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Pea Aphid

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1 Introduction

Aphids have long been of interest because of their complex life cycles, environmentally induced morphologies, and importance to agriculture. However, only recently has the resulting wealth of ecological and population genetic data begun to be supplemented by genomic and genetic mapping approaches. In 2004, the International Aphid Genomics Consortium, a collaboration of aphid researchers, chose to establish the pea aphid, *Acyrthosiphon pisum,* as the aphid of choice for the development of genomic resources. Here we introduce the pea aphid and discuss a number of biological questions for which the species is well suited. We then review previous mapping and quantitative trait loci (QTL) studies, ending with a discussion of the genomic tools that are currently available, including the recently initiated genome-sequencing project.

The pea aphid is one of approximately 4,400 species of aphids in the order Hemiptera. Pea aphids belong to the family Aphididae, subfamily Aphidinae, and are named after their host plants, members of the pea family Fabaceae (Leguminosae). The historical range of the pea aphid was palaearctic, but pea aphids are now distributed worldwide, introduced to North America within the last 125 years, presumably by transportation on their host plants (Blackman and Eastop 2000). Like other aphids, they are soft-bodied animals that have a pair of long antennae that stretch much of the length of their body, and a pair of cornicles on the dorsal fifth abdominal segment that excrete alarm pheromones in response to predators. They probe the plant surface with their proboscis and use a piercing stylet to tap phloem and passively ingest sap. Because this diet is carbohydrate-rich and deficient in a number of essential amino acids, aphids rely on obligate bacterial endosymbionts to meet their nutritional requirements. Adult pea aphids reach up to 4.5 mm in length, are pink or green, and can be winged or unwinged.

The pea aphid has a complex life cycle that seasonally alternates between sexual and asexual reproduction (Figure 1). In the spring, a female pea aphid emerges from an egg that has overwintered. This female is asexual and produces or "founds" a population of genetically identical females, which continue to reproduce asexually during the summer months. Asexual reproduction is accomplished via a modified meiosis in which the reduction division is effectively skipped and no recombination occurs (Blackman 1987; Hales et al. 2002). Daughter embryos complete embryogenesis within the mother's ovarioles and mothers give live birth to first instar nymphs, up to 12 per day. After progressing through four nymphal instars (approximately 10 days), the resulting adult females are capable of producing offspring. The short asexual generation time allows aphid clones to undergo many generations during the summer months and, along with their high fecundity, to quickly colonize host plants.

In the fall, asexual females respond to the cues of shortened day length and colder temperatures by asexually producing a generation of sexual females and males (MacKay et al. 1983; Lees 1990). Males, which are XO, are produced genetically by the random loss of one X chromosome due to a failure to attach to spindle fibers on the metaphase plate during the single maturation division (Orlando 1974; Blackman 1987; Wilson et al. 1997). Mating between sexual females, which are oviparous, and males produces an egg containing a female (XX) embryo, presumably because only sperm that carry an X chromosome are viable. The egg is specially adapted to withstand winter con-

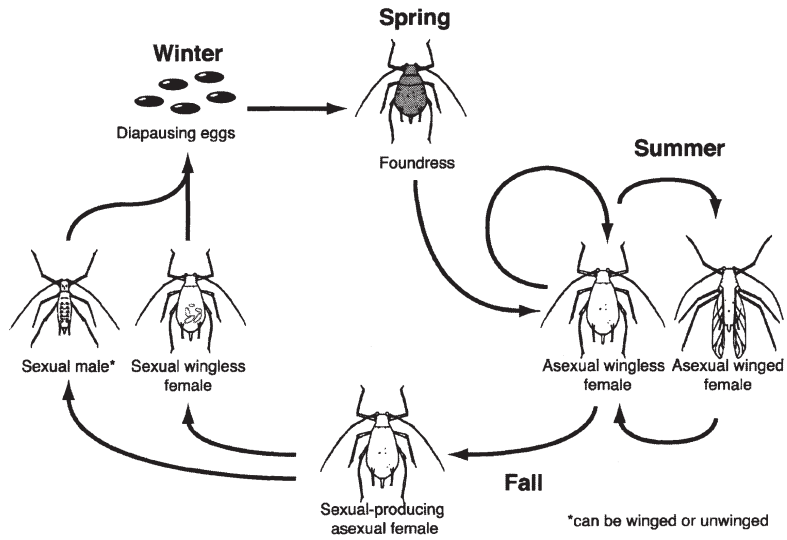

Figure 1. Life cycle of the pea aphid (copyright: A. Shingleton)

ditions and hatches approximately 100 days later, in the spring, allowing a newly emerged asexual female to found a new clone.

