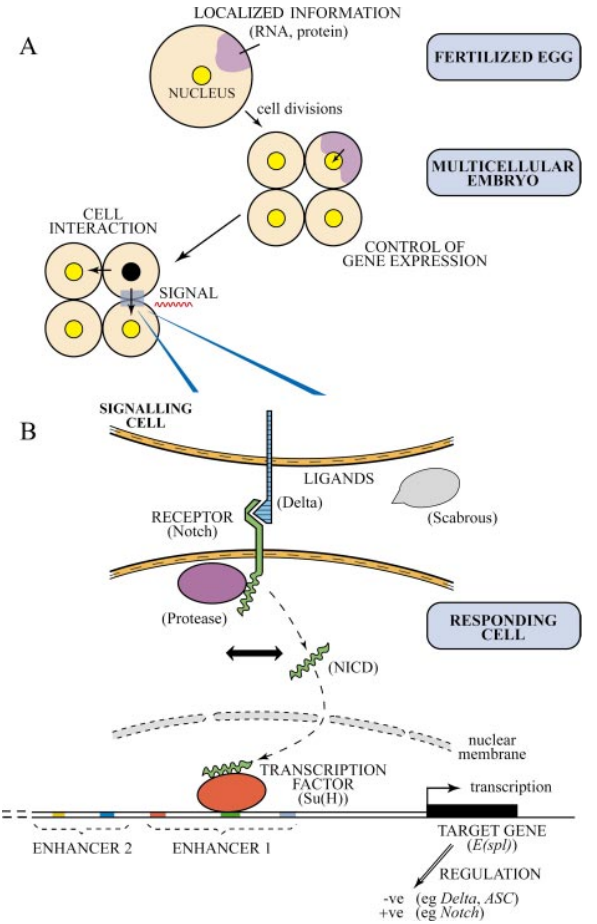

Figure 1 Developmental mechanisms. (*A*) Cartoon illustrating the two major ways in which cellular differences arise within an embryo. Typically, transcripts and/or proteins are localized in the egg cell as it forms in the ovary, and these control gene expression in embryonic cells derived from that portion of egg cytoplasm. Differences are then elaborated through cell signaling; signals may affect only immediate neighbors (as shown) or have different effects, depending on distance. (*B*) Cell signaling, illustrated by the conserved Notch pathway. The Notch receptor is unusual in being able to bind several ligands, including transmembrane Delta and secreted Scabrous, and in responding by being cleaved, generating a free intracellular fragment (Nintra or NICD) that enters the nucleus and activates the transcription factor [Su(H)]. This complex can then bind to specific sites (*color*) on enhancers in the *cis*-regulatory region of target genes [such as *E(spl)*], activating their transcription. In a more typical signaling pathway (e.g., Hedgehog) the bound receptor remains intact and initiates changes in a complex signal transduction pathway. Different signaling pathways interact extensively within the cell (*double-headed arrow*). Notch signaling inhibits neural development in the responding cell, as the transcription factor E(spl) then represses *Achete/Scute* (ASC) and *Delta* genes, while upregulating transcription of *Notch* itself.

