

8 A Framework for Studying the Evolution of Gene Networks Underlying Polyphenism: Insights from Winged and Wingless Ant Castes

Ehab Abouheif

Authors have been impressed by very different aspects of the complicated phenomena of polymorphism... one of these is physiological and ontogenetic, the other ethological and phylogenetic.

—Wheeler, 1910, 101–102

Since the 1980s, the evolutionary conservation of developmental regulatory genes and networks across metazoan phyla has largely been accepted as a new paradigm in evolutionary developmental biology (Hall, 1999; Holland, 1999; Wilkins, 2002). This paradigm has come mainly from comparing the expression of signaling molecules and transcription factors, such as *Hox* genes, across a broad range of metazoan phyla (McGinnis et al., 1984). Although many evolutionary and developmental biologists are now attempting to increase the phylogenetic resolution of their studies by examining closely related species (Kopp, Duncan, and Carroll, 2000), there remains a large gap in our understanding about how these conserved genes and networks produce developmental and evolutionary changes *within* species (S. F. Gilbert, 2001; Arthur, 2002).

Polyphenism, which is the ability of a single genome to produce alternative morphologies within a population in response to an environmental cue, is an important ecological trait (West-Eberhard, 1989; Moran, 1992). Polyphenism has evolved many times independently, and is phylogenetically widespread in plants and animals (Schlichting and Pigliucci, 1998). Its expression within populations (spines/no spines in rotifers, eyespots/no eyespots in butterflies, papilliform/molariform morphs in cichlid fish; Gilbert, 1966; Meyer, 1990; Brakefield et al., 1996) provides an excellent opportunity to understand how regulatory genes and their interactive networks respond to environmental cues to produce alternative morphologies during development and evolution (Evans and Wheeler, 2001). In this essay I build a conceptual and methodological framework for studying the evolution of gene networks that underlie polyphenism, and use wing development in ants as my main example.

Ants, Polyphenism, and the Development of Wings

Perhaps one of the most dramatic cases of polyphenism is found in an ant colony (Wilson, 1953). Depending on environmental cues received during development (temperature, photoperiod, or nutrition), an egg develops into either a winged queen or a wingless worker (Nijhout, 1994). Wing polyphenism in ants evolved once, 125 million years ago, when the ancestral ant colony became eusocial (Grimaldi and Agosti, 2000). This event has been implicated as key to the amazing evolutionary success of ants:

What unusual or unique biological traits led to the remarkable diversification and unchallenged success of the ants for over 50 million years? The answer appears to be that they were the first group of predatory eusocial insects that both lived and foraged primarily in the soil and in rotting vegetation on the ground. . . . The wingless workers can easily penetrate small, remote cavities less accessible to flying wasps, which are burdened with wings and bulky thoraces. (Hölldobler and Wilson, 1990, 29)

It is quite remarkable that, at the level of adult anatomy, ant wing polyphenism has remained virtually unchanged. All known ant species are eusocial, and with the exception of a few parasitic genera, all possess a worker caste that lacks wings entirely (Wheeler, 1910; Hölldobler and Wilson, 1990).

The regulatory gene network that specifies and patterns wings in the fruit fly, *Drosophila melanogaster* (figure 8.1), has been conserved among holometabolous insects (Carroll et al., 1994; Carroll, Grenier, and Weatherbee, 2001) for the past 300 million years (Labandeira, 1998). Genes known to operate in this network are represented by A–Z in figure 8.1. During the middle stages of embryogenesis, a set of interacting signaling molecules and transcription factors (A–F) establish a cluster of about 20 cells in the ectoderm as precursors of the leg and wing imaginal discs. Late in embryogenesis, a second set of interacting gene products (A, D, E–H, J, K in figure 8.1) divides this proliferating population of cells into two separate clusters, which will give rise to three pairs of leg and two pairs of wing imaginal discs. During the last larval instar, the wing imaginal precursor cells proliferate into full-size imaginal discs. A third set of interacting gene products (A, C, E, J, L, M, O–R, T–V, X, Z in figure 8.1) subdivides and compartmentalizes cells within the disc, imparts a wing-specific identity to them, and activates downstream target genes that pattern detailed structures of the wing, such as the veins and bristles, during metamorphosis (Carroll et al., 2001).

