Genetic mapping of *aphicarus* – a sex-linked locus controlling a wing polymorphism in the pea aphid (*Acyrthosiphon pisum*)

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We have initiated research to determine the genetic basis of a male wing polymorphism in the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae). Previous studies showed that this polymorphism is controlled by a single biallelic locus, which we name *aphicarus* (*api*), on the X chromosome. Our objectives were to confirm that *api* segregates as a polymorphism of a single gene on the X chromosome, and to obtain molecular markers flanking *api* that can be used as a starting point for high-resolution genetic and physical mapping of the target region, which will ultimately allow the cloning of *api*. We have established an F$_2$ population segregating for *api* and have generated X-linked AFLP markers. The segregation pattern of *api* in the F$_2$ population shows that the male wing polymorphism segregates as a polymorphism of a single gene, or set of closely linked genes on the X chromosome. Using a subset of 78 F$_2$ males, we have constructed a linkage map of the chromosomal region encompassing *api* using seven AFLP markers. The map spans 74.1 cM and we have mapped *api* to an interval of 10 cM. In addition, we confirmed X linkage of our AFLP markers and *api* by using one X-linked marker developed in an earlier study. Our study presents the first mapping of a gene with known function in aphids, and the results indicate that target gene mapping in aphids is feasible.
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Introduction

Many organisms have the capacity to switch between developmental pathways that result in distinctly different phenotypes. The primary factors regulating the expression of such alternative phenotypes may be exclusively genetic (polymorphism) or their action may further depend on environmental signals (often called polyphenism). Alternative phenotypes are usually specialized for different tasks or habitats and exhibit corresponding differences in physiology, morphology and behaviour (Moran, 1992; Roff, 1996). The production of alternative phenotypes enables an organism to conduct alternative life styles by exploiting different resources, which may allow a division of labour among phenotypes specialized for different tasks. The production of alternative phenotypes is thus considered to be adaptive in many instances. Moreover, alternative phenotypes may play significant roles in the evolution of novelty and rates of speciation (West-Eberhard, 1989). However, despite the widely recognized importance of alternative phenotypes in many biological phenomena, the genetic basis underlying such discrete phenotypic variation has rarely been elucidated.

Wing polymorphism and polyphenism of insects represent well-studied examples of alternative phenotypes (Harrison, 1980; Roff, 1986; Zera and Denno, 1997). Insects across a wide range of taxa have evolved the capacity to develop flight-capable (fully winged) phenotypes or flight-incapable (wingless or short-winged) phenotypes. The winged phenotype is generally responsible for long-range dispersal and exhibits a lower fecundity than the wingless, sedentary phenotype. This trade-off between flight capability and fecundity appears to be the driving force maintaining intraspecific variation in wing morphology (Zera and Denno, 1997). In some species, the alternative adopted during development may depend on the alleles present at a single locus. More commonly, the production of alternative phenotypes appears to be under polygenic control and to be condition-dependent, that is, specific environmental conditions induce the developing animal to adopt one of the alternatives (Roff and Fairbairn, 1991). (Note that both wing polymorphism and wing polyphenism are often referred to in the literature as wing polymorphism.)

From a physiological perspective, wing polyphenism/polymorphism is now one of the best-understood life history trade-off models (Zera and Harshman, 2001). In contrast, we have limited information on the genetic mechanisms controlling the production of such alternative phenotypes. Most importantly, we do not know which genes play a direct role in causing divergence of alternative developmental pathways. In general, it is difficult to identify such critical genes in a polyphenic

switch because most currently used assays, such as differential gene expression analysis, generate candidate genes that are likely to result from phenotype-specific expression after developmental divergence. Nevertheless, recent studies using this approach have provided the first genetic insights into polyphenic development (eg Evans and Wheeler, 1999; Miura et al, 1999). Similar problems arise when adopting a candidate gene approach: as alternative phenotypes generally differ in a suite of morphological characters and because the time point of developmental divergence is often known only at the phenotypic level, the selection of potential candidate genes to be analysed is difficult. Hence, it is more feasible to identify genes critical for the regulation of alternative phenotypes where the underlying genetic determinants can be mapped using classic genetic analysis. To our knowledge, however, no studies have so far attempted to map any genes involved in the regulation of insect wing polymorphisms.

The pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphididae), like most aphid species, exhibits a life cycle involving several parthenogenetic generations alternating with a single sexual generation (cyclical parthenogenesis). During the parthenogenetic mode of reproduction, females display a wing polyphenism. Environmental cues, such as crowding or low host plant quality, trigger the production of winged females that disperse and colonize new host plants (eg Lees, 1966). In the sexual generation, females are always wingless, while males are either winged or wingless. This phenotypic difference among pea aphid males is under exclusively genetic control, thus representing a wing polymorphism (Smith and MacKay, 1989; Caillaud et al, 2002).

Initially, Smith and MacKay (1989) reported the occurrence of three types of pea aphid clones collected in nature: clones producing only winged males, clones producing only wingless males and clones producing an equal proportion of winged and wingless males. As aphid sex determination is of the XX/X0 (female/male) type (Blackman, 1987), Smith and MacKay (1989) proposed that the male wing polymorphism segregates as an X-linked factor, most likely as a single locus with two alternative alleles. Males produced by females homozygous at the locus would therefore produce either only winged or only wingless males. In contrast, heterozygous females would produce an equal proportion of winged and wingless males. Caillaud et al (2002) tested the genetic model proposed by Smith and MacKay (1989) by analysing the segregation pattern of the male wing polymorphism in F$_1$ and F$_2$ generations derived from parental clones producing either winged or wingless males only (ie clones presumptively homozygous for the alternative alleles at the locus). The segregation pattern of the polymorphism was consistent with X linkage and a biallelic single locus control. The X linkage of the locus was further demonstrated by the linkage of X-linked microsatellite markers with the phenotypic polymorphism in heterozygous F$_1$ clones.

The male wing polymorphism in the pea aphid provides a relatively simple, suitable system to dissect the molecular genetic basis underlying the expression of alternative phenotypes. We have named this gene *aphicarus*, abbreviated *api* (after the tragic figure from Greek mythology, Icarus, whose wax and feather wings melted when he flew too close to the sun). In this study, we generated amplified fragment length polymorphism (AFLP) markers for the pea aphid X chromosome using an F$_2$ mapping population segregating for the male wing polymorphism. Our major goals of this mapping project were to confirm X linkage of *api*, to determine whether *api* segregates as a polymorphism of a single gene, and to obtain molecular markers flanking *api* that can be used as a starting point for high-resolution genetic and physical mapping of the target region, which will ultimately allow the cloning of *api*.

Materials and methods

Phenotype of winged and wingless males
In the pea aphid, the winged male is fully winged and has functional flight muscles. In comparison to the wingless male, the winged phenotype exhibits heavier sclerotization of head and thorax, better-developed compound eye ocelli, longer antennae with more rhinaria, and the siphunculi and cauda may sometimes be longer (eg Kring, 1977; Kawada, 1987; Miyazaki, 1987). Furthermore, alternative phenotypes may differ in body length and body weight, with the winged phenotype usually being smaller. The morphological differences between winged and wingless phenotypes correlate with differences in life history traits. For example, the winged male shows an increased postembryonic developmental time due to a prolonged fourth larval instar.

Terminology
We use the abbreviations *api^w* and *api^{wl}* to refer to the alleles of *aphicarus* causing winged and wingless males, respectively. Note that it is not possible to determine whether there is a dominance relationship between these alleles, since males are haploid for the X chromosome (and therefore *aphicarus*) and this phenotype is expressed only in males. In a previous study, the *api* locus was referred to as 'WgMa' locus (Caillaud et al, 2002).

