Gene expression and the evolution of insect polyphenisms

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Summary

Polyphenic differences between individuals arise not through differences at the genome level but as a result of specific cues received during development. Polyphenisms often involve entire suites of characters, as shown dramatically by the polyphenic castes found in many social insect colonies. An understanding of the genetic architecture behind polyphenisms provides a novel means of studying the interplay between genomes, gene expression and phenotypes. Here we discuss polyphenisms and molecular genetic tools now available to unravel their developmental bases in insects. We focus on several recent studies that have tracked gene-expression patterns during social insect caste determination. *BioEssays* 23:62–68, 2001. Published 2001 John Wiley & Sons, Inc.†

Introduction

The production of predictably structured organisms from single cells has intrigued biologists for centuries. Biologists have also looked to shared patterns in development to find insights into the evolutionary history of organisms.(1) The overall predictability of the developmental process has tended to overshadow plasticity within species as a source of physiological or morphological variation. Developmental plasticity can be divided into two related phenomena. (1) Environmental variables may elicit reaction norms or continuous phenotypic variation with a genotype. (2) Environmental variation can result in discrete phenotypic classes or polyphenisms.(2) Developmentally, the critical difference between reaction norms and polyphenisms lies not in the discreteness of the outcome but in the complexity of the underlying regulatory mechanism. In both cases, morphological differences reflect differences in gene expression. For reaction norms, an environmental factor such as temperature directly alters gene expression while, for polyphenisms, a token stimulus, such as a change in hormone level, intervenes between the environmental signal and gene expression.(3) Table 1 describes the salient features of reaction norms and polyphenisms, and places these into the continuum of developmental events.

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This review focuses on the processes that shape polyphenisms in insects, and the tools available to study them. Insects reveal a special evolvability in regard to plasticities and polyphenisms, relative to other animal groups.(1,4) In many insect species, environmental variation during larval development leads to a continuum of variation in one or a few morphological traits. Grasshoppers, for example, develop more robust head capsules, with greater bite strength, in response to increasing food toughness.(5,6) A classic example of a more complex, polyphenic, trait involves dimorphic oak caterpillars, whose morphologies differ in response to diet and time of year. Here, caterpillar morphs are so strikingly different from each other that they were previously regarded as distinct species.(7,8) Finally, social insect castes provide stunning examples of complex and diversified polyphenism(9) (Fig. 1). Indeed, the related phenomena of plasticity and polyphenism form the cornerstones of insect sociality, making the social insects an exceptionally rich group for exploring the evolution of polyphenisms.

We highlight new molecular-genetic tools that can contribute to determining the mechanisms behind phenotypic plasticity. Improved efficacy of these tools reflects advances in two areas: the application of improved methods for detecting differential gene expression in non-model systems(10) and the advent of genome-level analyses of patterns of gene expression.(11,12) Most importantly, these advances allow the genetic study of polyphenisms in a diversity of organisms, including social insects and other taxa showing extremely divergent polyphenisms.

Recent advances in understanding the molecular bases of insect polyphenisms

The expression of a polyphenism begins when one or more signals are transduced into physiological or cellular responses that result in a developmental switch. This switch is governed by the interplay of hormone secretion, hormone titer, threshold of sensitivity to hormone, timing of the hormone-sensitive period, and specific cellular responses to hormones.(3) Downstream from this critical reprogramming, differential gene expression results in the development of organisms with different characteristics. A great challenge remains to follow the cascade of events from a developmental switch to the distinct differences in the polyphenic forms.

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Table 1. Mechanisms of developmental variation across and within organisms. Shaded cells indicate primary stimuli behind a specific class of variation.

Class of variation	Genomic differences	Allelic differences	External environment	Specific signal	Developmental switch
Cell differentiation				■	■
Polyphenism			■	■	
Reaction norm			■		
Genetic polymorphism		■			
Species-level traits	■				

Development in holometabolous insects can be viewed as a form of sequential polyphenism from larvae through the pupal stage to adults, allowing interesting comparisons with other polyphenisms. Conveniently, one of the premiere model systems for studying the hormonal and molecular genetic bases of pattern formation in development is *Drosophila melanogaster*, a holometabolous insect. Levels of the steroid hormone ecdysone change over a tenfold range during the 30 hours that span the transition from larva to pupa in *Drosophila*.[13] Microarray analysis of seven time points during these 30 hours revealed two general patterns of gene expression that appear to be linked to ecdysone levels. One group of genes is highly expressed before the late larval ecdysone pulse, then loses expression during this pulse. The other major category has the reverse pattern; genes are not expressed until near the late larval ecdysone pulse, when they are induced.[12] This pattern supports a model in which hormones orchestrate a wide variety of changes in diverse tissues, by changing the level of gene transcription. Genes important during *Drosophila* development can be used to predict the roles of their orthologs in other insect taxa, helping to place polyphenisms into a broad developmental context.

