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Evolution and molecular mechanisms of wing plasticity in aphids

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Aphids present a fascinating example of phenotypic plasticity, in which a single genotype can produce dramatically different winged and wingless phenotypes that are specialized for dispersal versus reproduction, respectively. Recent work has examined many aspects of this plasticity, including its evolution, molecular control mechanisms, and genetic variation underlying the trait. In particular, exciting discoveries have been made about the signaling pathways that are responsible for controlling the production of winged versus wingless morphs, including ecdysone, dopamine, and insulin signaling, and about how specific genes such as *REPTOR2* and *vestigial* are regulated to control winglessness. Future work will likely focus on the role of epigenetic mechanisms, as well as developing transgenic tools for more thoroughly dissecting the role of candidate plasticity-related genes.

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Introduction

Many organisms have evolved the ability to respond to changing environmental conditions by altering development to produce adaptive phenotypes. This developmental phenotypic plasticity can be highly advantageous, allowing short-term adjustments to changing environmental circumstances. Phenotypic plasticity has been studied for decades with respect to the factors that promote its evolution (e.g. [1–3]) as well as its potential costs and limits (e.g. [4,5]). Far less-understood, however, are the molecular and physiological mechanisms that are responsible for the determination and development of different plastic phenotypes. Identifying these mechanisms is a prerequisite for understanding how they properly function, have evolved, and may constrain or facilitate future modifications.

A renewed focus on the role of plasticity in evolution [6] has invigorated studies of its mechanistic bases in a variety of insects and noninsects. Important discoveries have been made, for example, in a nematode of the genus *Pristionchus* that generates alternative feeding-type mouth forms in response to the environment, where sulfation-related enzymes [7–9] act as molecular switches controlling phenotype determination. In another example, ecdysone signaling controls *Bicyclus* butterfly plastic seasonal color differences [10 and references therein]. But these are just two examples; a wider view of the exciting ongoing work in this field can be gained by reading other articles of this issue.

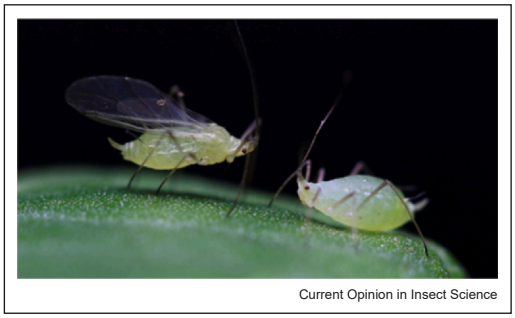
Our focus here will be on the wing plasticity of aphids, in which dramatically different winged and wingless morphs (Figure 1) are produced from identical genotypes depending on environmental conditions. The wingless morphs are specialized for maximizing reproductive output, while the winged morphs are capable of long-range dispersal. Below, we will review recent studies that have provided insight into the evolution and mechanistic basis of the aphid wing plasticity.

Aphid wing plasticity, an overview

The aphid wing plasticity occurs naturally during the spring and summer months. During this time, females reproduce asexually and give live birth to clonal daughters via a modified meiosis that bypasses recombination [11]. Cues that induce the production of winged instead of wingless morphs are varied but are generally indicators of a deteriorating environment such as tactile stimulation (often caused by high densities) and low food quality [12]. For some species, this is transgenerational: the aphid mother senses environmental cues and her daughters are winged or wingless, while in other species, a developing aphid nymph can sense the environmental cues and alter their target adult phenotype [12].

Winged or wingless is shorthand for an integrated, adaptive suite of morphological, physiological, behavioral, and

Figure 1



Genetically identical winged (left) and wingless (right) pea aphid (*Acyrthosiphon pisum*) females. Photo by Omid Saleh Ziabari.

life-history trait differences. In addition to the wing structure and associated wing musculature, there are other, finer-scale morphological differences associated with winged morphs. These include more sensory organs (rhinaria) on the antennae [13] and larger antennal and optic lobes in the brain [14]. Wingless morphs, in contrast, complete development more quickly, are more sedentary, and have higher fecundity [15]. Recent -omic analyses have revealed extensive physiological divergence between the winged and wingless morphs. These studies have interrogated alternative splicing differences in adult pea aphids [16], long noncoding RNA and protein expression differences between the penultimate (fourth) nymphal instar and adult brown citrus aphids [17] and pea aphids [18], and gene expression differences across development in the bird cherry-oat aphid [19]. These studies provide a wealth of information as to how a single genome can be shaped to produce functionally distinct morphs, with concomitant physiological functions.