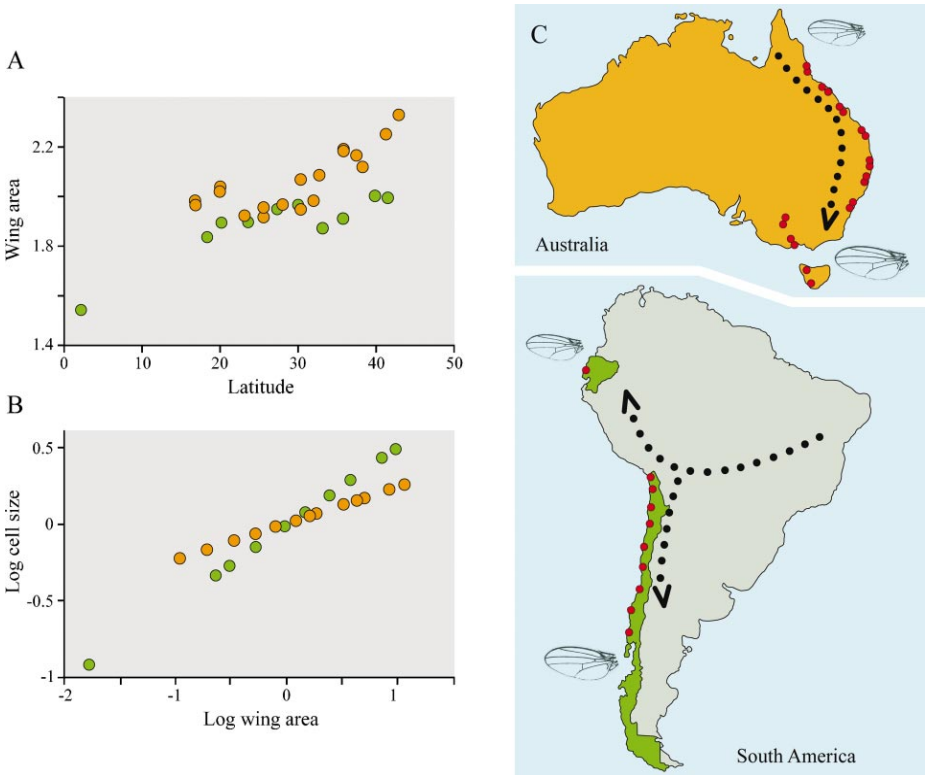

Figure 2 Size variation of *Drosophila melanogaster* along latitudinal clines. (*A*) Strong latitudinal clines for wing size are found on both the Australian (*orange*) and South American (*green*) continents. Flies from the different populations [red circles in (*C*)] were reared under standard temperature conditions. Rearing temperatures were different for the Australian (18°C) and the South American (25°C) populations, causing the general differences in size between continents in (*A*). Therefore, for (*B*), values for the traits were first standardized within continent. (*B*) The slope of the relationship between log(cell size) and log(wing area) is an estimate of the contribution of cell size to variation in wing area. Clearly, in South America (*green*), cell size contributes much more to the latitudinal variation in size than in Australia (*orange*). Log(wing area) and log(cell size) were first individually regressed on latitude and the predicted values saved. These predicted values are plotted in the graph to avoid confounding effects of interpopulation deviation from the clinal relationship. (*C*) The sample sites of *D. melanogaster* populations on each continent are shown as red circles, together with the inferred colonization routes by *D. melanogaster* in black (David & Capy 1988). Data are taken from James et al. (1995) and Zwaan et al. (2000); only those for females are shown, but closely similar patterns were observed for males.

