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*Review*

# Aphid wing dimorphisms: linking environmental and genetic control of trait variation

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Both genetic and environmental factors underlie phenotypic variation. While research at the interface of evolutionary and developmental biology has made excellent advances in understanding the contribution of genes to morphology, less well understood is the manner in which environmental cues are incorporated during development to influence the phenotype. Also virtually unexplored is how evolutionary transitions between environmental and genetic control of trait variation are achieved. Here, I review investigations into molecular mechanisms underlying phenotypic plasticity in the aphid wing dimorphism system. Among aphids, some species alternate between environmentally sensitive (polyphenic) and genetic (polymorphic) control of wing morph determination in their life cycle. Therefore, a traditional molecular genetic approach into understanding the genetically controlled polymorphism may provide a unique avenue into not only understanding the molecular basis of polyphenic variation in this group, but also the opportunity to compare and contrast the mechanistic basis of environmental and genetic control of similar dimorphisms.

**Keywords:** genetic accommodation; gene expression; pea aphid; polymorphism; polyphenism; wing

## 1. INTRODUCTION

How environmental and genetic variation influence phenotypic variation remains a fundamental question in biological investigations. The confluence of evolutionary and developmental biology in the past few decades has begun to reveal how genetic variation affects morphological variation within and between species. Less well understood at the molecular level is how organisms adjust their morphology in response to the environment. Phenotypic plasticity, as this is called, is taxonomically widespread.

Plasticity may play an underappreciated role in the evolution of morphological diversity. Some have hypothesized that when a new morphological variant arises in a population, it is more likely due to environmentally dependent expression of standing genetic variants rather than to new allelic forms (Matsuda 1987; Stearns 1989; Pigliucci & Murren 2003; West-Eberhard 2003). Over time, natural selection fine-tunes this variation via selection on modifying alleles, with the end product being the new variant genetically fixed or maintained as a morphological polymorphism under genetic or environmental control (this multi-step process, termed genetic accommodation, is reviewed by West-Eberhard 2003; Braendle & Flatt 2006).

Given the potential importance of plasticity in the evolutionary process and its ubiquity, it becomes quite interesting to try to disentangle the gene networks that make it possible. The distribution of plastic effects is often continuous and therefore challenging to analyse using traditional molecular and developmental approaches. Instead, for these types of investigations, we can focus on polyphenism, an extreme form of phenotypic plasticity in which a single genotype produces discrete alternative morphologies. Polyphenism is prevalent in the animal kingdom (reviewed in West-Eberhard 2003). Examples include caste polyphenisms of social insects (Wilson 1971) and seasonal colour morphs in caterpillars and butterflies (Shapiro 1976; Brakefield & Larsen 1984). Despite decades of work, we are only beginning to understand the molecular mechanisms underlying polyphenic development, although many polyphenisms involve changes in the timing or level of hormones (reviewed in Nijhout 1999).

In this review, I will focus on another familiar polyphenism: the winged and unwinged morphs of aphids. This system is particularly instructive for two primary reasons. First, as I will illustrate below, within their life cycle, some aphids display both a polymorphism (used here to describe phenotypic differences caused by alternative alleles at a locus or loci) and a polyphenism for the same alternative phenotypes, winged or

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unwinged morphs. Hence, a traditional developmental genetic examination of the polymorphism could provide an avenue into discovering the molecular pathways used to achieve the polyphenism. Second, phylogenetic patterns suggest evolutionary transitions between monomorphism and polymorphism or polyphenism, and possibly between polyphenism and polymorphism. The system could eventually provide insight into how, at the mechanistic level, trait variation can transition between environmental and genetic control.

First, I will present background information on wing dimorphisms and later focus on investigations into the wing morph control mechanisms. Although I will write 'environmental' versus 'genetic' as dichotomies for simplicity, it is with the understanding that all phenotypes are the result of an interaction between the genotype and the environment.

## 2. WING DIMORPHISM IN APHIDS

### (a) *Winged dispersers and unwinged reproductives*

Many insects produce both flight capable and flight-deficient forms. Here, I will limit my discussion to aphids, but wing polymorphisms have a long history of study from ecological, evolutionary and physiological points of view (see reviews in Harrison 1980; Roff 1986; Zera & Denno 1997). Many aphids exhibit discretely different winged and unwinged morphs, an example of which can be seen in figure 1 for the pea aphid, *Acyrthosiphon pisum* (although not discussed here, some species display more continuous variation between winged and unwinged morphs such as long- and short-winged morphs). The morphs are distinguished not just by whether they have wings, but also by additional morphological differences, their behaviour and their life history. The unwinged morphs lack wings and the wing musculature of the thorax. The winged morphs possess heavily sclerotized thoraces, more convex compound eyes and more sensory organs called rhinaria on their antennae; they also possess ocelli (Kring 1977; Miyazaki 1987; Tsuji & Kawada 1987; Ishikawa & Miura 2007). Behaviourally, unwinged morphs are more sedentary (Sack & Stern 2007).

As with wing dimorphisms in other species, the morphs exhibit trade-offs related to dispersal and reproduction. Fortunately, aphids are cyclical parthenogens (see §2*b*), so a single aphid clone can hedge its bets by producing both morphs. Winged aphids of course have the ability of flight, which enables them to migrate to new host plants if aphid densities become high or if the quality of the host plant deteriorates. But this advantage comes with a downside: winged aphids have lower reproductive output, producing less offspring overall and producing offspring that are generally smaller when compared with unwinged aphids (Wratten 1977).