In the ancestral ant colony, the expression of one or more genes within this “wing-patterning” network had to be interrupted to halt wing development in sterile soldiers and workers, but at the same time had to retain the ability to specify and pattern wings in reproductive queens and males. Because each gene within the network has multiple responsibilities and cannot be silenced by mutation in its protein-coding regions, this flexibility in gene expression most probably evolved through an *epigenetic mechanism*. Epigenetic regulation of these wing-patterning genes may occur by any of the following:

1. Direct or indirect regulation via the endocrine system, such as by juvenile hormone or ecdysone (Nijhout, 1994)
2. DNA methylation, which is the imprinting of particular nucleotides within coding or noncoding DNA (Jones and Takai, 2001)

3. RNA silencing, which is the destruction of the RNA transcripts of particular genes in the genome (Matzke, Matzke, and Kooter, 2001).

Although the mechanisms responsible for the epigenetic regulation of these genes are unknown, current research suggests that the endocrine system plays a major role (Talbot, Swyryd, and Hogness, 1993; Emery, Bédian, and Guild, 1994; Thummel, 1996; Bender et al., 1997; Harshman and James, 1998).

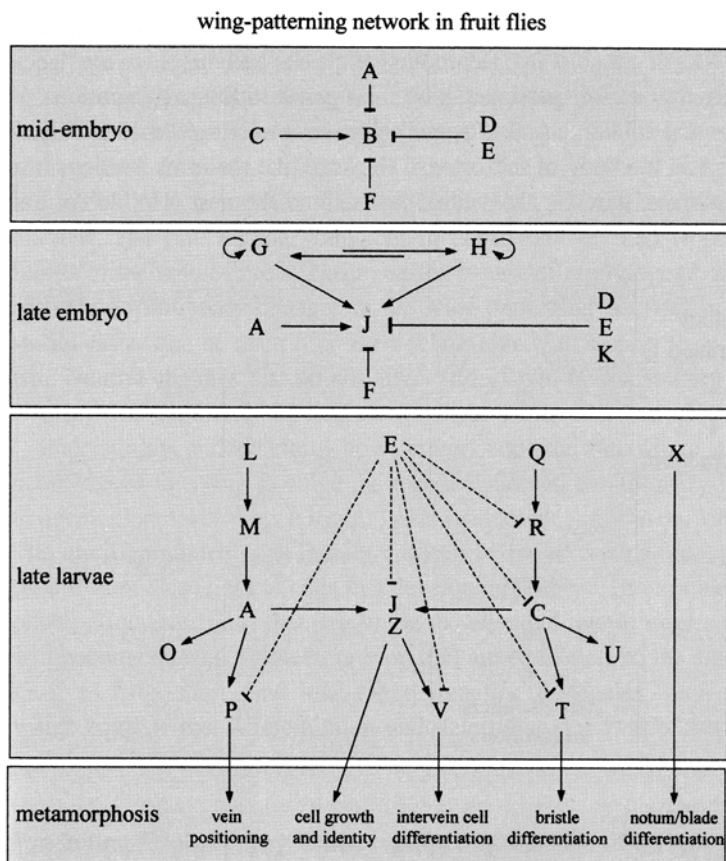


Figure 8.1

A simplified representation of the wing-patterning network in *Drosophila melanogaster*. Dashed lines indicate regulatory interactions specific to the hindwing disc; arrows indicate activation, and bars indicate repression. See text for details.

The Ant Colony as Superorganism

Before establishing an empirical framework for comparing the network in winged and wingless castes across ant species, it is important to consider how evolutionary changes in gene expression (favorable, neutral, or deleterious) are transmitted to future generations within ant species. The *superorganism concept*, originally proposed by Wheeler (1911), has been revived as an important idea (Wilson and Sober, 1989; Hölldobler and Wilson, 1994; Bourke and Franks, 1995). This concept, which equates an entire colony with an individual or “superorganism” (figure 8.2), is essential for understanding the evolutionary dynamics of gene networks in ant colonies. Reproductive queens and males are the “germ line” of the colony; only they can disperse and pass their genes to future generations. In contrast, sterile workers and soldiers are the “soma”; they have sacrificed their ability to reproduce in order to act as the body of the colony. They are like the arms and legs of a colony; they gather resources, care for the young, and defend the nest (Hölldobler and Wilson, 1990).