Recent successes using methods to identify differentially expressed genes in social insects, both during developmental divergence and downstream after further differentiation,[10,14,15,16] represent an additional breakthrough in our ability to dissect the developmental mechanisms underlying divergence of forms in polyphenic organisms. First, new techniques for isolating differentially expressed genes, including cDNA subtraction and differential-display techniques, can be used to screen social insect genomes for genes involved with both regulatory and downstream steps on the route toward polyphenisms. Previous methods, including radiolabeled translation product assays[17,18] offered hints for the

Figure 1. Major and minor workers, and the larvae that lead to these castes, in a carpenter ant, *Camponotus* sp. (Mark Moffett).

differential expression of genes during social insect development, despite being unable to characterize these genes. Second, genome-wide studies of expression in other insects, including *Drosophila*, provide a rich source of potential homologues that can be screened in social insects. As one example, juvenile hormone-responsive genes isolated from *Drosophila* might be involved in similar ways in reproductive caste determination and development in social insects. Several genes with caste-biased expression in honey bees show sequence similarity to genes whose expression is affected by hormones in Drosophila.[19] Further, gene-expression studies of metamorphosis[12] and other major developmental events in *Drosophila* will almost certainly clarify processes important for the evolution of phenotypic variation in polyphenic species.

Finally, the honey bee, *Apis mellifera*, has emerged as a bona fide "genetic organism", thanks to a fairly complete physical map,[20] available DNA[21] and cDNA gridded libraries, and an abundance of data on the genetic components of behavior and physiology.[22] While this system still lacks attributes that would push it into the realm of established genetic systems, including a demonstration of transgenesis or a viable tissue-culture protocol, honey bees show traits such as male haploidy and an efficient method for artificial insemination that make them unique among insects for studies of genetic mechanisms.

We, and several other groups, are studying the molecular genetic basis of caste determination and differentiation in honey bees (Fig. 2). Queen and worker honey bees show an alternative, rather than sequential, polyphenism, as both forms undergo complete metamorphosis. Differences between adult queens and workers involve principally the size and function of various organs. Using subtractive hybridization and genetic arrays, we sampled gene expression at six time points from embryos and undetermined larvae through the point of caste determination. In the first screening, we identified seven differentially expressed loci, two of which might play a central role in caste determination. The others are more likely to be involved in early downstream differentiation.[10] Array-based expression patterns for a much wider sample of genes suggest that workers and younger, bipotential, larvae, are more similar in gene expression to each other than to queen-destined larvae[19] (Fig. 3). This bias may reflect common effects of hormones on larval gene expression. Workers and younger larvae share similar titers of both ecdysteroids and juvenile hormone, while queens show much higher titers of these molecules.[23] Further, in vitro assays of gene expression in worker ovarian tissues suggest a significant effect of the ecdysteroid makisterone A on the expression of several genes.[16] Additional trends are emerging with respect to the functional groups of caste-biased genes. As one example, an overabundance of genes with queen-biased expression in larvae is involved with metabolism and respiration,[14,19] a result consistent with caste-biased changes found in larval respiration rates.[17] Workers, in contrast, show higher expression of storage protein genes, indicating a greater allocation toward storage in workers than queens during the caste determination stage. This counter-intuitive result may reflect the costs of a high-stakes race between queen larvae to develop quickly and gain direct fitness as heads of the colony. Developing queens that emerge first, even if only by a matter of hours, almost always beat rival queens in gaining control of colony reproduction.[24] While these results beg for comparative data using larvae of other social and solitary insect species, the common trends that are emerging indicate that gene-expression assays will be a critical tool for describing both the nature and timing of social insect caste determination.

Figure 2. Honey bee colony with cells for developing worker larvae surrounding a single queen-rearing cell, in center (Jay Evans).

Five key questions addressable by genetic assays

(1) How are polyphenisms transduced?

Polyphenisms typically allow organisms to adjust or shift their development in response to some sort of environmental cue.