Unwinged aphids have a faster development time and larger body size (Dixon & Howard 1986), but the unwinged females have limited migratory ability. They can only drop off a plant and walk away if they encounter a predator or if their food source deteriorates. The trade-off between the morphs likely relates directly to where they invest their energy, with unwinged morphs using nutrients to develop quickly and produce offspring rather than to build wings and flight muscles and to maintain energetically costly flight performance as adults. In fact, to alleviate this trade-off, some species histolyze their wings following migration, allocating resources back into offspring production. Excellent and much more thorough treatments of the optimal partitioning of resources between winged and unwinged morphs can be found in Dixon *et al.* (1993) and Dixon (1998).

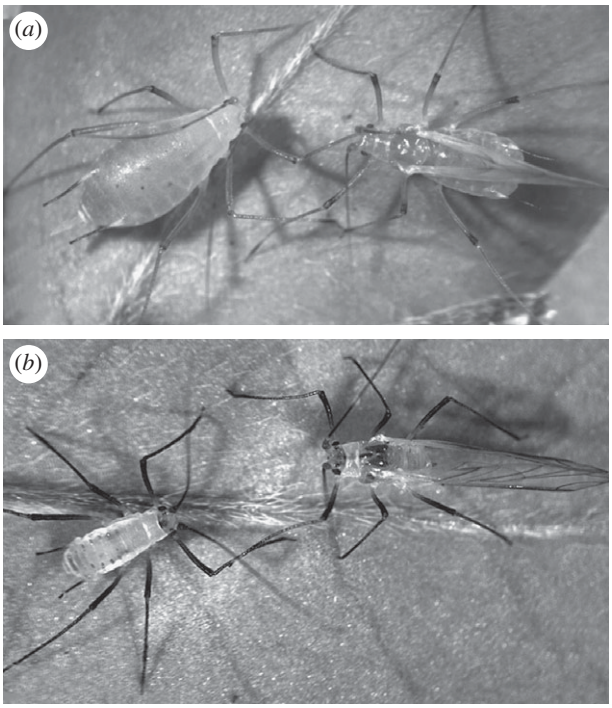


Figure 1. (*a*) Unwinged and winged females and (*b*) unwinged and winged males of the pea aphid. Note that the two morphs differ by more than whether they have wings. For example, the winged morphs have heavily muscled, well-defined thoraces relative to the unwinged individuals.

Although throughout this article I will refer to the morphs as winged or unwinged, this is just a shorthand to describe *systemically* different phenotypes.

### (b) *Dynamic use of the winged and unwinged morphs across the aphid life cycle and phylogeny*

There are approximately 4400 species of aphids (Blackman & Eastop 2000), geographically distributed throughout primarily temperate regions and belonging to the extant families of Aphididae, Phylloxeridae and Adelgidae. The latter two families, together called the Phylloxeroidea (figure 2), comprise only about 5 per cent of aphid species. The third family, Aphididae, is likely over 200 Myr old and the alternation between parthenogenetic and sexual reproduction is basal to this group (Moran 1992). There is little phylogenetic resolution at the subfamily level, likely due to a rapid radiation following their host plant switch from gymnosperms to angiosperms during the Cretaceous (von Dohlen & Moran 2000; Martinez-Torres *et al.* 2001). I present a simplified phylogenetic hypothesis