Genotyping at the *api* locus
The *api* genotype for all aphid clones was determined by inducing the production of males in parthenogenetic clones (Caillaud et al, 2002). We induced the production of sexual morphs by transferring 3–4 third-instar nymphs from long-day conditions (L:D 16:8, 20°C) to short-day conditions (L:D 13:11, 17°C). Sexual morphs started appearing approximately 8 weeks after transfer. Clones that produced only wingless males were typed as *api^{wl}/api^{wl}*, clones that produced only winged males were typed as *api^w/api^w* and clones that produced both male morphs were typed as *api^w/api^{wl}*. Clones producing at least five males all of a single morph were considered to be homozygous for the corresponding *api* allele.

F$_2$ mapping population
Two parental clones, PBR8 and LSR1, were used to generate the F$_2$ mapping population. PBR8 is homozygous for *api^w* and LSR1 is homozygous for *api^{wl}* (Caillaud et al, 2002). The parental clones originated from the same population and were collected in the summer of 1998 in alfalfa fields (*Medicago sativa*) in Tompkins county, NY, USA. Parental, F$_1$ and F$_2$ clones

were maintained in the laboratory on *M. sativa* or *Medicago arborea* at 20°C, L:D 16:8 and cultured as previously described (Caillaud *et al*, 2002; Miura *et al*, 2003).

Crosses were performed as described by Via (1992) and Caillaud *et al* (2002). Briefly, virgin females isolated as nymphs from the sexual stock cultures were mated to males in Petri dishes. All fertilized eggs produced throughout the life of the females were harvested, surface sterilized and placed in an incubator under daily cycles of 4°C during a 10 h day and 0°C during a 14 h night. After 100 days of this cold treatment, eggs were removed from the incubator and the hatchling progeny were reared in Petri dishes containing alfalfa foliage. The hatching rates for eggs

resulting from the different crosses (see below) were relatively high for aphids (cf Via, 1992), ranging from 82 to 87%.

Figure 1 depicts the crosses performed. F_1 hybrids were obtained from reciprocal crosses between sexuals of parental clones PBR8 and LSR1: female PBR8 × male LSR1 ($F_{1\text{-}P \times L}$ hybrids) and female LSR1 × male PBR8 ($F_{1\text{-}L \times P}$ hybrids). To produce the F_2 generation, we randomly selected one $F_{1\text{-}P \times L}$ and one $F_{1\text{-}L \times P}$ hybrid and reciprocally crossed them (Figure 1):