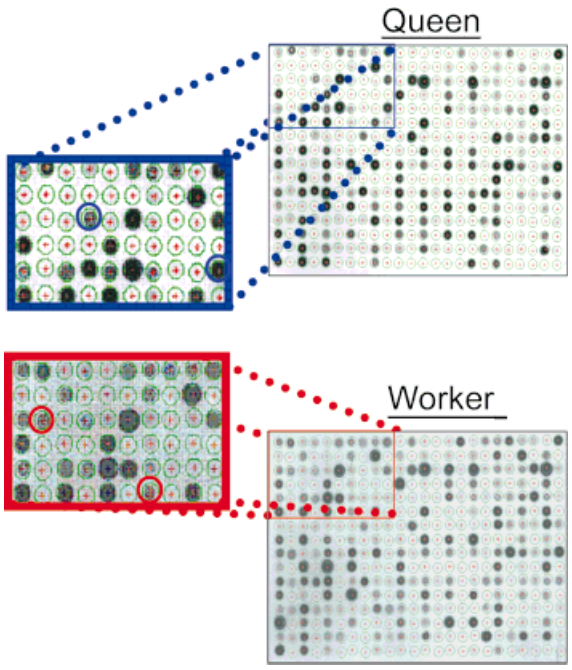

Figure 3. Genetic array showing genes expressed differentially by honey bee workers and queens during caste determination.

Physical environmental variables such as day length and humidity are involved in inducing some seasonal forms in aphids and butterflies.[25,26] Biotic factors such as population density and predator presence mediate development and subsequent morphology in a variety of arthropods.[27–29] Nutrition can be an excellent cue to predict environment and is used by such diverse insects as the oak catkin caterpillar,[7] horned scarabs,[30] and honey bee queens.[10]

Although environmental cues are important in some systems, other polyphenisms arise in the absence of cues, or at any rate with insensitivity to the expected types of cues. These cases seem to be confined to systems in which the fitnesses of individuals with different phenotypes depend on the actions of others. For example, some aphid soldiers[31] and the defender morphs in the parasitic wasp *Copidosoma*[32] are produced within the same clones constitutively. The fact that a single clone of organisms produces these alternative morphs indicates that regulation does not have an allelic basis and, as such, reflects a proper polyphenism. Further study is needed to determine the specific factors that either initiate a soldier pathway in one or a few individuals in a clone, or shut it down in the majority.

Token stimuli involved in transduction commonly include juvenile hormone and ecdysone, as well as neurohormones.[3,33] Indeed, any substance that can be used as a signal to coordinate developmental events throughout the body could, in theory, play this role. By looking at a diversity of systems controlled by the same substance, we may be able find additional clues as to the identity of common mechanisms. These clues could arise from common expression patterns of entire suites of genes, as determined by genetic arrays, or by similarities in sequences affecting the transcription of co-expressed genes. We may subsequently be able to determine why juvenile hormone has been adopted by so many insects as a means of regulating developmental decisions that involve large-scale changes in patterns of growth.

(2) What is the physiological relationship between reactions norms and polyphenisms?

Have polyphenisms evolved from reaction norms or are they of completely different evolutionary origin? As stated earlier, reaction norms are believed to reflect the direct response of genes to environment variation while polyphenisms reflect the integration and transduction of a token stimulus (often a hormone) that mediates a fork between different developmental pathways.[3] Comparative approaches within and between lineages will help determine similarities between the transducing mechanisms of these two classes of phenotypic plasticity. For example, identification of gene-by-gene interactions can show whether environmental signals temporally upstream of the polyphenic switch are similar to those that result in reaction norms for similar morphological systems. These approaches will also help determine whether there are general rules for how token stimuli are incorporated into the developmental regulation of polyphenisms. The phase change between solitary and gregarious forms in migratory locusts provides one system in which the functional and evolutionary relationship between environmental variables, reactions norms and hormonal coordination could be explored. Morphological as well as physiological aspects of these phase changes accumulate over several generations. Humidity, density, background color and maternal effects can each affect grasshopper color patterns, apparently via different mechanisms.[34,35]

(3) How are regulatory networks that govern the development of similar morphs using different environmental cues and token stimuli related to each other?

Many insects show convergence to similar polyphenic morphologies employing seemingly unrelated processes. For example, aphids commonly show polyphenism in both wing expression (winged versus wingless) and mode of reproduction (live-bearing versus egg-producing, parthenogenetic versus sexual). In the aphid, *Aphis fabae*, two winged forms serve as an example of contrasting development controls. Winged aphids can be induced by both decreasing day length (fall form) and crowding (summer form). The former

effect can be mimicked with application of juvenile hormone[36] while the latter cannot. Instead, the proximate cue behind summer wing development appears to come from neurohormones. In both cases, wing tissue is the target. Are the control mechanisms regulating this switch homologous and, if so, at which point in the decision-making process? By tracing gene-expression patterns throughout development, we should be able to identify shared genes involved in the production of common phenotypes as well as the inevitable differences present during the distinct developmental switches themselves.

