ary divergence of *Brassica* and *Arabidopsis*, such as aberrant maturation of the *Brassica* SRK protein in *A. thaliana* stigmas or its inability to interact productively with *Arabidopsis*-derived downstream targets.

The large number of genetically well-characterized *S* haplotypes that are available in *Brassica* species has been critical for identification of the SRK and SCR SI recognition proteins. However, the relatively laborious transformation methods and rudimentary state of genome studies in *Brassica* make further studies of the SI response difficult. The availability of *A. thaliana* strains that express SI provides new opportunities for exploiting the tools of this tractable model plant for structure-function studies of SRK and SCR as well as for the genetic and molecular dissection of the SRK-mediated signal transduction pathway.

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Evolution of the Gene Network Underlying Wing Polyphenism in Ants

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Wing polyphenism in ants evolved once, 125 million years ago, and has been a key to their amazing evolutionary success. We characterized the expression of several genes within the network underlying the wing primordia of reproductive (winged) and sterile (wingless) ant castes. We show that the expression of several genes within the network is conserved in the winged castes of four ant species, whereas points of interruption within the network in the wingless castes are evolutionarily labile. The simultaneous evolutionary lability and conservation of the network underlying wing development in ants may have played an important role in the morphological diversification of this group and may be a general feature of polyphenic development and evolution in plants and animals.

Polyphenism, which is the ability of a single genome to produce two or more alternative morphologies in response to an environmental cue, is an ecologically important and phylogenetically widespread feature of plants and animals (*1*). Yet almost nothing is known about its developmental genetic basis. Wing polyphenism in ants is a dramatic case: Depending on environmental cues, the regulatory gene network underlying wing development either produces a queen with fully functional wings or is halted to produce a wingless soldier or worker (*2*, *3*). We examined the expression of several ant genes orthologous to those from the wing-patterning network in *Drosophila melanogaster* (Fig. 1) to determine how the expression of this network changes during the development and evolution of winged and wingless ant castes.

The wing-patterning network has been largely conserved across holometabolous insects (*4*) for 300 million years (*5*). Therefore, we predicted that this network would be conserved in reproductive castes (queens and males), which produce wings. Fossil and phylogenetic evidence strongly supports a single origin of wing polyphenism in ants: A wingless worker caste was present in the earliest known

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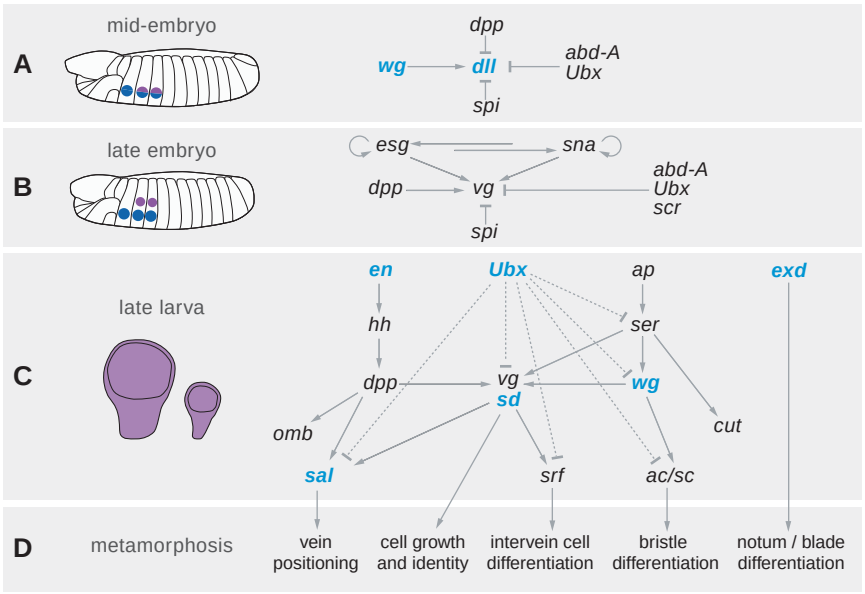