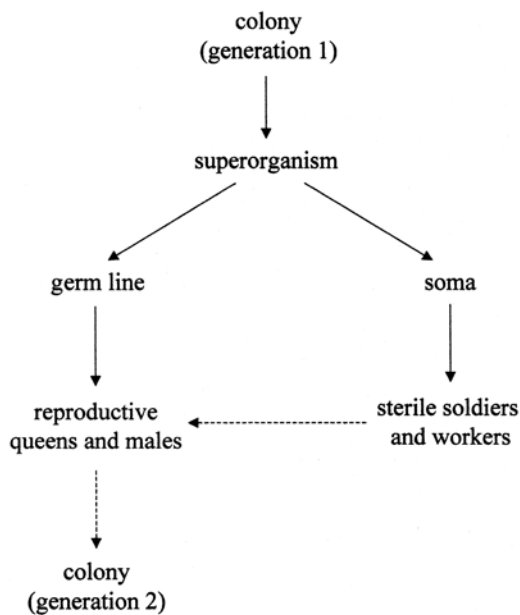


Figure 8.2

Germ line and soma distinction in a superorganism. Favorable or neutral evolutionary changes in gene expression (with respect to fitness) within the wing-patterning network in the reproductive and sterile caste are transmitted through germ line of the colony.

Colonies of leaf-cutter ants from the genus *Atta* best illustrate the superorganism concept. After returning from her nuptial flight, a queen tears off her wings, excavates an underground nest, and begins laying thousands of eggs. These eggs eventually form four sterile worker castes, each with a specialized role in cultivating a fungal garden from which they sustain themselves. Some workers only cut and transport leaves, others only defend and cultivate the garden itself. The size range of these workers is quite amazing, the largest worker caste being at least 300 times bigger than the smallest (Hölldobler and Wilson, 1994).

Evolutionary changes in the wing-patterning network, which increase the fitness of the superorganism, are ultimately transmitted through the germ line of the colony (figure 8.2). In reproductive queens and males, changes are likely to be favorable if they increase the fitness of a colony by improving dispersal ability and mating success. In sterile soldiers and workers, however, changes are likely to be favorable if wing development is halted in such a way as to increase the ability of soldiers and workers to gather resources, defend the nest, and care for the young. In so doing, soldiers and workers ultimately increase fitness of the whole colony by increasing fitness of reproductive queens and males. Alternatively, evolutionary changes in the wing-patterning network in both reproductive and sterile castes can be neutral or even deleterious with respect to fitness of the superorganism. Neutral changes that do not affect fitness will be transmitted through the germ line; changes that negatively affect fitness will be eliminated through selection.

Interestingly, understanding how networks operate and evolve within “superorganisms” is analogous to trying to solve the mechanistic and evolutionary basis of morphogenesis in developmental biology (Gregg, 1942; Hölldobler and Wilson, 1990; S. F. Gilbert, 1997). The undifferentiated eggs laid by a queen in an ant colony are equivalent to a totipotent (undifferentiated) field of cells in a developing embryo. Environmental cues (temperature, photoperiod, nutrition) that determine the eventual fate of eggs and larvae (whether they will become queens, soldiers, or workers) are equivalent to the signals that a field of cells receives from short- and long-range signaling molecules, such as the *hedgehog* gene, which triggers the differentiation and determines the fate of particular cells within the embryo.