Among other phenomena, the pea aphid is a model system for examining polyphenisms, which are environmentally induced, discrete, alternative morphologies. Displayed by a wide variety of insects, polyphenisms are typically adaptive, allowing insects to cope with environments that change in predictable ways. In the case of the pea aphid, the principal polyphenisms are the so-called wing and reproductive polyphenisms.

In the summer months, asexual females typically develop without wings. However, under stressful conditions, such as when the plant becomes overcrowded, they produce offspring that develop wings and can fly to a new host plant. Details of the wing polyphenism and the genomics and genetic approaches that have been taken to better understand it are discussed below. The reproductive polyphenism, already described as part of the life cycle, refers to the alternative production of sexual and asexual females as determined primarily by day length. Although sexual and asexual females possess subtle differences in external morphology, the principal difference between the two is the presence of either large, yolk-filled, haploid oocytes or much smaller, developing diploid embryos within the ovarioles. The developmental decision for the females to become either sexual or asexual is made during embryogenesis based on the day length-induced state of the mother. In the case of either polyphenism, pea aphids offer the advantage that the alternative morphologies can be found among members of the same clone, which possess identical genotypes, greatly simplifying analysis of the phenomena.

1.1 Agricultural Importance

Aphids are best known as agricultural pests. As a group, they are estimated to be responsible for the annual loss of hundreds of millions of dollars worth of crops in the USA alone (Oerke 1994; Morrison and Peairs 1998). Their high fecundity and short generation time result in large populations that can destroy crops. Feeding alone can result in either plant death or cosmetic damage that can make a crop undesirable. The primary damage caused by aphids, however, is due to their ability to vector devastating plant viruses (Nault 1997; Blackman and Eastop 2000; Nault et al. 2004). Aphids are efficient virus vectors due in part because the winged morphs disperse widely. Winged morphs can traverse large areas via active flight or via passive migratory flights in upper air currents, traveling as far as 1,000 km in a single flight (Robert 1987). They are, therefore, capable of traveling from one agricultural field to another, spreading viral diseases as they disperse.

The pea aphid specifically is classified as a mild agricultural pest on alfalfa and clover, vec-

toring more than 30 viral diseases (Blackman and Eastop 2000). As a member of the Macrosiphini, pea aphids are closely related to most major pest aphid species, including the peach-potato aphid (*Myzus persicae*) and the Russian wheat aphid (*Diuraphis noxia*). Hence, investigations into potential means of controlling pea aphid populations are likely to be applicable to these and other aphid pest species.

1.2 Breeding Objectives

Pea aphids are relatively easy to rear in the laboratory. They can be housed in small Petri dishes containing a single leaf of *Medicago arborea* inserted into agar containing fertilizer, or in closed buckets containing whole alfalfa plants. The asexual phase of the life cycle is highly amenable to laboratory culture: lines of interest can be kept as clones indefinitely, without recombination, in incubators replicating summer-like conditions. If a sexual generation is required, for genetic crosses, for example, individuals can be placed in an incubator replicating fall-like conditions (Via 1992). Resulting eggs are subsequently placed in incubators mimicking winter-like conditions.

Here we briefly describe three aspects of pea aphid biology, the wing polyphenism, host plant specialization, and bacterial symbioses, that have been examined from a genetic mapping, quantitative trait loci (QTL), and genomics perspective, respectively. Specific studies representing the latter approaches will be described when we revisit each of these topics later in the chapter. Although we have chosen to focus on these three areas, they are by no means the only aspects of pea aphid biology that are likely to be amenable to future genomics or genetic mapping approaches (for excellent reviews about aspects of pea aphid biology, see Heie 1980; Minks and Harrewijn 1980; Moran 1992; Blackman and Eastop 1994, 2000; Dixon 1998).