Although the wing plasticity is generally discontinuous, with only winged and wingless morphs, a recent study revealed that intermediate, seemingly maladaptive morphs are surprisingly common among some pea aphid genotypes [20]. These morphs exhibit wing asymmetries, for example, wings on one side but not the other. Future study of these asymmetric morphs could reveal which aspects of the alternative morphs are most developmentally integrated and therefore presumably most important for morph function, and which are less developmentally coupled.

Evolution of aphid wing dimorphisms

Aphid wing dimorphisms have an interesting evolutionary history with respect to the two sexes. The asexual female wing plasticity evolved early in aphid evolution, and most aphid species display this plasticity (reviewed previously in [15,21]). Male aphids also exhibit winged and wingless morphs. Males are produced as part of the sexual generation in the fall, when sexual females and males mate and produce overwintering, diapausing eggs. Male wing polymorphism is rare, occurring in only ~4% of aphid species and having evolved multiple times [22]. Most species produce winged *or* wingless males, not both, and there have been many transitions between the two wing morph phenotypes over evolutionary time [22]. It is possible that the persistence of the female wing plasticity across the aphid phylogeny has facilitated these many male wing morph transitions: if a species loses one of the morphs in males, it can be re-evolved at a later date because the genome retains the ability to produce both morphs (because the asexual females are always producing both morphs).

Compared with females, far less is known about tradeoffs between male winged and wingless morphs, although in pea aphids, the wingless males reach reproductive maturity faster and have larger testes, while the winged males have an advantage in competitive matings [23]. Interestingly, the male wing dimorphism in pea aphids is *not* a phenotypic plasticity, but rather is controlled genetically by a single locus on the X chromosome: wingless males have a 120-kb insertion at this locus containing a duplicated, expressed *follistatin* gene, while winged males do not [24]. It is remarkable that a single aphid species, the pea aphid, exhibits both an environmentally induced wing dimorphism in asexual females and a genetically controlled one in males. The male genetic dimorphism evolved relatively recently [22,24] compared with the female plasticity, suggesting that this might be a case of genetic assimilation, the phenomenon by which trait variation originally induced by environmental variables loses its environmental responsiveness [25].

The ecological and life-history contexts of winged versus winglessness vary considerably across and within aphid species [21]. For example, in some gall-forming genera, only winged migrants are produced during the asexual part of the life cycle [26]. On the other hand, the egg-laying females of most species are wingless. Other ecological contexts that impact the prevalence of winged or wingless morphs include ant tending, galling, diet breadth of host plants, and host alternation [22,27].

Proximate basis of the wing plasticity

Wing plasticity is relatively widespread in insects, and a growing literature has begun to reveal how these plasticities work at the molecular, mechanistic level (see especially recent work in planthoppers, e.g. [28–30]; also see previous reviews in [31–33]), In aphids, this process must traverse a sequence of molecular events that culminates in morph determination (the 'decision' of an aphid to be winged or wingless) and subsequent, downstream effects that contribute to morph-specific development (the developmental achievement of that

morph once determined). When considering studies addressing the proximate basis of the aphid wing plasticity, it is important not to attribute a role in morph determination to genes that function only in the development of a morph-specific trait. For example, RNA-interference (RNAi) knockdown of genes important for wing development may result in aphids with reduced or missing wings [34]. However, such developmental functions in the wing machinery are distinct from a function in wingless morph determination, if the remaining suite of winged-morph-specific characters remains unchanged after gene loss-of-function.

Hormones play a prominent role in the aphid wing plasticity, as with likely many plasticities [35]. Juvenile hormone has long been implicated, but no role for this hormone in the aphid wing plasticity has been unequivocally established [15,31]. Rather, ecdysone signaling seems to be a key player, promoting winglessness [36,37], and is hypothesized to be the maternal-to-embryo signal critical for the transgenerational plasticity response of pea aphids. Similarly, higher levels of dopamine are associated with increased production of wingless offspring in pea aphids [36], and adding dopamine via injection resulted in more wingless offspring [38]. Given that dopamine acts upstream of ecdysone signaling in *Drosophila* [39], dopamine signaling may be responsible for the early stages of the environmental cue integration in pea aphids.