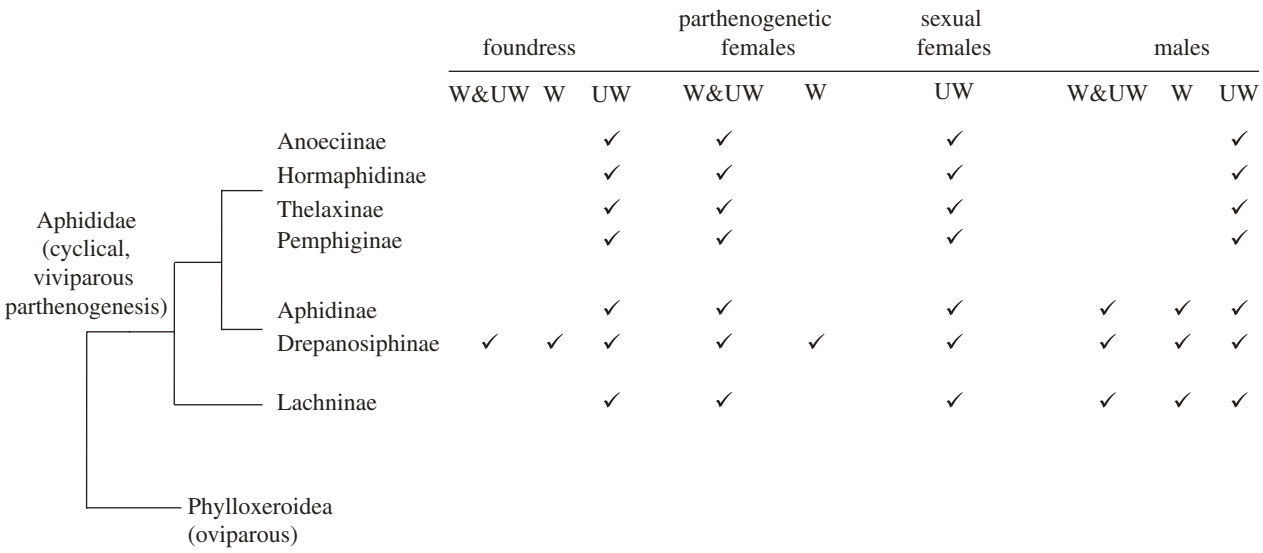


Figure 2. Phylogeny of several aphid subfamilies, simplified from Ortiz-Rivas *et al.* (2004). To the right of the phylogeny are columns showing the presence of winged (W) and unwinged (UW) morphs during stages of the life cycle in each group. A checkmark indicates that at least one species in the subfamily is monomorphic if 'W' or 'UW' is marked, or at least one species is dimorphic if 'W&UW' is marked. Data compiled from multiple sources (Hille Ris Lambers 1966; Stroyan 1977; Heie 1980, 1982, 1986, 1992, 1993, 1995; Blackman & Eastop 1994, 2000). See table S1 in the electronic supplementary material for a species-by-species listing.

of the relationships between the subfamilies as determined by Ortiz-Rivas *et al.* (2004) in figure 2.

(i) *Life cycle*

The occurrence of winged and unwinged morphs varies across the complex and diverse aphid life cycle, with both winged and unwinged morphs occurring during some stages and only a single morph (monomorphism) in others (Hille Ris Lambers 1966; Miyazaki 1987). Most species alternate between live-bearing parthenogenetic generations in the spring and summer and a sexual egg-bearing generation in the autumn. A complete consideration of aphid life cycles and their variations can be found in Moran (1992).

The parthenogenetic female fundatrix that emerges from an egg in the spring is typically unwinged. These asexual females give live birth to genetically identical daughters via a modified meiosis that bypasses genetic recombination and segregation of homologous chromosomes (Blackman 1987). The parthenogenetic generation time is short, of the order of 10 days or so in some species, and consequently many generations occur during the spring and summer months. Parthenogenetic generations exhibit both winged and unwinged morphs, with mothers producing winged daughters after they experience certain environmental cues such as declining food quality, increased crowding and increased interspecific interactions (reviewed in Müller *et al.* 2001; Braendle *et al.* 2006). For example, in the vetch aphid (*Megoura viciae*), if unwinged females are raised individually and then placed together as adults in groups of ten, they will produce winged daughters, whereas those that remain isolated do not (Lees 1967). Because the winged and unwinged morphs of a single genetic clone are determined by environmental circumstances, this is referred to as the *wing polyphenism*.

Most aphid species live in temperate climates, and adults are incapable of surviving subzero temperature. In the autumn, aphids produce a sexual generation that reproduces oviparously, resulting in an egg that diapauses through the cold winter months. Most sexual females are monomorphic unwinged, and no sexual females are dimorphic (Hille Ris Lambers 1966; Miyazaki 1987). Males can be winged or unwinged, with some species producing both and others only one of the morphs. Although in most of the male dimorphic taxa the underlying cause of the dimorphism is unexamined, the male dimorphism of species in the Macrosiphini (Aphidinae) seems to be owing to *genetic*, and not environmental, causes (Hille Ris Lambers 1966; Smith & MacKay 1989). The male dimorphism is thus not a polyphenism, but rather a genetically determined polymorphism.

Host-alternating species have a more complicated life cycle: a parthenogenetic female, usually unwinged, emerges from an egg on the primary host plant. She produces a lineage of females, some of which must eventually be winged for dispersal to the secondary host plant. On the secondary host, multiple parthenogenetic generations can persist, but at the end of the summer season they again migrate back to their primary host for sexual reproduction and egg deposition. To attain this necessary second migration, these species generate a winged parthenogenetic female that returns to the primary host where she produces unwinged sexual females and unwinged or winged males, or some mix of winged and unwinged parthenogens and sexuals.

The beautifully detailed monographs by Heie (1980, 1982, 1986, 1992, 1993, 1995) describe the biology and morphology of aphid species from the major taxonomic groups. All the species in which he described the wing morphology for all major life cycle stages are listed in table S1 in the electronic

supplementary material and summarized in figure 2 (the few exceptions to the generalities presented in figure 2 can be found in Hille Ris Lambers (1966)).

(ii) *Evolutionary patterns*

Most species use both wing morphologies to varying degrees across the life cycle (figure 2). Two broad patterns emerge.

First, environmental control of wing phenotypes probably evolved early. Many aphid species exhibit stage-specific morphs such as unwinged foundresses, winged migrants and unwinged sexual females. Their limited temporal expression implies environmental contributions to their phenotypes.