Fig. 1. The wing-patterning network in *D. melanogaster*. During embryogenesis (**A**), interacting signaling molecules and transcription factors establish a cluster of about 20 ectodermal cells as precursors of both the leg and wing imaginal discs (*11*) (blue/purple circles). (**B**) A second set of interacting gene products then divides these cells into separate clusters that give rise to three pairs of leg (blue) and two pairs of wing (purple) imaginal discs (*11*). During the last larval instar (**C**), the wing precursor cells proliferate into full-sized imaginal discs (purple). A third set of interacting gene products then patterns these discs, imparts a wing-specific identity, and activates downstream target genes that pattern detailed structures, such as veins and bristles (**D**) (*18*). Genes examined in this study are shown in blue. Dashed lines indicate regulatory interactions specific to the hindwing disc, arrowheads indicate activation, and bars indicate repression.

ant fossils (*6*), and all ~10,000 extant ant species possess a wingless worker caste (*7*). Therefore, we predicted that all ant species would share a common mechanism to interrupt wing development in sterile castes (workers and soldiers), which do not produce wings.

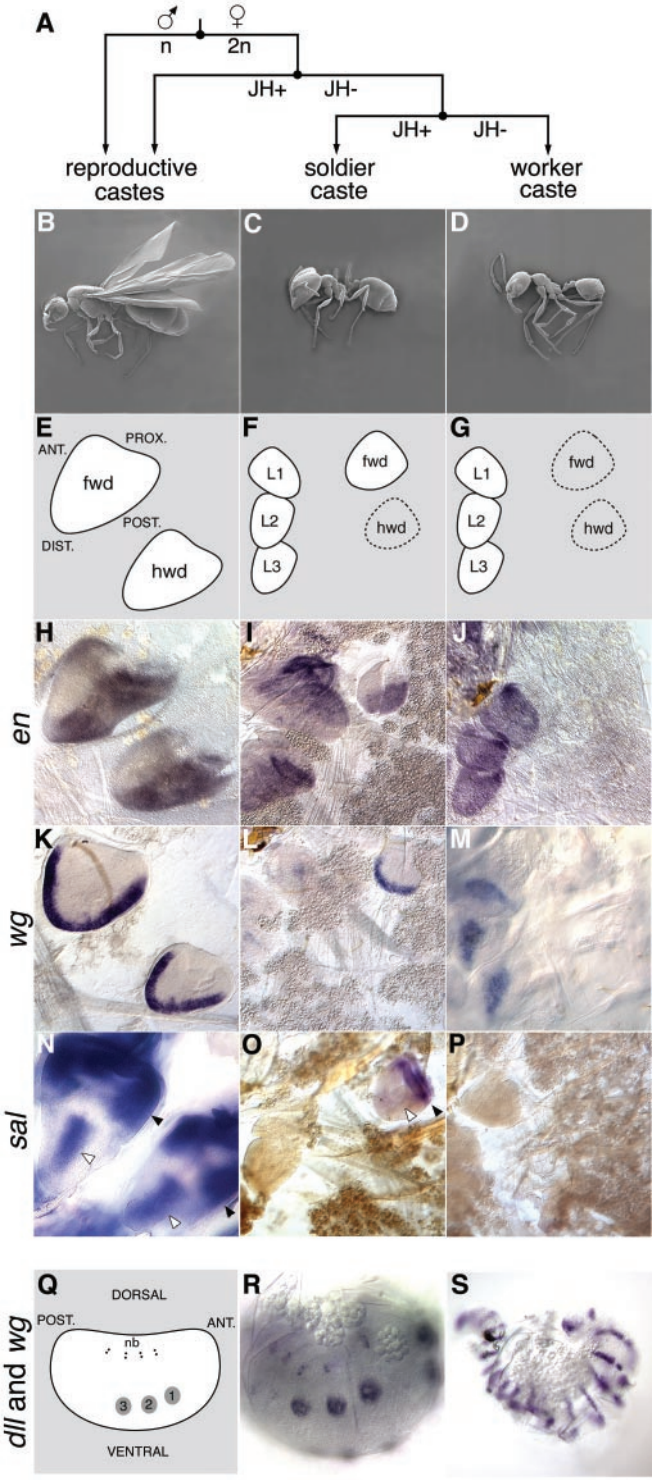

Fig. 2. Polyphenic gene expression profiles in *P. morrisi*. (**A**) Caste determination. We confirmed that castes in *P. morrisi* are determined at three switch points during development (*8*, *19*). The first switch point is genetically controlled: Fertilized eggs develop into diploid females, and unfertilized eggs develop into haploid males. The second switch point is environmentally controlled: If embryos experience appropriate shifts in photoperiod and temperature, they experience a pulse of juvenile hormone (JH) during embryogenesis and develop into queens; if not, they develop into sterile workers. The third and final switch is also environmentally controlled: If worker larvae experience the appropriate diet, they experience a pulse of JH and develop into soldiers; if not, they become workers. (**B** to **D**) Scanning electron micrographs of adults. (**E** to **G**) Diagrams indicating the position of the forewing disc (fwd), hindwing disc (hwd), and the three leg discs (L1, L2, and L3); anterior is at the top. (**H** to **P**) Polyphenic gene expression profiles in three castes. Wing imaginal discs in ants are unlike those of *Drosophila* in that they are folded along their dorsal-ventral margin and do not specify part of the body wall (*4*, *19*). The first column shows gene expression in the fore- and hindwing discs of final instar reproductive castes [(H), (K), and (N), and fig. S1, Q, T, and W], whereas the second and third columns show expression in the wing primordia of soldiers [(I), (L), and (O), and fig. S1, R, U, and X] and workers [(J), (M), and (P), and fig. S1, S, V, and Y], respectively. Absence of gene expression was not an artifact, because it was evident in the leg discs or central nervous system within the same individual [for example, (J) and (M)]; and wing discs of reproductive larvae processed alongside those of soldiers and workers showed the expected expression. (**Q**) Diagram of a *Pheidole* embryo, indicating the position of appendage disc primordia (1, 2, and 3) as well as the neuroblasts (nb). This is a lateral view; anterior is to the right. (**R**) Embryonic expression of DLL: ventrolateral view. (**S**) Expression of *wg* in each future segment along the anterior-posterior axis: ventrolateral view. For additional genes, see supporting online material (figs. S1 and S2).