Insulin/insulin-like growth factor signaling (IIS) has also long been known as a regulator of a variety of plasticities [31], including controlling differences between long- and short-winged morphs of the planthopper [29,30] and the soapberry bug [40]. It is also important for the aphid wing plasticity. Wingless-destined pea aphid embryos exhibit higher expression levels of genes regulated by Forkhead Box O (FoxO) [41]. Because insulin signaling represses FoxO, this result implies that insulin signaling is decreased in wingless- compared with winged-destined embryos. Indeed, a subsequent study in pea aphids found that IIS activation alleviates FoxO-mediated repression of several wing development genes, including the wing 'master' gene *vestigial* [42]. Shang et al. [43] found that IIS modulation is important for wingless morph determination, this time in both the pea aphid and in the brown citrus aphid. This modulation is achieved via miRNA-9b-mediated inhibition of the gene *ATP-Binding Cassette subfamily-G member 4* and subsequent loss of *Insulin-Like Peptide 3* expression. Importantly, this mechanism appears to be critical both for transgenerational morph determination and post-embryonic wingless morph development, and may involve a non-canonical IIS pathway.

Still other studies have provided insight into the specific genes responsible for wingless morph development. Yuan et al. [44] found that a novel duplicate of the *Repressed by TOR* gene (*REPTOR2*) is upregulated in wingless pea aphid nymphs, and its action led to autophagy of developing wing buds. All pea aphid nymphs have wing primordia at birth, so wing bud loss is an important stage for morph differentiation [45]. Fan et al. [46] found that *vestigial*, which is critical for wing growth across insects, is downregulated in developing wingless morphs of the bird cherry-oat aphid. Further, they discovered that this downregulation of *vestigial* is modulated posttranscriptionally by miRNA-*147b*, not by modulating the upstream regulators of *vestigial*. This latter study is particularly interesting because it shows how *vestigial* is directly affected, avoiding the likely pleiotropic effects that would have occurred if *vestigial's* regulators were also modulated. In this case, direct regulation of *vestigial* may bypass pleiotropic constraints on the evolution of phenotypic plasticity in this system.

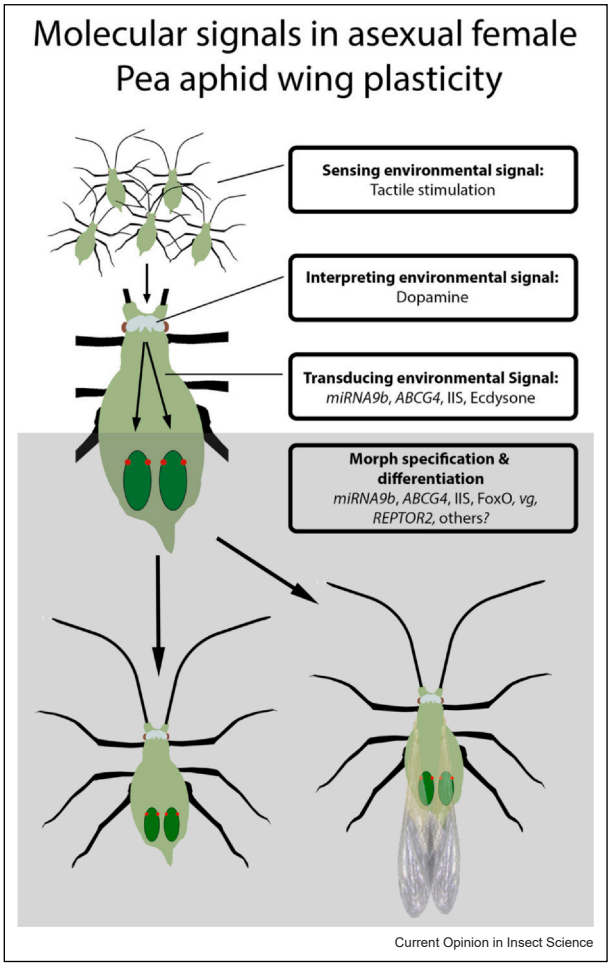
Much, therefore, has been learned about the molecular mechanisms involved in the aphid wing plasticity (summarized in Figure 2). Future studies will work to connect the pieces of the puzzle, from environmental cue reception (perhaps the least-understood part of the process) to alternative morph development. Another open question is how the wing plasticity evolved from a wing monomorphic ancestor. It is intriguing that the signaling mechanisms important for aphid wing plasticity such as ecdysone, IIS, and TOR (Target Of Rapamycin), are known to play many, often interacting roles in reproduction, behavior, metabolism, metamorphosis, and stress response in *Drosophila* (e.g. [47–49]). Thus, the pea aphid wing plasticity could have evolved from preexisting, integrated networks controlling metabolic, gonadotropic, and metamorphic alterations that occur in response to environmental stress.