Second, dimorphism has evolved from monomorphism. Aphid ancestors were winged, so the unwinged morph is an evolutionarily derived condition (Blackman 1974). Most species produce both winged and unwinged parthenogenetic females. The female wing polyphenism thus evolved early and is generally maintained across the family. The pattern in males is more complex. Most species have monomorphic males. Species in the Anoeciinae, Hormaphidinae, Thelaxinae and Pemphiginae seem to exclusively produce unwinged males (Heie 1987; Blackman & Eastop 2000). Genera within the Aphidinae and Drepanosiphinae have species that produce only unwinged morphs, others that produce only winged morphs and a handful that are dimorphic (table S1 in the electronic supplementary material). For example, of the non-host-alternating species in the genus *Aphis* (Aphididae) described by Heie (1986), I counted 30 species with unwinged males, 16 species with winged males and only one species with winged and unwinged males. Smith & MacKay (1989) estimated that about 90 per cent of European aphid species produce monomorphic males. Dimorphism in males has therefore evolved multiple times even within the Aphidinae. Further, the production of winged males, unwinged males or both, is highly labile across the phylogeny, in that closely related species can be monomorphic for opposite wing morphologies. This pattern suggests a lability to the developmental processes underlying the morph decision.

Thus, a single aphid genome allows wing morphology to be determined by an environmental trigger during some stages and genetic control during others. Given that all aphids were ancestrally winged, this implies that both environmental and genetic controls of wing morphology dimorphisms have evolved in this system. What remains unclear is the order of their appearance. For example, it is possible that the different temporal deployment of the two morphs emerged first, with the default winged state produced during some stages and the disrupted unwinged state during other stages. Once the alternative morphologies were established in the genetic programme, the polyphenism could have emerged by evolving an environmentally sensitive choice between the two options. The male polymorphism could have evolved by placing the same mechanism responsible for the switch between unwinged and winged females under genetic control. If this were the case, it would be a form of genetic accommodation, and a particularly interesting case of genetic accommodation because a developmental plasticity during one point in the life cycle (females, summer) would be under genetic control at other points in the life cycle (males, autumn).

### (c) *The pea aphid: one species, two dimorphisms*

A well-studied species that exhibits a wing dimorphism during the parthenogenetic, summer portion of the life cycle as well as with males during the sexual, autumn portion of the life cycle is the pea aphid (*A. pisum*, in the Aphidinae subfamily). As with other species of aphids, parthenogens are typically unwinged, but if they encounter crowded circumstances on their host plant they will produce winged daughters. Males are produced asexually in the autumn in response to shortened day length and lower temperatures (MacKay *et al.* 1983; Via 1992), and are formed when they receive only one X chromosome during the division of an ooctye (Orlando 1974; Blackman 1987). Hence, the sole genetic difference between male offspring and their mother is that they carry only one of her X chromosomes. Females are thus XX and males XO.

A pea aphid clone collected from nature produces either all winged males, all unwinged males or winged and unwinged males in an equal ratio. Based on this observation, and the fact that males have only one X chromosome, Smith & MacKay (1989) hypothesized and Braendle *et al.* (2005*a*) later confirmed that the winged state of males is determined by a locus on the X chromosome. This locus was later named '*aphicarus*' (*api*) after Icarus, the tragic figure of Greek mythology whose wax-cemented feather wings melted when he flew too closely to the Sun (Braendle *et al.* 2005*a*). We do not know when during development *api* acts to determine male-morph type, and mapping of this locus is ongoing.

Like many polyphenisms, there is genetic variation for the polyphenic response, both in the pea aphid (Markkula 1963; Weisser & Braendle 2001; Hazell *et al.* 2005) and in other species (MacGillivray & Anderson 1958; Blackman 1979; Groeters 1989). This variation exists over space and time and is likely subjected to natural selection. A proposed selective pressure for winged-morph production in females and males is the persistence of host plants, with more winged morphs produced to adapt to short-lived host plants and less-winged morphs for long-lived host plants (Markkula 1963; Groeters 1989; Frantz *et al.* 2009).

Does genetic variation at the *api* locus contribute to the variation in the polyphenic response? To explore this question, Braendle *et al.* (2005*a*) produced an F2 mapping population by crossing a clone homozygous for the *api* unwinged allele to one homozygous for the *api* winged allele and then crossing the F1 generation. The *api* genotype of each F2 clone was assessed by scoring the proportion of winged and unwinged males produced in a sexual generation. Parthenogenetic females of these clones were crowded to determine their polyphenic response as measured

by the number of winged offspring they produced. The response was compared among clones that were homozygous for the unwinged *api* allele, heterozygous or homozygous for the winged *api* allele. The results were quite striking: clones with at least one copy of the *api* unwinged allele produced more winged daughters than females homozygous for the *api* winged allele (Braendle *et al.* 2005*b*). Thus, a large amount of genetic variation for the female polyphenism maps near the *api* locus, although we do not know if this variation is due to *api* itself or to a linked locus. Further, this linkage is in reverse phase such that clones that produce winged males in the sexual phase of the life cycle are less likely to produce winged females during the parthenogenetic phase of the life cycle, raising the intriguing possibility that the sexual and parthenogenetic generations balance the need for dispersal using the same locus.

Because of the presence of both polyphenism and polymorphism and the observed linkage between them, we can use the pea aphid system to compare and contrast the mechanistic basis of the two dimorphisms with the ultimate goal of informing how an evolutionary transition between polyphenism and polymorphism might occur.