Predicting Evolutionary Conservation and Change of a Gene Network

Given what is known about the conservation of developmental regulatory genes, the biology of ant colonies, and the evolutionary origin of ant wing polyphenism, one can begin to make empirical predictions about the pattern one should observe when comparing the network in winged and wingless castes across ant species. Because the wing-

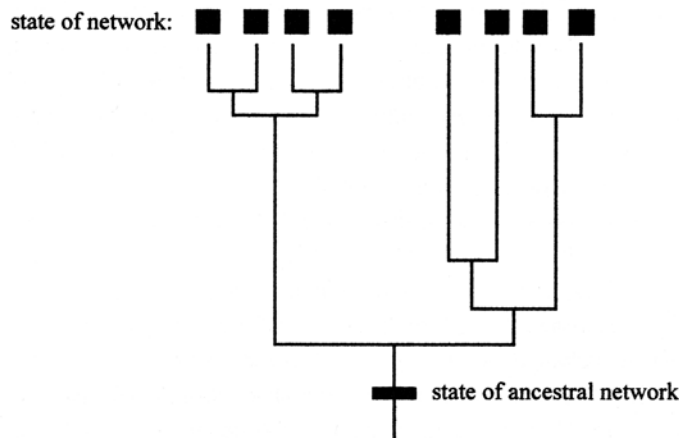


Figure 8.3

Hypothetical phylogeny of eight species, showing evolutionary conservation of the wing-patterning network. The black bar shows the ancestral state of the network, and the black boxes show the state of the network in each extant taxon. It is predicted that the wing-patterning network will be largely conserved in the winged reproductive castes of ant species.

patterning network (figure 8.1) has been largely conserved among holometabolous insects for the past 300 million years, one might predict that the expression of genes within this network will also be conserved in winged reproductive castes (queens and males) in all ant species (figure 8.3). In wingless sterile castes (soldiers and workers), one might predict that the network is interrupted at the same point(s) in all ant species (figure 8.4a). This prediction is based on two important facts: that the wing polyphenism evolved just once, 125 million years ago, and that genes within the wing-patterning network must play the dual role of specifying and patterning wings in queens and males, but halting wing development in soldiers and workers.

If this prediction is right, and no evolutionary change in points of interruption is observed within the wing-patterning network among ant species, then there may be historical constraints (i.e., any combination of selective, genetic, developmental, ecological, or mechanical constraints) restricting the evolution of points of interruption within the network. As a consequence of these constraints, little or no correlation is expected between points of interruption and their phylogenetic history (i.e., both closely and distantly related species will share similar interruption points), or between points of interruption and the ecology or life history of an ant species (figure 8.4a).

An alternative to this historical constraint hypothesis is that points of interruption within the network evolve via drift or natural selection. As long as wings are completely sup-