We first asked whether the expression of six genes that regulate wing development in *Drosophila* [*Ultrabithorax* (*Ubx*), *extradenticle* (*exd*), *engrailed* (*en*), *wingless* (*wg*), *scalloped* (*sd*), and *spalt* (*sal*) (Fig. 1C)] is conserved in winged queens and males of *Pheidole morrisi* (*8*, *9*). As predicted, the expression patterns of all six genes were similar to what has been observed in flies (*Drosophila*) (*10*, *11*) and butterflies (*Precis*) (*4*, *12*) (Fig. 2, H, K, and N; fig. S1, H, K, and T). Once embryos receive the appropriate genetic, environmental, and hormonal cues (Fig. 2A), they develop into winged males or queens and initiate a wing-patterning network that is largely conserved with that in other holometabolous insects.

We next examined the expression of these six genes in the sterile soldier and worker castes of *P. morrisi*, which lack wings. Worker larvae of many ant species possess vestigial wing imaginal discs (*13*). In *P. morrisi*, soldier larvae develop large vestigial forewing discs but no visible hindwing discs (Fig. 2F), whereas the worker larvae develop neither fore- nor hindwing discs (Fig. 2G). It was therefore unclear whether the wing-patterning network is initiated and then interrupted in vestigial discs or whether vestigial discs are simply undifferentiated cells. In the large soldier forewing disc, we found that the expression profiles of *Ubx*, *exd*, *en*, *wg*, and *sd* were very similar to those in winged queens and males (Fig. 2, I and L; fig. S1, I, L, and U). In contrast, the medial stripe of *sal* expression was absent from the soldier forewing disc (Fig. 2, N and O, white arrows), although it was present in the hinge region, which does not contribute to the wing (Fig. 2, N and O, black arrows). Once larvae receive the appropriate environmental and hormonal cues (Fig. 2A), they develop into soldiers. Although patterning is initiated within the large forewing disc, including the establishment of compartments (*en*) and the specification of wing margins (*wg*), the expression of *sal*, the most downstream gene examined, is interrupted. Thus, patterning within this disc is nearly completed, despite the fact that it does not produce a wing.

We found no expression of the genes examined in the expected position of the soldier hindwing disc (Fig. 2, I, L, and O; fig. S1, I, L, and U) and none in the expected positions of the worker fore- and hindwing discs (Fig. 2, J, M, and P; fig. S1, J, M, and V). The patterning network of these wings is probably interrupted much earlier, during embryogenesis (Fig. 1, A and B). We therefore examined the embryonic phase of *wg* and *distal-less* (*dll*) expression to bracket this interruption. The expression of both genes was conserved in *P. morrisi* embryos (Fig. 2, R and S) relative to that in *Drosophila* (*14*), indicating that interruptions to wing patterning occur between mid-embryogenesis (Fig. 1A) and the last larval instar (Fig. 1C). Together, our findings from *P. morrisi* demonstrate that

interruption of the wing-patterning network occurs at different points in the two wingless castes in one species, and even between fore- and hindwing discs within a single caste.