## 3. TOWARDS AN UNDERSTANDING OF THE MOLECULAR GENETIC BASIS OF THE WING POLYPHENISM

### (a) *Moving aphids into the genomic era*

A number of years ago, researchers pursuing divergent topics in aphid biology joined together to form the International Aphid Genomics Consortium and agreed upon the pea aphid as the laboratory aphid of choice to focus the development of genomic resources. In 2007 and 2008, the approximately 525 Mb pea aphid genome was sequenced at 6X coverage by the Human Genome Sequencing Center at Baylor College of Medicine. A first assembly was released in 2008, along with a database (Aphidbase.com) for integrating the sequence and annotation information (Gauthier *et al.* 2007). There are also currently close to 170 000 expressed sequence tags for the pea aphid (Sabater-Muñoz *et al.* 2006), approximately 28 000 in the green peach aphid, *Myzus persicae* (Ramsey *et al.* 2007) and thousands more covering a range of other aphid species.

### (b) *The developmental basis of the wing polyphenism*

Effectively targeting genomic resources requires an understanding of the developmental basis of the polyphenism. Parthenogenetic aphids are viviparous, meaning that they give live birth. Daughter embryos develop serially along the ovarioles, completing embryogenesis within the mother before they are born as first instar nymphs. This close and continued association creates a unique opportunity for the mother to convey information about her environmental circumstances directly to her daughters before they are even born. Indeed, all experimental evidence in the pea aphid suggests that it is the mother that perceives cues such as crowding and transmits this information to her daughter embryos. Only aphids that have *not yet been born* are competent to respond to this unknown signal and set in motion events that are morphologically visible two nymphal stages (several days) later (Sutherland 1969). Post-natal nymphs cannot be induced to produce wings in the pea aphid. If nymphs are crowded, they give rise to no greater proportion of winged offspring than control groups. (However, in other species of aphids such as *A. craccivora* (Johnson 1965) or *M. persicae* (Sutherland & Mittler 1971), the winged morphology can be induced up to the third nymphal instar (Hille Ris Lambers 1966; Lees 1966; Müller *et al.* 2001). This suggests some flexibility in the timing and possibly the mechanism of the developmental determination of the wing polyphenism switch.)

The events leading to the production of winged offspring are thus obviously multi-step: the mother must perceive the environmental stimulus; she must convert that stimulus into a transmissible signal; that signal must make its way to the daughter embryos growing in the mother's ovarioles; and the daughter embryos must respond to that signal. The molecular mechanisms underlying all steps in the process are unknown, important, interesting and therefore excellent targets for study.

In species that have been examined, all aphids appear to be born with wing buds (Turner & Baker 1916; Davidson 1927; Johnson & Birks 1960). Thus, the winged state is not only the phylogenetically basal state, but also the default developmental state. Unwinged aphids must somehow disrupt this default developmental plan. Externally, winged and unwinged morphs are not distinguishable until the third nymphal instar when the wing buds can be seen as bumps on the thorax. Histological examinations, however, can discern differences as early as the second nymphal instar (Ishikawa & Miura 2007).

### (c) *Assaying gene-expression differences between winged and unwinged morphs*

(i) *The wing development gene repertoire of the pea aphid*

We can now begin to leverage the emerging pea aphid genomic resources to study the wing polyphenism at the molecular level. To date, we have a fairly thorough knowledge of the morphological sequence of events that characterize winged and unwinged morphs. But what are the molecules underlying those morphological events? At what point is the wing determination network interrupted to result in an unwinged morph? The most complete examination of wing patterning, growth and differentiation has occurred in *Drosophila melanogaster* (Campbell *et al.* 1993; Sturtevant & Bier 1995; Weatherbee *et al.* 1998; Butler 2003; Ren *et al.* 2005; Kiger *et al.* 2007). However, *Drosophila* is an indirect-developing insect, with much of its wing development occurring during the dramatic reorganization that happens at pupation. In contrast, the pea aphid is a direct developer, born with wing buds that grow relatively slowly over the nymphal instars. There are over 300 Myr of evolution that separate aphids and flies. Can we use *Drosophila* as a model when investigating the wing patterning of the pea aphid?

Brisson *et al.* (in press) searched for homologues of 22 wing development genes (as identified in *Drosophila*) in the pea aphid genome. These included genes that function in anterior–posterior axis determination, dorsal–ventral axis formation, segmentation and wing hinge growth. The majority of these genes have roles that are not specific to wing development, but rather are highly pleiotropic across development. All 22 of these genes were found in the pea aphid, suggesting that despite the large differences of developmental processes between holometabolous and hemimetabolous insects, many of the major components of wing development studied in *Drosophila* are conserved in the pea aphid genome. Two of the wing genes in the pea aphid exhibited duplications. Specifically, there are two *apterous* (*ap*) and four *decapentaplegic* paralogues. The pea aphid genome exhibits quite a large number of duplications (Sabater-Muñoz *et al.* 2006; IAGC submitted).

Brisson *et al.* (in press) also examined the expression levels of 11 of these wing development genes via quantitative PCR (qPCR) at the embryonic stage, and at each of the four nymphal instars for both winged and unwinged parthenogenetic females. One *ap* orthologue showed a significantly higher level of expression in the winged morphs relative to the unwinged morphs during the early nymphal instars. This intriguing result suggests that *ap* may play a proximate role in differentiating the morphs, perhaps with lower levels of *ap* expression in the unwinged morphs leading to mispatterning of the dorsal–ventral axis of the wing bud. The limitation of this approach is that it focuses specifically on only one aspect of the polyphenism: wing development. As previously mentioned, the two morphs exhibit systemic differences that go well beyond the presence or absence of wings.