Winged and Unwinged Morphs

As described above, asexual females typically develop without wings. However, under stressful conditions such as a decline in host plant quality or an overcrowded plant, they produce offspring that develop wings and can fly to a new host plant (Sutherland 1969). Other stressors that have been documented to induce winged offspring include exposure to predators (Dixon and Agarwala 1999; Podjaseket al. 2005) and parasitoids (Sloggett and Weisser 2002). In the pea aphid at least, such cues, rather than directly influencing developing nymphs, are instead first perceived by the mother, who then somehow transmits a permissive signal to develop with wings to her embryos before they are born (Sutherland 1969).

The sexual males produced in the fall are also found as winged and unwinged forms. Though in contrast to the environmentally cued wing dimorphism in females, wing production in males is determined by an unidentified X-linked genetic polymorphism at the *aphicarus* (*api*) locus (Smith and MacKay 1989; Caillaud et al. 2002; Braendle et al. 2005a). The male wing dimorphism is, therefore, referred to as a polymorphism. Interestingly, genetic variation for the female polyphenism is linked to the *api* locus (Braendle et al. 2005b).

Although referring to the female polyphenism as a "wing dimorphism" is convenient shorthand, many aspects of the phenotype in fact differ between winged and unwinged individuals (Kring 1977). For example, winged morphs have ocelli on the vertex of their head and greatly expanded thoraces with flight musculature, whereas unwinged morphs do not. Also, the cuticle of the winged morph is more heavily sclerotized than that of the unwinged morph and the dimensions of the legs and siphunculi differ. Moreover, winged individuals are active and fly to new host plants, whereas unwinged morphs are sedentary. Winged females also have reduced fecundity relative to unwinged morphs (MacKay and Wellington 1975; MacKay et al. 1983).

The existence of two distinct morphologies is generally thought to be a trade-off between resources dedicated to reproduction in the unwinged morph versus resources dedicated to dispersal in the winged morph (reviewed in Zera and Denno 1997). The pea aphid provides a unique advantage for studies of wing dimorphism, which are common in insects, by exhibiting both environmental (the female polyphenism) and genetic (the male polymorphism) mechanisms of determination. Investigations into the molecular basis of the genetically determined polymorphism may thus reinforce our understanding of the molecular basis of the extreme phenotypic plasticity shown by the polyphenism.

Host Plant Specialization

The pea aphid is also well suited for examining the genetics of adaptation and speciation. Although some clones of pea aphids are generalist feeders, others prefer to feed on particular host plants such as pea, alfalfa, or clover (Via 1991; Sandstrom 1994). This specialization may have preceded their introduction to North America (Birkle and Douglas 1999; Simon et al. 2003; Frantz et al. 2006). Especially well studied are the host races that specialize on either alfalfa (*Medicago saliva*) or red clover (*Trifolium pretense*) in North America (Via 1991; Caillaud and Via 2000). When a pea aphid encounters a host plant, it decides whether it is an acceptable food source by probing it several times with its stylet (Caillaud and Via 2000). Upon acceptance, the aphid will settle on a plant for feeding. Because aphids tend to breed where they spend their time feeding, this creates a situation where gene flow between the two host plant specialists is low (Via 1994, 1999). These pea aphid host plant specialists may, therefore, represent incipient species produced by sympatric speciation (Hawthorne and Via 2001).

Bacterial Symbioses

Pea aphids have been exceptionally well studied with regard to their bacterial endosymbionts. The species of bacteria that aphids require to provide them with essential amino acids, *Buchnera aphidicola,* resides within specialized cells of the aphid called bacteriocytes (Buchner 1965; Braendle et al. 2003) and is transmitted vertically, from mother to daughter. This obligate relationship between aphids and *Buchnera* is an ancient one, dating to 150 to 250 million years ago (Munsonet al. 1991). As with other obligate endosymbionts, the genome of *Buchnera* is highly reduced (approximately 650 kb; Gil et al. 2002), but retains genes required for the synthesis of essential amino acids (Baumann et al. 1999; Shigenobu et al. 2000).