This result raised the possibility that the polyphenic expression of the wing-patterning network may be much less conserved than we had initially expected. To test this possibility, we examined the expression of three genes, *Ubx*, *exd*, and *en* (Fig. 1C), in the winged and wingless castes of three additional ant species: *Neoformica nitidiventris*, *Crematogaster lineolata*, and *Myrmica americana* (*8*). Unlike *P. morrisi*, these species each possess only one wingless worker caste (*7*). In addition, the morphology of rudimentary wing imaginal discs in worker larvae differs among all four species (Fig. 2, E to G; Fig. 3, E to H; fig. S2, K and L) (*15*). Workers of *N. nitidiventris* and *M. americana* possess fore- and hindwing discs that are reduced, although to different degrees (Fig. 3F and fig. S2L), whereas workers of *C. lineolata* possess small epidermal invaginations called "wing pads" (Fig. 3H).

The expression profiles of *Ubx* and *exd* were conserved in winged queens and males, as well as in the vestigial wing discs of workers in all three species (Fig. 3, I to P; fig. S2, Q, R, W, and X). Surprisingly, *en* expression, which plays an important role in establishing the posterior compartment of the wing (*16*), was absent from the vestigial wing discs of *N. nitidiventris* workers (Fig. 3R) and from the vestigial wing pads of *C. lineolata* workers (Fig. 3T). Thus, interruption of the network in workers of *N. nitidiventris* and *C. lineolata* is further upstream than in the soldier forewing disc of *P. morrisi* but further downstream than in the soldier hindwing and both minor worker wings of *P. morrisi*.

These results provide clear evidence that the polyphenic expression of several genes within the wing-patterning network differs among four closely related ant species, despite the fact that the wing polyphenism in ants evolved just once. When our results are placed in a phylogenetic context (Fig. 4), three important conclusions emerge. First, points of interruption within the wing-patterning network of wingless worker castes are evolutionarily labile. Second, this evolutionary lability occurs over relatively short time scales (that is, 20 million to 90 million years), despite the fact that the network has been largely conserved across holometabolous insects, including winged ant castes, for the past 325 million years. Third, dissociations between the morphological reduction of discs and the interruption of gene expression have evolved in worker larvae independently of phylogenetic history. Therefore, one cannot predict from phylogenetic history or from rudimentary disc morphology where the network has been interrupted. This evolutionary lability and dissociation underlying ant wing polyphenism are remarkable given that wing polyphenism evolved just once and that, within an ant species, all of the wing-patterning genes that are interrupted in workers must still retain the ability to specify and pattern the wings in queens and males, as well as the legs or central nervous system in all castes (Fig. 2M). We predict that lability and dissociation may be general evolutionary patterns underlying polyphenism, because regulatory genes and networks are modular and can be changed independently of other developmental processes (*17*, *18*).

The wing-patterning network was first interrupted during the evolution of eusociality in ants, somewhere in the lineage leading to all extant ant species. The evolutionary mechanism driving developmental and genetic changes in the network since this event is not clear. Our findings rule out phylogenetic constraint, because polyphenic gene expression differs so strikingly between species. We cannot, however, distinguish between the relative influence of two other evolutionary mechanisms: natural selection and drift. Natural selection may be playing an active role in determining the most efficient route to halting wing development and may operate directly on different genes in different species because of their unique ecology or life history. Natural selection may also operate indirectly through selection on a closely linked developmental process, favoring different interruptions through correlated changes. Alternatively, as long as natural selection ultimately prevents wings from being produced, some points of interruption may evolve through a