(ii) *The transcriptional basis of the wing dimorphisms*

Transcriptional profiling of polyphenisms via microarrays, cDNA sequencing, subtractive hybridization and (at least in the very near future) RNA sequencing is becoming an accessible form of investigation in non-model systems, and has been used to characterize the gene-expression states of alternative morphologies in termites (Scharf *et al.* 2003), bees (Evans & Wheeler 1999; Pereboom *et al.* 2005; Judice *et al.* 2006), parasitic wasps (Donnell & Strand 2006) and social wasps (Hoffman & Goodisman 2007). A particular advantage of gene-expression profiling in the aphid wing polyphenism is that the confounding effect of genotype can be controlled by using aphids of the same clone. Ghanim *et al.* (2006) were the first to examine an aphid wing polyphenism via transcriptomics, using an array of *M. persicae* cDNA sequences to interrogate expression differences between winged and unwinged parthenogenetic adult females. They identified 31 unique sequences that differed between the two (of an estimated 4000 Unigenes on the array). These included many hypothetical or unknown proteins, nine genes with products involved in ATP metabolism and synthesis, a LIM-like protein and a cuticle protein (table 1). qPCR analysis showed strong expression of the *M. persicae* mitochondrial adenine nucleotide translocase (ANT) orthologue in the thoraces of winged individuals. Ghanim *et al.* (2006) hypothesized that ANT is associated with the flight muscles of the thorax and the energy necessary for flight. Similarly, *M. persicae* OS-D, with a putative role in chemoreception, showed higher expression levels in the antennae and legs of the winged morphs, presumably because flight requires greater chemosensory ability. Finally, they showed that the *M. persicae takeout-like* (TOL) gene, a putative member of the TOL and *takeout* gene family of *Drosophila*, exhibited higher expression in winged morphs, with particularly high expression levels in abdomens and heads.

Brisson *et al.* (2007) used a microarray approach to characterize the differences between winged and unwinged morphs in the pea aphid, which as explained above (and unlike *M. persicae*) presents unwinged and winged morphs in both males and females. The pea aphid microarray contained 1734 known, unique cDNAs (Wilson *et al.* 2006). They found 141, 131, 142 and 353 genes with differential transcript accumulation between winged and unwinged morphs in fourth instar females, fourth instar males, adult females and adult males, respectively. The majority of these genes were at higher expression levels in winged relative to unwinged morphs, such as genes involved in muscle and energy production and metabolic functions (table 1). For example, the expression level of the pea aphid homologue of *flightin*, a component of the indirect flight muscles (Vigoreaux *et al.* 1993), was dramatically higher in the winged morph. A number of the differentially expressed genes were the same between the pea aphid and *M. persicae* (table 1). Like Ghanim *et al.*'s (2006) study, many genes with differences were of unknown function.

Further, Brisson *et al.* (2007) asked: since the two morphs of the two sexes share morphological similarities, do they share transcriptional similarities as well? In other words, are they using a similar transcriptional programme to build the alternative adult body forms? At both developmental stages, it was found that the same genes expressed differentially between the morphs in the two sexes, with 54 genes in the fourth instar and 67 as adults. Further, even when exactly the same genes were not differentially expressed between both sexes, the general gene classes that were differentially expressed were the same. It was concluded that these gene-expression similarities suggest that despite the difference in control of the switch between the polyphenism and polymorphism (environmental versus genetic), the downstream events are fundamentally similar.

Based on these types of transcriptomic assays, we have learnt that alternative morphologies in various taxa are accompanied by alternative gene-expression states. And specifically in the pea aphid, we have learnt that there are fundamental similarities that differentiate the wing morphs in the two sexes, which further suggest that they share a mechanistic basis at some level. However, these types of studies analyse gene expression far downstream of the molecular events that control the switch between the two morphological outcomes. In many polyphenisms, it is difficult to study the period in which the upstream

Table 1. Transcripts showing differential accumulation between winged and unwinged morphs of both males and females (fourth instars or adults) of the pea aphid (*A. pisum*), or between winged and unwinged adults females of the peach potato aphid (*M. persicae*).[a]