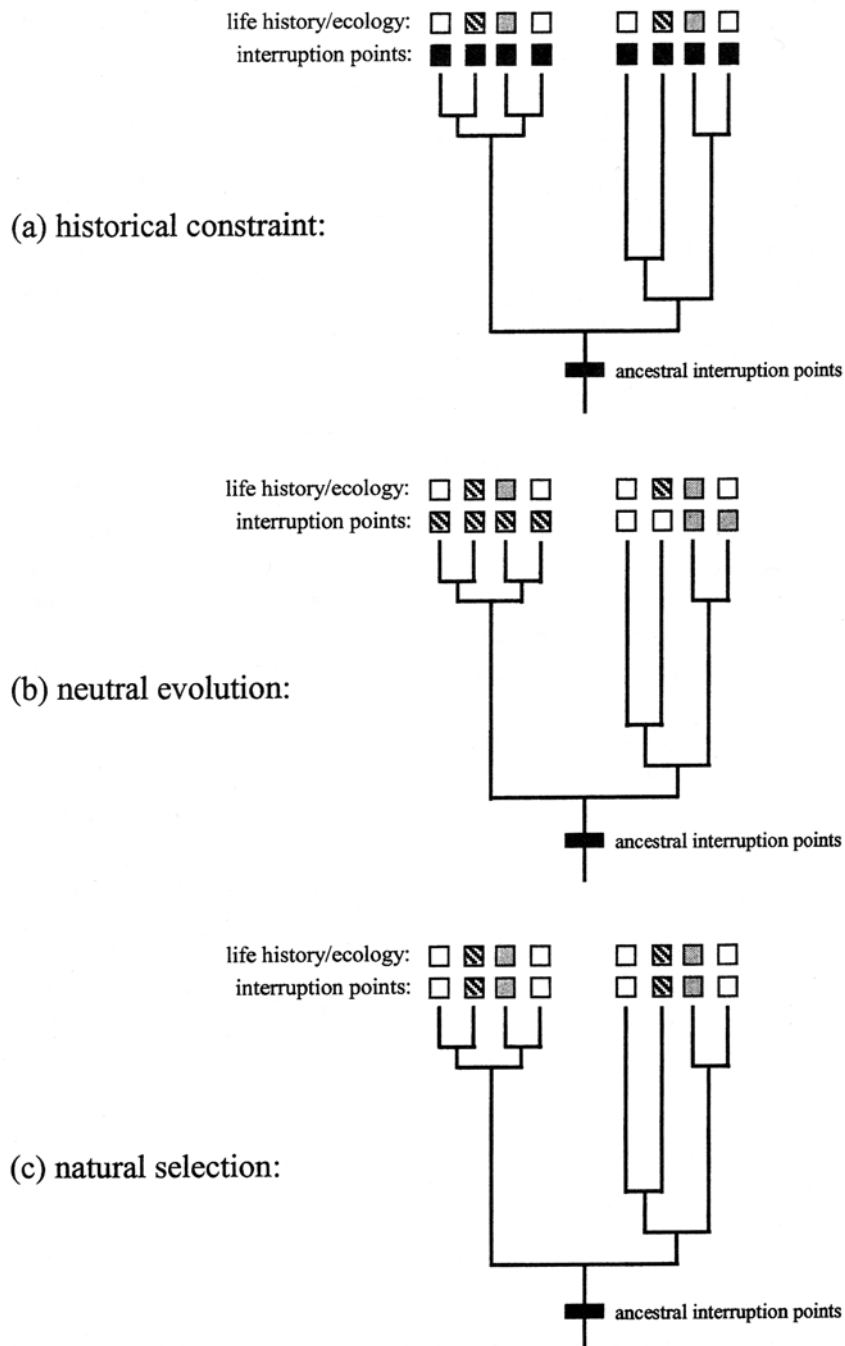


Figure 8.4

Hypothetical phylogeny of eight species demonstrating the patterns expected if points of interruption within the wing-patterning network in wingless castes are (a) historically constrained, (b) evolving neutrally, or (c) evolving via natural selection. The black bar indicates the ancestral state of interruption points; boxes indicate similarities and differences (clear, shaded, striped) in interruption points between taxa. Boxes also show similarities and differences (clear, shaded, striped) between the life histories or ecologies of these taxa. See text for details.

pressed during development, some points of interruption in wingless workers may be free to evolve neutrally. In this case, there will be a correlation between these points of interruption and their phylogenetic history. Neutral points of interruption will follow a random accumulation (Brownian motion) model of evolutionary change, and as a consequence, closely related species will be more similar in their points of interruption than distantly related species (figure 8.4b). This correlation may become weak, however, if the rate of neutral evolution of interruption points is faster than the rate of speciation. Because points of interruption are evolving neutrally with respect to fitness of the superorganism, little or no correlation is expected between points of interruption and the ecology or life history of an ant species (figure 8.4b).

Natural selection may also play an important role in driving the evolution of interruption points between ant species. Natural selection may operate directly on different genes within the network to find the most efficient route for suppressing wings in ants with different ecologies and life histories. Alternatively, selection may act on a linked developmental process or network, which would indirectly create correlated changes on genes within the wing-patterning network itself. Because the wing-patterning network is ultimately interrupted in such a way as to increase fitness of the superorganism in a particular environment, there will be a correlation between points of interruption and a species' life history or ecology (figure 8.4c). As a consequence, there should be little or no correlation between points of interruption and their phylogenetic history.

These predictions and alternative hypotheses can be distinguished empirically by using the comparative method (Brooks and McLennan, 1991; Harvey and Pagel, 1991). Several new statistical methods are available that can test correlations between interruption points and life history or ecology (Pagel, 1994, 1997; Lewis, 2001), as well as between interruption points and their phylogenetic history (Abouheif, 1999). The statistical power of this methodological approach to distinguishing between the hypotheses put forth in this essay will depend upon the robustness of the phylogeny used and the scale at which different species and genes within the network are sampled.