In addition to *Buchnera*, the pea aphid harbors at least five less well characterized facultative secondary symbionts of the *Rickettsia*, *Spiroplasma*, *Regiella*, *Serratia*, and *Hamiltonella* genera (Chen et al. 1996; Fukatsu et al. 2001; Moran et al. 2005). These symbionts affect traits such as resistance to elevated temperature (Montllor et al. 2002), parasitoid resistance (Oliver et al. 2003, 2005), host plant specialization (Tsuchida et al. 2004), and induction of winged forms (Leonardo and Mondor 2006).

1.3 Limitations of Genetic Linkage Mapping

As with many other systems, one of the greatest limitations of genetic mapping in the pea aphid is its long sexual generation time. Although stock populations can be induced by fall-like conditions to produce sexual individuals on a rolling basis, the eggs require about 100 days of winter-like conditions (alternating between 13 hours at 4°C and 11 hours at 0°C) to complete development (Via 1992). Unfortunately, rearing eggs at higher temperatures generally does not successfully speed up development: at 10 °C hatching success is significantly lower, and at 16°C embryos show severe malformations, with no embryos hatching (Shingleton et al. 2003). It is possible, however, to induce eggs to hatch sooner by shifting them to 16 °C following a critical period, though such eggs typically show decreased hatching rates: eggs transferred from 0–4°C to 16°C at day 49 instead of day 98 showed a 20% reduction in hatching rate (Shingleton et al. 2003).

2 Construction of Genetic Maps

The densest pea aphid genetic map to date was developed by Hawthorne and Via (2001) in order to study host plant specialization. They developed a linkage map of 173 dominant amplified fragment length polymorphism (AFLP) markers. These markers group into four linkage groups (Figure 2), agreeing with a previous report of four chromosomes in the pea aphid (Sun and Robinson 1966). Braendle et al. (2005a) developed an additional seven AFLP markers, all on the X chromosome (Figure 3).

A number of studies have identified microsatellites that are variable in the pea aphid (Caillaud et al. 2002,2004; Kurokawa et al. 2004). However, only a subset of these microsatellites have thus far been localized to any particular linkage group (Caillaud et al. 2002). Sabater-Munoz et al. (2006) identified 921 microsatellite repeats based on the expressed sequence tag (EST) collection (discussed below). It is likely that a portion of these will vary and hence be useful markers. Future map construction will undoubtedly be aided by the forthcoming genome sequence.

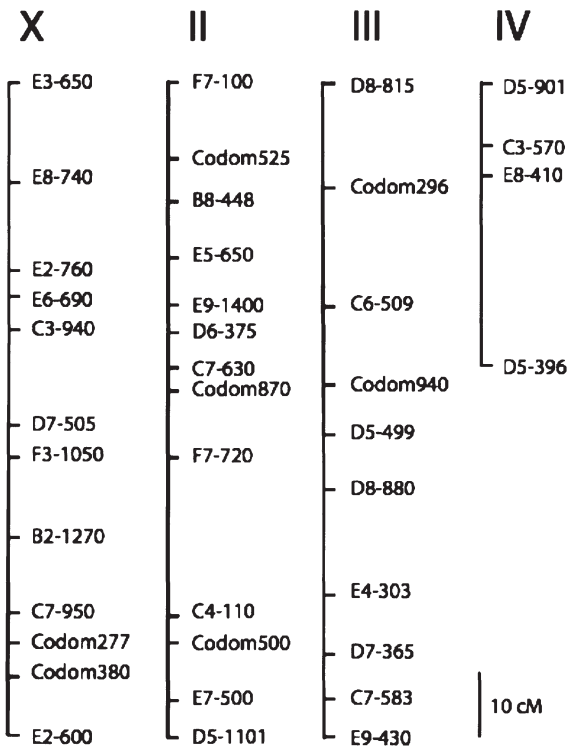

Figure 2. Map of the four linkage groups (X, II, III, IV) of the pea aphid clover specialist, illustrating the location of co-dominant markers (those with a prefix of "Codom") and AFLP markers (all others). An additional set of AFLP markers and their corresponding positions for the alfalfa pea aphid specialist can be found in Hawthorne and Via (2001). Figure modified from Hawthorne and Via (2001)