F_2 cross 1: $F_{1\text{-}P \times L}$ api^{w}/api^{wl} female × $F_{1\text{-}L \times P}$ api^{w} male
F_2 cross 2: $F_{1\text{-}P \times L}$ api^{w}/api^{wl} female × $F_{1\text{-}L \times P}$ api^{wl} male
F_2 cross 3: $F_{1\text{-}L \times P}$ api^{w}/api^{wl} female × $F_{1\text{-}P \times L}$ api^{w} male
F_2 cross 4: $F_{1\text{-}L \times P}$ api^{w}/api^{wl} female × $F_{1\text{-}P \times L}$ api^{wl} male
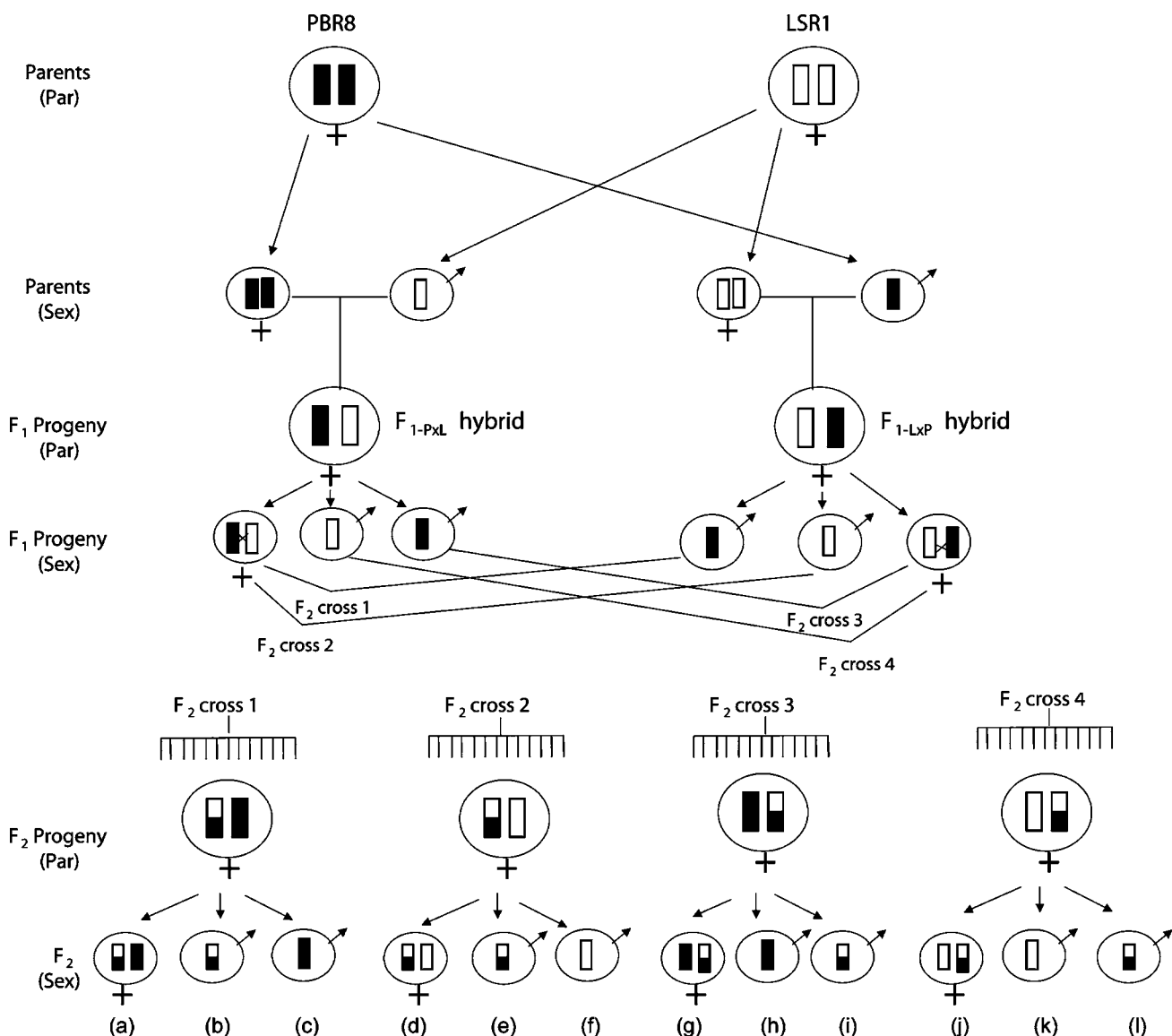

Figure 1 Schematic illustration of F_2 population segregating for *api* and selection of informative F_2 males for linkage analysis. The black and white bars represent the respective parental X chromosomes with different *api* alleles. Parental females of PBR8 are homozygous for api^{w} (black chromosome). Parental females of LSR1 are homozygous for api^{wl} (white chromosome). The parthenogenetic (par) females (large circles) are diploid for the X chromosome. The sexual (sex) generation (small circles) consists of sexual females, which are diploid for the X chromosome, and males, which are haploid for the X chromosome. The sexual F_2 generation consists of diploid females (a, d, g, j) and haploid males (b, c, e, f, h, i, k, l). F_2 males c, f, h and k inherited the nonrecombinant X chromosome from their F_1 father. In contrast, F_2 males b, e, i and l inherited an X chromosome containing informative recombination events.