A Real Example

Abouheif and Wray (2002) sampled the expression of several genes from the wing-patterning network in the winged and wingless castes of four ant species: *Pheidole morrisoni*, *Neoformica nitidiventris*, *Crematogaster lineolata*, and *Myrmica americana*. As predicted for the winged reproductive castes (figure 8.3), the network is largely conserved across all four species relative to what has been observed in flies (*Drosophila*) and butterflies (*Precis*) (Carroll et al., 1994, 2001; see figure 8.5). Surprisingly, however, the wing-patterning network was interrupted at different points in the wingless sterile castes of these species

(figure 8.5). In the rudimentary forewing disk in soldiers of *P. morrisi*, expression of the *spalt* gene (denoted by the letter *P* in figure 8.1) was interrupted. In contrast, in the rudimentary forewing and hind wing disk of *N. nitidiventris* and *C. lineolata*, expression of the *engrailed* gene (denoted by the letter *L* in figure 8.1) was interrupted. Furthermore, dramatic differences in gene expression were found between the wing primordia of the soldier and worker castes of *P. morrisi*, and even between the forewing and hind wing disks within the soldier caste of this species (figure 8.5). These observations indicate that points of interruption within the wing-patterning network in wingless ant castes are evolutionarily labile. Thus, the prediction that points of interruption are historically constrained can tentatively be rejected (figure 8.4a).

It is not clear, however, whether these differences arose via drift or selection on different genes within the network. With respect to the drift hypothesis, there is no apparent correlation between points of interruption and phylogenetic history of the species being compared (figure 8.5). And with respect to the selection hypothesis, there is no apparent correlation between points of interruption and an ecological trait, such as habitat (e.g., whether they live in sandy soil or in debris above ground; figure 8.5). Thus, only through greater phylogenetic sampling of more closely related ant species, as well as finer sampling of genes within the wing-patterning network, will one be able to distinguish between drift and selection.

From the Specific to the General: Data from Other Taxa

The conceptual framework and empirical findings presented here for ant species are applicable to other taxa. Data on gene expression underlying polyphenism in other insects, such as butterflies, honeybees, and termites, are accumulating quickly (Brakefield et al., 1996; Miura et al., 1999; Evans and Wheeler, 1999, 2000, 2001). Unfortunately, in each of these cases data are confined to a single species. There is, however, one polyphenism for which comparative data are available: the temperature-dependent sex determination system in several reptile species (Crews et al., 2001; Pieau, Dorizzi, and Richard-Mercier, 2001). Low temperatures (approximately 26° C) experienced during embryogenesis produce males, whereas high temperatures (approximately 30° C) produce females. The regulatory gene network underlying this environmentally regulated process in reptile species is composed of many genes that have been conserved across mammals and birds (Crews et al., 2001; Pieau, Dorizzi, and Richard-Mercier, 2001). Interestingly, some genes within this network have maintained similar expression profiles in males and females across reptile species, while others have changed dramatically. For example, the gene *WT-1* is upregulated in both males and females of alligator and turtle species. In contrast, the gene *SF-1*, which is a key regulator in this network, has changed dramatically. In alligators, *SF-1* is