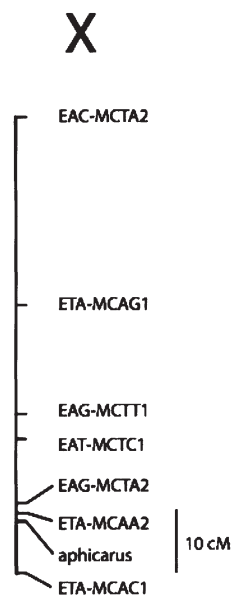

Figure 3. Genetic map of the pea aphid X chromosome near *aphicarus*, illustrating the location of AFLP markers. Figure modified from Braendle et al. (2005a)

3 Gene Mapping by Linkage Analysis

The previously discussed male wing dimorphism is the only trait in the pea aphid that has been mapped by classical linkage analysis. Clones collected from nature produce 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 and MacKay (1989) hypothesized that the winged state of males is determined by a locus on the X chromosome. This hypothesis was supported by later work by Caillaud et al. (2002), who showed that the trait is determined by a single locus and segregates accordingly in the F_2 generation of a mapping panel produced from an initial cross between a clone that only produced winged males, and a clone that only produced unwinged males. They further confirmed that the locus was on the X chromosome by showing that the trait co-segregated with three X-linked microsatellite markers. Braendle et al. (2005a) then detected AFLP markers flanking this locus (Figure 3), and named the locus *aphicarus* (*api*) after Icarus, the tragic figure of Greek mythology whose wax-cemented feather wings melted when he flew too closely to the sun.

4 Detection of Quantitative Trait Loci

The sole quantitative trait loci (QTL) study conducted in pea aphids aimed to identify the loci that underlie host plant specialization and mate choice. Hawthorne and Via (2001) reciprocally crossed an alfalfa specialist to a red clover specialist in order to test the hypothesis that specialization for a particular host plant and mate choice are genetically correlated, which would suggest a mechanism to

facilitate reproductive isolation. They measured fecundity on each of the two host plants (two traits) as a proxy for host plant specialization, and acceptance of each host plant (two traits) as a proxy for mate choice given that pea aphids mate where they feed.

Quantitative trait loci were identified on all four linkage groups that together explained 10-57% of the genetic variance in the four traits. On two of the linkage groups, they found four complexes of QTLs in close enough proximity to suggest that either the same locus might affect more than one of the traits or that there was tight linkage between the QTLs. These QTLs were all in the direction of promoting fecundity and acceptance on the host plant for which each aphid host race was specialized, while decreasing fecundity on the opposite host plant. They concluded that the genetic correlations between these traits could have facilitated divergence between the host plant specialists. Further studies using higher-resolution mapping will be necessary to confirm this result.

5 Advanced Work

5.1 Physical Mapping Efforts

A 6× coverage bacterial artificial chromosome (BAC) library has been constructed, consisting of 27,648 clones with an average insert size of 130 kb (Chris Amemiya, personal communication). As this library has only recently been completed (June 2006), it has not yet been used in any mapping attempts.

5.2 Sequencing Projects: ESTs and Whole-genome Shotgun

To date, over 67,000 expressed sequence tags (ESTs) from the pea aphid are publicly available in dbEST. These ESTs complement smaller numbers of ESTs from other aphid species, including *Myzus persicae* (approximately 14,000), *Aphis gossypii* (approximately 8,400), *Toxoptera citricida* (approximately 4,300), and *Rhopalosiphum padi* (approximately 500). In comparisons of the pea aphid to other Macrosiphini (including *Myzus*) and to Aphidini (including *Aphis*, *Toxoptera*, and *Rhopalosiphum*), nucleotide divergences for sequenced open reading frames of orthologous genes range from 5% to 10% and up to 15%, respectively (Moran et al. 1999; Von Dohlen and Teulon 2003).

A recent study analyzed 40,904 of the pea aphid ESTs derived from cDNA libraries made from antennae, bacteriocytes, digestive tracts, heads, parthenogenetic embryos, and multistage whole-bodies (Sabater-Munoz et al. 2006). These ESTs formed 12,082 contigs and singletons with an overall GC content of 33%. Of the unique transcripts 59% showed no homology with known proteins, although 25% of these transcripts are less than 300 bp and many did not have open reading frames (over 70% for transcripts < 1,000 bp and over 30% for transcripts > 1,000 bp). Of the unique transcripts that showed homology to known proteins, only 34% were present in *Drosophila melanogaster*. Further, 741 *D. melanogaster* genes showed similarity to more than one pea aphid contig, raising the possibility that gene duplications have occurred in the pea aphid.