Genetic variation for wing plasticity

A promising area of focus for ongoing and future research is examining genetic variation for the wing plasticity and determining the genetic variants responsible for that variation. As with other traits, plastic traits exhibit genetic variation that can be subject to natural selection [50,51]. Despite the importance of variation to the evolutionary process, little is known about the types of genes/genetic pathways that underlie variation in plastic responses.

Parker and Brisson [52] provided some initial insights. They found that a single pea aphid population harbored extensive variation for the wing plasticity, observing a continuum from genotypes that produced high proportions of winged offspring in response to high-density cues to genotypes that produced low proportions of winged offspring in response to those same cues. Subsequently, comparative transcriptomic analyses revealed

Figure 2



Summary of putative molecular mechanisms involved in the aphid wing plasticity. The top part of the model summarizes how the aphid mother likely senses and transduces information about her environment, while the bottom section (in gray) summarizes molecular signals involved in morph specification and/or differentiation.

laterally transferred genes of densoviral origin that had higher expression in those high-responding compared with low-responding lines. Interestingly, densovirus infection in another aphid species caused the production of winged offspring [53], implying that the gene had retained its function post genome transfer. In this example, the conclusion gave an interesting answer to the question of whether genes underlying the development of plastic traits are the same ones that control variation in plasticity: not only are the densoviral genes from outside the developmental genetic pathway for the aphid wing plasticity, they also are from outside aphids themselves.

Pea aphid biotypes, which are host-plant associated lineages associated with some degree of ecological isolation, exhibit a similar range of plasticity variation [54]. Parker et al. [54] investigated gene expression in clonal lines from two biotypes: one that produces a large number of winged offspring in response to crowding (high-responding), and one that produces mostly wingless offspring, regardless of crowding (low-responding). They found that the high-responding biotype line had a strong transcriptional response to a high- versus low-density environment, while the low-responding biotype had no differentially expressed genes with the same treatments. This result suggested that genotypes can lose their ability to assess their environment, and cannot respond to environmental density cues.

While these studies provide building blocks to understanding variation in aphid wing plasticity at a mechanistic level, many unresolved questions remain. Are the mechanisms underlying intraspecific variation in plasticity the same as the interspecific variation differences between species? Which type of variation is more likely to arise, for example, variation due to endocrine versus epigenetic or other factors? Is variation found more often at the environmental sensing, cue integration, or morph differentiation level? This lack of knowledge represents a significant roadblock to understanding how plastic responses evolve. The synthesis of intra- and interspecific studies on the developmental basis of plasticity will begin to resolve these outstanding questions.

Future directions

Much remains to be discovered about the molecular mechanisms underlying aphid wing plasticity. Given that morph differences emerge from the same genome, epigenetic analyses show great promise for future studies [55,56]. As noted in Richard et al. [57], epigenetic mechanisms such as chromatin accessibility are the likely effectors downstream of signaling cascades that set up the alternative transcriptional programs that eventually lead to the alternative morphs. There have been some recent, pioneering epigenetic studies in aphids. Richard et al. [58] revealed a sex-specific chromatin accessibility profile in pea aphids via Formaldehyde-Assisted Isolation of Regulatory Elements followed by deep sequencing (FAIRE-seq) and Mathers [59] did the same with DNA methylation.

Functional manipulations will become more critical as research groups become increasingly interested in probing a range of molecular mechanisms associated with the wing plasticity. Targeted mutations via the Clustered Regularly Interspaced Repeat-CRISPR associated protein 9 (CRISPR–Cas9) system are technically possible in aphids [60], but have not been widely implemented due in part to the low hatching rates following the obligate overwintering diapause period of aphid eggs. This is in addition to the common problems faced with CRISPR experiments, including the potential lethality or sterility of mutants and the difficulty of screening and identifying mutants. Still, CRISPR may provide a valuable means to functionally evaluate genes

for a role in morph determination via complete loss-of-function.

No transgenic lines of aphids have yet to be produced, but an established enhancer-reporter assay would be highly beneficial for dissecting gene networks responsible for plasticity. Specifically, a thorough dissection of the *cis*-regulatory elements at genes important for morph determination in the embryo is required to understand how they are activated or repressed by environmentally induced maternal endocrine signaling. Despite the inherent difficulties, the establishment of ever-more elegant molecular tools in nontraditional insect models (e.g. [61,62]) provides hope for developing these technologies in aphids. Combining established next-generation sequencing experiments with protein loss-of-function (RNAi or CRISPR) and enhancer-reporter assays, will be fruitful approaches for future studies aimed at piecing together the wing plasticity gene-regulatory network.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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