(4) Are both polyphenisms and polymorphisms that arise from allelic variation mediated by the same mechanisms?

Here, comparisons are aided by the fact that some polyphenisms (as well as plasticity) have both genetic and plastic components influencing development simultaneously. The control of wing length in crickets, for example, has both genetic and plastic components. Selection for either short or long wings yields lines that congenitally produce predominantly one form or the other.[37] Similarly, the soapberry bug, *Jadera haematoloma*, produces one short-winged morph and three long-winged morphs with different degrees of muscle histolysis. The genetic differences between the short- and long-winged forms, as well as phenotypic plasticity in response to food level, appear to reflect differences in production of juvenile hormone.[38] Such results would suggest that genetic and polyphenic wing morph variants arise through similar routes.

Other systems hint at a similar blurring between genotypic and environmental sources of phenotypic variation. Consider the phenomenon of temperature-dependent sex determination found in many reptiles, in which subtle temperature differences during embryogenesis drive embryos to develop into males or females. Here, the difference between sex determination mediated by temperature versus genotype may be relatively small. In the first case, temperature affects the activity of enzymes that determine whether female- or male-inducing hormones will be produced from the precursor testosterone during embryogenesis. In the second, genome-level differences on the sex chromosomes determine which of these hormones will be synthesized.[39] By the same logic as above, genes involved with downstream effects of sex determination might be shared across taxa with either genetic or environmental sex determination, while genes acting early in this switch are likely to be of independent origin.

(5) Do developmental mechanisms constrain polyphenic caste determination in social insect colonies?

In social insect colonies, the integration of behavior, physiology and morphology across colony members confers an organismic character to the colony as a whole.[40,41] This concept has been used to argue that, when members of different castes (e.g., queens and workers) become codependent, individual castes are "released" from certain selection pressures, allowing them to quickly evolve novel traits.[42] The developmental mechanisms by which colony members diverge are important in that both the timing of commitment to a particular caste and the cues needed for this commitment can affect colony fitness. As one example, increased morphological specialization can lead to a reduction in the number of roles individuals in each caste can perform.[43,44] The extent to which larvae are committed during development to a specific caste might limit the ability of colonies to track the needs imposed by short-term environmental changes.[9] Finally, while insect colony members cooperate in diverse ways, there is great potential for conflict among individuals over which colony members reproduce and which play more altruistic roles.[45–47] The extent to which potential conflicts manifest themselves must depend in part on the developmental mechanisms that control reproductive polyphenisms.[48] A further motivation to determine the timing and nature of caste determination is to understand more fully the means by which conflicts in the colony are realized and the "victors" of these conflicts are determined. Both "diagnostic" genes, which are expressed overwhelmingly by members of a single caste, and quantitative differences in expression across entire suites of genes should be useful for characterizing when castes are determined and the flexibility, if any, of this determination.

Concluding remarks

We anticipate a burst of research aimed at defining the genetic features that underlie the developmental and evolutionary biology of polyphenisms. At this time, the functions of genes that are overexpressed or underexpressed along each developmental trajectory can only be inferred by sequence homologies with known genes. Next, gene function during polyphenic development will be established by more direct assays of gene function such as RNA inhibition[49–51] or ectopic expression using recombinant baculoviruses.[52]

A parallel goal will be to determine the key genes in the complex mesh of gene networks that govern holometabolous metamorphosis. Here, studies of polyphenism will benefit from genome-wide assays of expression in *Drosophila*[12] and other model systems, since these studies have also identified clusters of developmental genes with covarying levels of expression and related functions. Genetic arrays derived from normalized or subtractive cDNA libraries[10,53,54] should speed the identification of suites of genes that are related to polyphenic development. The complicity of the genes that have already been identified in honey bees during polyphenic development can be further supported by measuring their

expression after application of hormones and other specific cues involved with a particular developmental switch.

Insect polyphenisms provide diverse opportunities to study the evolution of developmental processes. Framing polyphenisms in the context of development allows us to ask both how different species evolve new morphologies, and how a single species evolves two different, alternative morphologies for a given stage. Indeed, an understanding of polyphenisms can complement the study of developmental processes at scales ranging from individual cells to different taxonomic groups (Table 1). Social insects, thanks to well-defined environmental cues and their unparalleled range of polyphenisms, offer novel opportunities to study the genetic mechanisms behind polyphenisms. By exploiting these opportunities and placing them into the context of developmental genetics, social insect researchers might reciprocate for the altruism of many geneticists who have shared insights from more traditional developmental models.

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