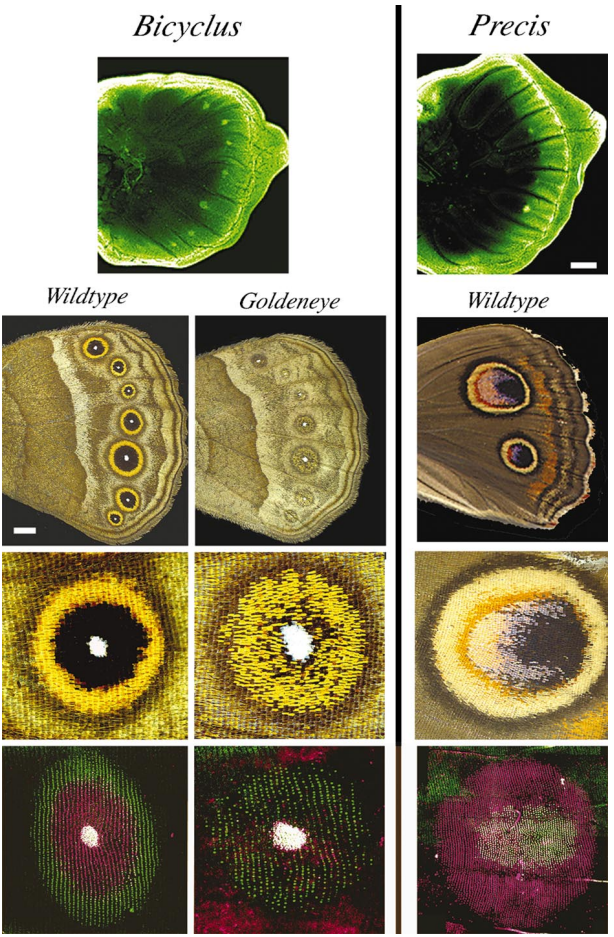

Figure 3 Butterfly eyespot formation and gene expression patterns. Left and right parts show the species *Bicyclus anynana* and *Precis* (= *Junonia*) *coenia*, respectively, with additional images for the *Goldeneye* mutant of *B. anynana*. The top row shows hindwing imaginal discs from wild-type final instar larvae, antibody stained to reveal *Distal-less* (*Dll*) expression (scale bar = 0.4 mm). Note the spots of strong *Dll* expression that correspond to the future signaling foci and the position of eyespots in the adult hindwing (second row, scale bar = 2 mm). The third row shows individual eyespots on the adult hindwing with, below, double labeling 16 h after pupation revealing rings of expression of *engrailed* (*en*)/*invected* (*green*) and *spalt* (*purple*). Both proteins are coexpressed in a central spot (the focus) in *B. anynana*, and the mutant also shows a change in expression corresponding to a near absence of black scales in the adult eyespot. The relationship between the expression of *en* and *spalt* and the scale pigmentation differs across species. Images from Brakefield et al. (1996) and Brunetti et al. (2001) courtesy of Craig Brunetti, Sean Carroll, Julie Gates, Steve Paddock, and Jayne Selegue.

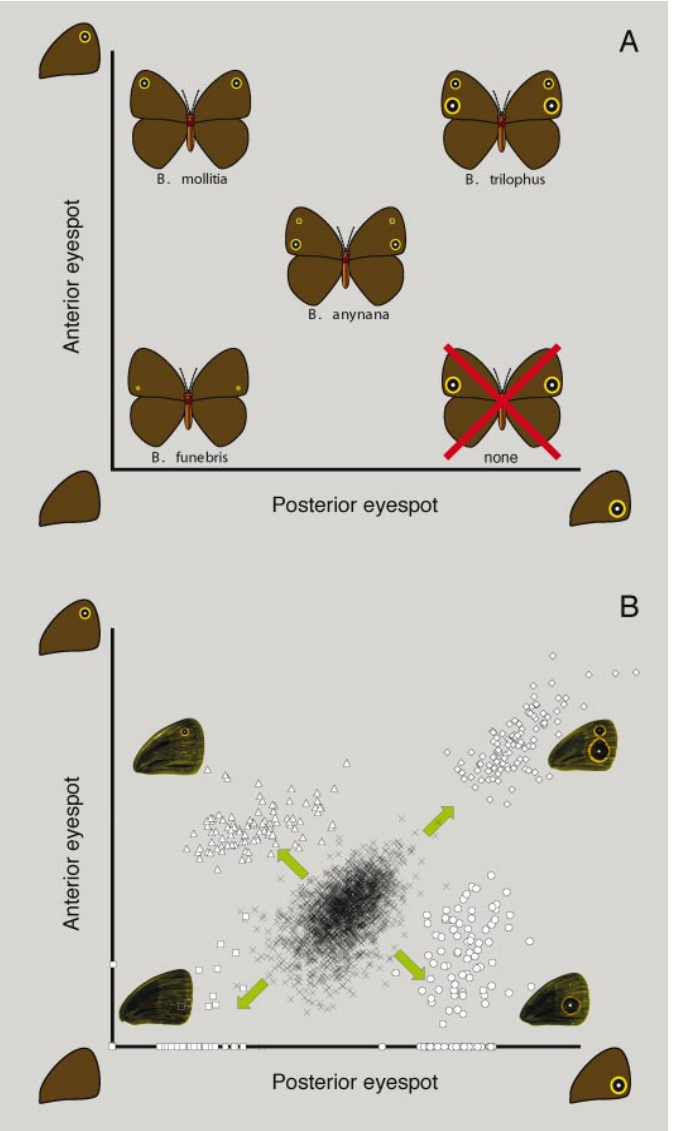

Figure 4 Analysis of a potential evolutionary constraint. (*A*) Occupation by species of the butterfly genus, *Bicyclus*, of morphological space for the pattern of sizes of the forewing eyespots. Names of representatives from among the 80 or so species are given. (*B*) Responses obtained over 25 generations of artificial selection in replicate lines of *B. anynana*. Crosses show female individuals at generation 0 from the base population with other symbols those at generation 25 following selection in the direction of the green arrows. Responses were gradual in each direction of selection. Results show that butterflies similar to each corner pattern in (*A*) were produced from standing genetic variation in a single laboratory stock, including one morphology not seen in any extant species (redrawn from Beldade et al. 2002b).

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