| gene description | *Acyrthosiphon pisum* | *Myzus persicae* | transcript levels higher in winged morphs | transcript levels higher in unwinged morphs |
|---|---|---|---|---|
| ATP binding/microtubule motor | | ✓ | ✓ | |
| ATP synthase subunits | ✓ | ✓ | ✓ | |
| ATP-binding cassette transporter | | ✓ | ✓ | |
| cytochrome-*c*-oxidase subunit | ✓ | ✓ | ✓ | |
| cytochrome-*c*-like protein | ✓ | ✓ | ✓ | |
| DNA-directed RNA polymerase II | | | | |
| subunit | ✓ | | ✓ | |
| elongation factor Tu | | ✓ | ✓ | |
| exosome component-8 | ✓ | | ✓ | |
| flightin | ✓ | | ✓ | |
| glycerol-3-phosphate dehydrogenase | ✓ | | ✓ | |
| guanosine monophosphate reductase | ✓ | | ✓ | |
| helicase | ✓ | | ✓ | |
| heme-based aerotactic transducer | | ✓ | ✓ | |
| histidine kinase | | ✓ | ✓ | |
| LIM-like protein | | ✓ | ✓ | |
| mitochondrial ADP/ATP translocase | | ✓ | ✓ | |
| muscle actin | ✓ | | ✓ | |
| myosin 1 light-chain-like protein | ✓ | | ✓ | |
| NADH-ubiquinone oxidoreductase | ✓ | | ✓ | |
| OS-D-like protein | | ✓ | ✓ | |
| ribosomal protein | ✓ | ✓ | ✓ | |
| Rieske iron–sulphur protein-1 | ✓ | | ✓ | |
| succinate dehydrogenase activity | ✓ | ✓ | ✓ | |
| takeout-like protein | | ✓ | ✓ | |
| triosephosphate isomerase 1b | ✓ | | ✓ | |
| ubiquinol–cytochrome *c* reductase | | | | |
| complex proteins | ✓ | | ✓ | |
| cuticle protein | ✓ | ✓ | | ✓ |
| GDP-L-fucose synthetase | | | | |
| guanine nucleotide binding protein | ✓ | | | ✓ |
| membrane alanyl aminopeptidase *N* | ✓ | | | ✓ |
| pyridoxal kinase | ✓ | | | ✓ |
| ribosomal protein | ✓ | | | ✓ |
| trehalase | ✓ | | | ✓ |

[a]See Brisson *et al.* (2007) and Ghanim *et al.* (2006) for more details.

switch between pathways occurs because it appears to depend on the accumulation of events over a period of time. As we know fairly precisely when and where the events of the pea aphid polyphenic switch are occurring (see §3*b*), transcriptional profiling of those times and tissues holds promise for capturing the changes occurring at the upstream, determining events.

### (d) *Hormones and the wing polyphenism*

Hormones have long been implicated in the control of polyphenisms, either through changes in their timing or level of expression, or through changes in the timing or level of expression of hormone receptors in different tissues (Nijhout 1999). For example, in the cricket genus *Gryllus*, decreased juvenile hormone (JH) esterase levels cause reduced JH degradation in

the last nymphal instar. The resulting higher JH titre, in combination with lower ecdysteroid titre, leads to the development of short-winged morphs rather than of long-winged morphs (Zera *et al.* 1989; Zera 2003). Similarly, in the honeybee, *Apis mellifera*, workers feed larger amounts of royal jelly protein to the larvae designated to be queens. This increased nutrition activates an endocrine response that elevates JH levels and eventually results in reproductive queens rather than sterile workers (reviewed in Hartfelder & Engels 1998).

In aphids, hormones are strong candidates in mediating the maternal response to crowding: hormone signals can traverse the haemolymph and affect the organism at the systemic level, and the polyphenism is marked by systemic differences between the morphs. An excellent review of the extensive literature investigating hormonal effects on the aphid wing polyphenism can be found in Braendle *et al.* (2006). Briefly, JH was considered a strong candidate because the unwinged morph looks like a juvenilized wing morph. However, studies investigating JH, for example, attempting to inhibit the production of winged progeny by application of JH, have provided equivocal results (reviewed in Braendle *et al.* 2006). In some aphid species, precocene II (PII), a plant-derived compound that putatively interferes with JH production, when applied to mothers can induce production of winged progeny (reviewed in Braendle *et al.* 2006). Two pieces of evidence suggest that this effect is not mediated by JH. First, although PII is able to induce winged progeny in pea aphids, it fails to induce precocious development, the classic JH-mediated trademark of precocenes (Hardie *et al.* 1996). Second, co-application of JH fails to reverse the wing-inducing effects of PII (Gao & Hardie 1996). Thus, the mode of PII action on wing induction remains unknown. Recently, Schwartzberg *et al.* (2008) directly measured JH titres from parthenogenetic females that had been exposed to wing-inducing cues versus those that were not, and discovered no difference between the two groups. However, it could be that titres remain the same, but that JH is regulated differently by JH esterase (which breaks down JH) or JH binding proteins. More likely is that JH is regulated in a tissue-specific manner.

### (e) *DNA methylation and the wing polyphenism*

Interestingly, the pea aphid wing polyphenism shows *trans*-generational effects. Unwinged parthenogenetic females can produce a large proportion of winged offspring when crowded. Those winged daughters, when presented with wing-inducing stimuli, produce predominantly *unwinged* offspring and thus seem to have lost their ability to respond to the environmental signal (Sutherland 1969). The unwinged grand-daughters, in turn, exhibit a return to 'normal' in that they can produce a large proportion of winged offspring again. Generational changes that can be reset are the hallmark of regulation by epigenetic mechanisms such as histone modification or methylation of cytosine residues in DNA. Indeed, DNA methylation could be a general regulator of plasticity (Moczek & Snell-Rood 2008) and was recently implicated in the regulation of the bee caste polyphenism. Kucharski *et al.* (2008) used RNAi to silence the expression of DNA methyltransferase 3, a key component of de novo DNA methylation. This silencing resulted in the majority of larvae emerging as queens, and none as workers.