Sabater-Munoz et al. (2006) also found tissue-specific gene expression in the bacteriocyte- and parthenogenetic embryo-derived libraries relative to the other six libraries. The bacteriocytes, which house the endosymbiotic bacteria, exhibited gene expression associated with amino acid metabolism and defense reactions, while 75% of the unique transcripts from the parthenogenetic embryo library had no known homology with *Drosophila*.

A pea aphid cDNA micro array has been constructed, consisting of quadruplicate spots of approximately 1,750 unique ESTs from pea aphids, and 117 unique genes from the bacterial endosymbiont *Buchnera aphidicola*. The pea aphid/*Buchnera* microarray has been used to study the heat shock response of the two organisms in parallel (Wilson et al. 2006) and to study the transcriptional basis of the winged and unwinged morphs of both females and males (Brisson et al. 2007).

Whole-genome shotgun sequencing of the 525-Mb pea aphid genome (Spencer Johnson personal communication) at 6× coverage was initiated in June 2006 at the Baylor College of Medicine, Human Genome Sequencing Institute, with funds provided by the National Human Genome Research Institute; http://www.hgsc.bcm.tmc.edu/project-species-i-Pea%20Aphid.hgsc?pageLocation=Pea%20Aphid & http://www.aphidbase.com/.

The genome will be assembled and computer-annotated in 2007. The strain used in the sequencing effort is the New York LSR1 line, which has been inbred for one generation and has had its secondary endosymbionts removed via ampicillin treatment. The strain, referred to as LSR1.G1.AC (for LSR1, one generation of inbreeding, antibiotic-cured), was also heat treated at 30 °C for four days prior to DNA extraction in order to decrease the amount of DNA contributed by its primary obligate endosymbiont, *Buchnera aphidicola.*

6 Future Foci

The recent and continuing acquisition of ESTs, a newly constructed BAC library, and a soon to be completed genome sequence will surely be a boon for those interested in the molecular and genetic processes underlying the phenomena exhibited by aphids. For example, the study of aphid insecticide resistance, which has thus far focused primarily on *Myzus persicae,* should benefit by easing the identification and cloning of insecticide targets and detoxifying enzymes that have been implicated in resistance more generally in insects (Ishaaya 2001) and more specifically in *Myzus* (Field et al. 1988; Field and Foster 2002). Our comparative genomics picture of arthropods is also likely to be enriched by the addition of one of the first genomes from a hemimetabolous insect (in addition to the hemipteran *Rhodnius prolixus* which is currently scheduled to be sequenced). Finally, the fact that the genomes of both *Medicago trunculata* — http://www.medicago.org/genome/ , one of the pea aphid's primary host plants — and *Buchnera* — http://buchnera.gsc.riken.go.jp/ , its primary endosymbiont — are also available should grant researchers the ability to explore the interactions of these organisms at a depth not previously possible.

Efforts must now focus on accurately annotating the genome and developing post-genomics technologies, including the development of whole-genome microarrays. Also needed are technologies that will allow us to test gene function, such as RNA interference (RNAi) and transgenesis. To date, there is one report of successful RNAi, in which a gene that is abundantly expressed in the adult salivary gland is knocked down by small interfering RNAs (siRNAs) injected directly into the hemolymph (Mutti et al. 2006). Apparently siRNAs are able to move into salivary gland cells, whereupon they exert their effects. It remains to be seen if this will be possible for other organs and tissues of the adult or the developing embryos found in asexual females. Transgenesis allows one to both misexpress a gene as well as test the regulative ability of its putative enhancers. To date, there are no reports of successful transgenesis in aphids, though successes in several other insect groups with at least two vectors (Wimmer 2003; Pavlopoulos et al. 2004) suggest that it is almost certainly possible. It is our hope that increasing interest in the pea aphid will facilitate such developments in the near future. Interested parties are encouraged to sign on to the aphid genomics list server — http://www.eco.princeton.edu/mailman/listinfo/aphidgenomics — to keep abreast of future developments.

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