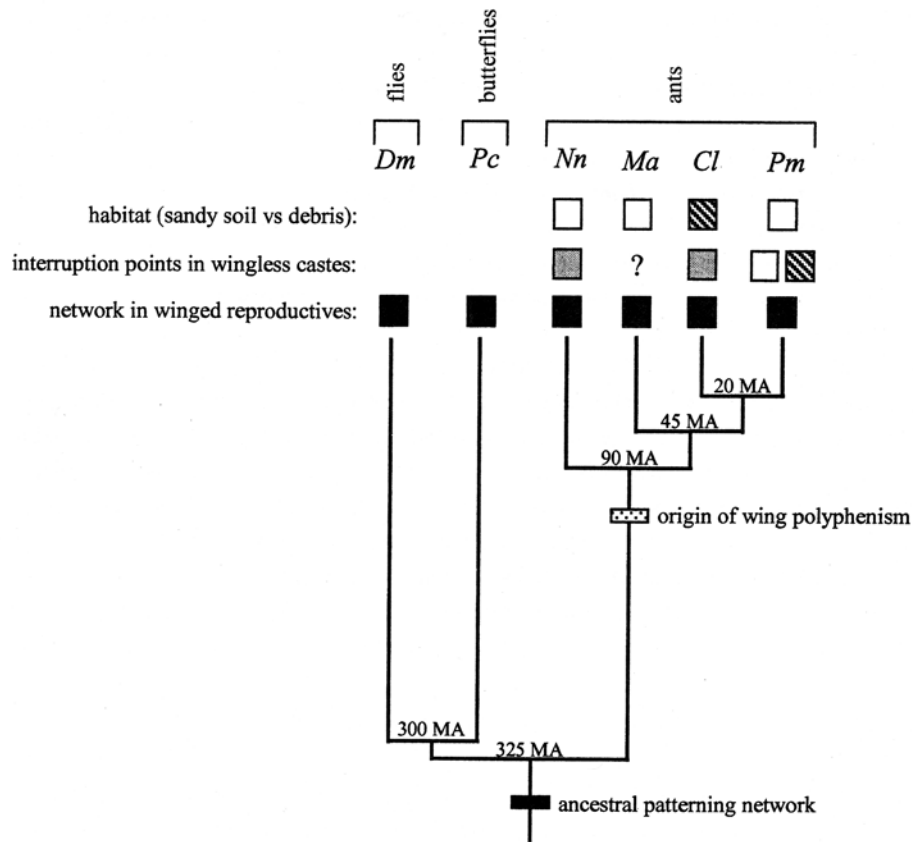


Figure 8.5

A real example from Abouheif and Wray (2002). The phylogeny shows the relationship of four ant species (*Nn*, *Neofornica nitidiventris*; *Ma*, *Myrmica americana*; *Cl*, *Crematogaster lineolata*; *Pm*, *Pheidole morrisi*) and their relationship to flies (*Dm*, *Drosophila melanogaster*) and butterflies (*Pc*, *Precis coenia*). This phylogeny is based on molecular sequences of the *wingless* gene, and the minimum divergence time estimates between taxa are based on their first appearance in the fossil record. The black bar indicates the ancestral wing-patterning network in these species, and the stippled bar indicates the origin of wing polyphenism in ants. Boxes indicate similarities and differences (clear, shaded, and striped boxes) of the wing-patterning network in winged reproductive and wingless sterile castes. The network is largely conserved in holometabolous insects, including winged reproductives, as indicated by the black boxes. In contrast, points of interruption within the network in wingless caste differ among the four species as indicated by the clear, shaded, and striped boxes (note that there are two boxes in *Pheidole morrisi* because there are two castes, soldier and worker). There is no apparent correlation between points of interruption and phylogenetic history, or between points of interruption and habitat (i.e., whether they live in sandy soil or in debris above ground). Thus, it is not clear whether the evolutionary lability in interruption points between these four ant species arose via drift or selection.

downregulated in males and upregulated in females, whereas in turtles the opposite pattern is observed (Crews et al., 2001; Pieau, Dorizzi, and Richard-Mercier, 2001). Thus, if temperature-dependent sex determination evolved just once in these reptile species, then, like ants, the underlying network appears to be evolutionarily labile. Thus, the historical constraint hypothesis can tentatively be rejected. Once again, more comparative data will be required to distinguish whether the network underlying temperature-dependent sex determination in these reptile species is evolving by drift or selection.

Prospects and Challenges

Uncovering the evolution of regulatory gene networks underlying polyphenism will undoubtedly open a new window from which to view the complex interaction between environment, genotype, and phenotype, and its role in generating morphological diversity. The alternative hypotheses and methodological approach proposed in this essay provide a framework for studying this complex interaction. Perhaps one of the greatest challenges for the future of evolutionary and developmental biology will be to generate the data needed to understand how the gene networks that underlie polyphenism evolve, as well as to understand the epigenetic regulation of these networks. Pursuing these challenges will open a new chapter in evolutionary developmental biology, and will take us closer to integrating the study of environment, development, and evolution.

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