We are only beginning to learn about the potential of gene-expression regulation by methylation in aphids. The pea aphid genome sequence contains the full complement of DNA methyltransferases (two *Dnmt1*s, a *Dnmt2*, a *Dnmt3* and a divergent *Dnmt3*-like gene), the enzymes necessary for maintenance of methylated cytosines and de novo cytosine methylation. Using mass spectrometry, Walsh *et al.* (in press) determined that the percentage of methylated cytosines in the pea aphid genome is 0.69 per cent ($\pm 0.25\%$) (methylation in insects is generally much lower than in vertebrates (Field *et al.* 2004)). Further, Walsh *et al.* (in press) identified 12 genes that exhibited methylation, with all the methylated cytosines observed in coding regions. If this trend of methylation in coding regions holds, given the approximately 30 per cent GC content of the genome (Sabater-Muñoz *et al.* 2006; IAGC submitted), that approximately 8 per cent of the genome consists of coding sequences, and a 0.38–0.80% rate of methylated cytosines (Walsh *et al.* in press), a high proportion of the coding sequences could be regulated by changing their methylation state.

Thus, we now know that methylation occurs in the pea aphid. What remains to be determined is the role of methylation in regulating gene expression in aphids. The E4 esterase gene was previously reported to be methylated in insecticide-resistant green peach aphids (Field *et al.* 1989; Field 2000). This gene appears to be upregulated when it is methylated, even though methylation in vertebrates associates with gene silencing (Eden & Cedar 1994). Whether the role of methylation in invertebrates is fundamentally different to its role in vertebrates remains an open question.

A more relevant question here is whether methylation patterns correlate with wing morphology. Walsh *et al.* (in press) used bisulphite sequencing to identify methylated sites in the genes coding for JH binding protein, JH epoxide hydrolase and JH esterase binding protein. They sequenced these genes from the heads of winged and unwinged parthenogenetic females as well as from the embryos of those females. All three genes contained methylation sites, but only JH binding protein exhibited differences between the morphs, with methylation at one site decreased by 50 per cent in winged heads when compared with unwinged heads. qPCR of the *Dnmt*s from RNA samples obtained from heads of unwinged adult parthenogenetic females that had been crowded (induced for the polyphenism) showed an upregulation of both maintenance methyltransferases (*Dnmt1a*&*b*) and of *Dnmt2* relative to a control, although only the *Dnmt2* result was significantly different (Walsh *et al.* in press). Further, Dombrovski *et al.* (2009) injected unwinged asexual females with DNA methyltransferase inhibitors and showed that they produced significantly less winged offspring relative to controls.

Together, these results are beginning to hint at the hypothesis that methylation plays a role in regulating the wing polyphenism, perhaps in a tissue-specific manner and perhaps through the use of JH-related genes.

### (f) *Other possible mechanisms controlling the wing polyphenism*

There are other mechanisms that could be involved in the polyphenic switch that have, to date, been completely unexplored in the pea aphid. The switch may be mediated by, for example, a transcription factor or microRNA sensitive to environmental conditions or to hormonal titres that could induce the use of alternative pathways. Another possibility is alternative splicing, the process where different exons are spliced from the precursor mRNA transcripts to produce alternative mature mRNAs and thus different proteins. This is a common phenomenon among eukaryotes (Kim *et al*. 2007), and has been proposed as a mechanism underlying phenotypic plasticity (Marden 2008). As genomic tools progress in the pea aphid, we will be able to interrogate this type of mechanism. For example, we can create exon-specific microarrays and hybridize tissues from induced and uninduced females in a manner that is analogous to studies that have identified an abundance of alternatively spliced transcripts in *Drosophila* (Stolc *et al*. 2004; Telonis-Scott *et al*. 2009) and humans (Xu *et al*. 2002; Johnson *et al*. 2003). Analogous results could be obtained by using next-generation sequencing approaches.

## 4. CONCLUSIONS

Recent years have seen breakthroughs in revealing the proximate causes of polyphenisms, sometimes aided by the sequenced genomes or transcriptomes of the species of interest. For example, the bee genome project revealed the existence of the full gene complement of methylation machinery (Wang *et al*. 2006), which subsequent studies have shown to be important in caste formation (Kucharski *et al*. 2008). In the phase polyphenic locust that alternates between solitarious and swarming gregarious phases, serotonin mediates the switch between the two (Anstey *et al*. 2009), resulting in a host of gene-expression changes between the two morphs (Kang *et al*. 2004).

This is an exciting time for pea aphid biologists. The genome-sequencing project has opened up a world of resources and we are only just beginning to aim these resources at deciphering the molecular mechanisms that switch the default, winged state to the derived, unwinged state and the genetic pathways that underlie the development and growth of the alternative morphs. The pea aphid system holds a great deal of promise in this respect because in addition to the wing polyphenism that most aphids display, the pea aphid also exhibits a male wing polymorphism. Further, genetic variation for the wing polyphenism co-segregates with the male polymorphism gene in a mapping population. The next step will be to map and clone the *api* locus to determine what it is, when it is expressed, how it acts and what its role is in the female polyphenism. Hence, a traditional developmental genetic examination of the polymorphism is likely to provide an avenue into discovering the molecular basis of the polyphenism.

Finally, one of the gaps in our theory of genetic accommodation is the exact mechanism by which the genotype captures the phenotype (Braendle & Flatt 2006; Moczek 2007). The pea aphid uses both environmental and genetic control of similar trait variation and may therefore serve as a model for studying the genetic links between polymorphisms and polyphenisms as well as polymorphisms and monomorphisms.

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