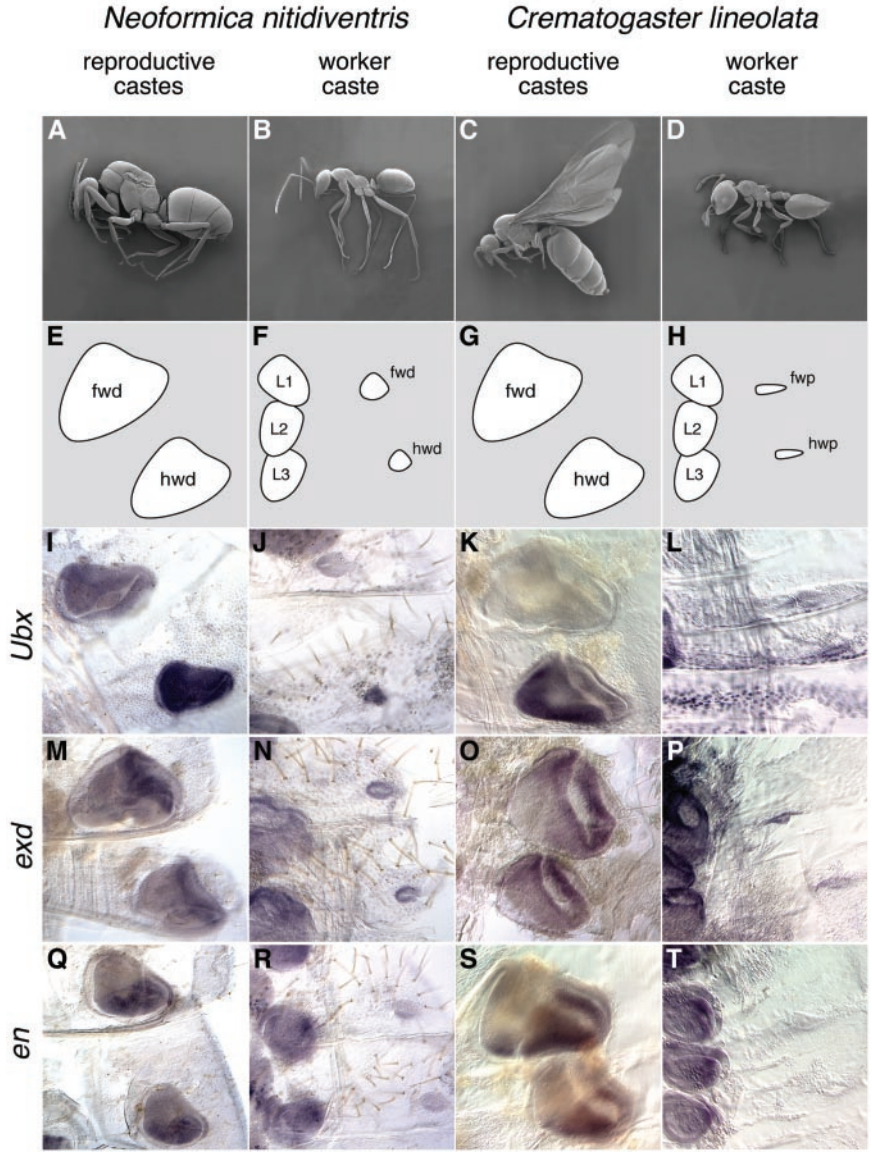

Fig. 3. Polyphenic expression profiles of three wing-patterning genes in final instar larvae of *N. nitidiventris* and *C. lineolata*. (**A** to **D**) Scanning electron micrographs of adult queens and workers. (**E** to **H**) Diagrams of wing discs (fwd, hwd) or pads (fwp, hwp) in final instar larvae as for Fig. 2. (**I** to **T**) Expression of UBX, EXD, and EN in reproductives and workers. For *Myrmica americana*, see supporting online material (fig. S3).

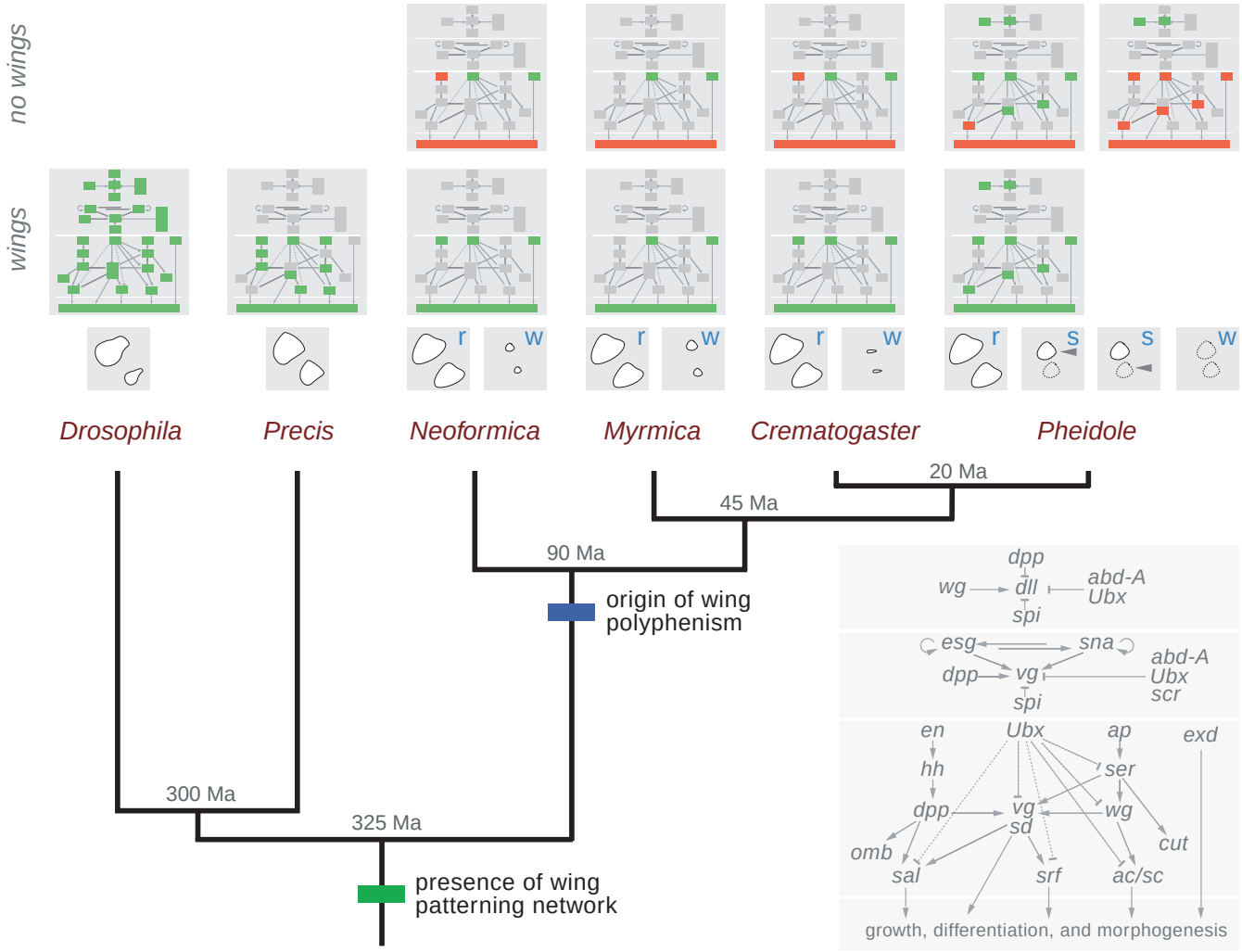

Fig. 4. Evolutionary history of ant wing polyphenism. Phylogenetic relationships among ant species are based on a 1.1-kb fragment of *wg* (*9*); all phylogenetic analyses (*8*) supported this topology with strong (>70) bootstrap support. Minimum divergence time estimates are based on the fossil record (*5*, *20*). Wing polyphenism evolved just once, approximately 125 million years ago (blue bar). The wing-patterning network has been largely conserved across holometabolous insects for 325 million years (green bar). Diagrams of reproductive and worker disc morphology within each species are shown in the first row above the phylogeny (for *P. morrisi*, arrowheads refer to either the soldier forewing or hindwing). Network diagrams for winged reproductive castes are shown in the second row above the phylogeny; network diagrams in wingless sterile castes are shown in the third row. Within each panel, conserved gene expression is indicated in green, and interrupted expression is indicated in red; genes not examined are shown in gray. Note the dissociation between phylogenetic history, rudimentary disc morphology, and points of interruption. Points of interruption have evolved over relatively short time scales, particularly in contrast to the long-term conservation of the wing-patterning network among holometabolous insect orders.

neutral process over time. We can only begin to distinguish between these possibilities through sampling of additional genes and species. This approach will broaden our understanding of the developmental, genetic, and evolutionary basis underlying polyphenisms (*17*) and in so doing will illuminate the complex interaction and relationship between the environment, phenotype, and genotype.

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Figs. S1 to S3

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