A total of approximately 500 F$_2$ hybrids were generated, and we determined the *api* genotype of a random sample of 170 F$_2$ hybrids. However, of these clones, only a subset of F$_2$ males was used for the following reasons. First, we screened F$_2$ males rather than F$_2$ females because males are haploid for the X chromosomes. For X-linked markers, we were thus able to avoid the problem of dominance in scoring AFLPs. Second, we only used F$_2$ males that inherited the X chromosome from their F$_1$ mother. As paternally inherited X chromosomes are nonrecombinant due to male haploidy of the X chromosome, only F$_2$ males with a potentially recombinant X chromosome from their F$_1$ mother are informative for meiotic mapping. In Figure 1, F$_2$ males c, f, h and k inherited the nonrecombinant X chromosome of their F$_1$ father, thus males of this type were not used for mapping. In contrast, F$_2$ males b, e, i and l all harbour a maternally inherited X chromosome potentially containing informative recombination events. Consequently, in F$_2$ clones homozygous at *api*, the parental origin of the X chromosome in males could not be determined and these males were excluded from further analysis ($N = 89$). Of the remaining informative F$_2$ hybrids (ie clones heterozygous at *api*, $N = 81$), 78 F$_2$ male descendants could be used for marker analysis: wingless males (*api^{wl}*) from two crosses (F$_2$ cross 1: $N = 5$; F$_2$ cross 3: $N = 48$) and winged males (*api^w*) from two crosses (F$_2$ cross 2: $N = 19$; F$_2$ cross 4: $N = 6$). Note that differences in the number of F$_2$ progeny between crosses arose from differential production of sexual forms that were used to generate the F$_2$ population (the differences therefore do not reflect differences in viability).

DNA extraction

Aphids used for AFLP analysis were stored in acetone (Fukatsu, 1999) at -20°C prior to DNA extraction. From each F$_2$ clone, DNA was extracted from 1–5 adult males using the Qiagen DNeasy Tissue kit (QIAGEN, Chatsworth, CA, USA). DNA was eluted in 20–100 μl TE buffer (20 μl per individual). The concentration of the eluted DNA was estimated by running a fraction of the sample on a 2% agarose gel.

AFLP assays

AFLP assays were performed as described previously (Vos *et al*, 1995), using the AFLP Analysis System II (AFLP Small Genome Primer Kit) (GIBCO BRL). AFLP fragments were generated according to the manufacturer's protocol with minor modifications as outlined below. For each reaction, an equal amount of DNA, that is, 3–9 μl, of the eluted DNA was digested using *Eco*RI (6-bp recognition site) and *Mse*I (4-bp recognition site) restriction endonucleases in a reaction volume of 12.5 μl. Following ligation of adaptors, 2.5 μl of this template was amplified in a 25 μl volume using a primer pair based on the sequences of the *Eco*RI and *Mse*I adapters with one selective nucleotide at the 3′ end. The PCR products of the preselective amplification were diluted 25-fold, and 2.5 μl of this stock was used for selective amplifications. The primers used for selective amplifications had two and three additional selective nucleotides (*Eco*RI + 2 primers and *Mse*I + 3 primers). Prior to selective amplification, *Eco*RI primers were end-labelled with ^{33}P. PCR reactions were carried out in a reaction volume of 10 μl

over 13 cycles with a denaturation step for 30 s at 94°C, an annealing step for 30 s at 65°C with a temperature decrease of 0.7°C per cycle, and an extension step of 1 min at 72°C, followed by 30 cycles with a denaturation step for 30 s at 94°C, an annealing step for 30 s at 56°C, and an extension step for 1 min at 72°C. All PCR amplifications were performed using a Techne Genius thermocycler.

Gel analysis

Prior to gel electrophoresis, reaction products of the selective amplification were mixed with an equal volume (10 μl) of formamide dye (98% formamide, 10 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) and denatured at 90°C for 3 min. After preheating an 8% LongRanger polyacrylamide gel (BioWhittaker Molecular Applications) containing 7 M urea and 1 × TBE for 30–60 min, 3 μl of each sample was loaded onto the gel and run for 2 h at 70–80 W in 1 × TBE buffer (at a temperature of 40–50°C). After electrophoresis, gels were dried on a gel drier (BioRad) and exposed to Biomax MS Kodak Film for 2–6 days.

AFLP reactions for each primer pair combination were run on a single gel and included the male F$_2$ progeny ($N = 78$), parental females ($N = 2$) and F$_1$ females ($N = 2$). We visually scored AFLP fragments as present or absent and excluded ambiguous or missing samples. To ensure accurate scoring, we scored all polymorphic bands twice, and presumptive X-linked polymorphisms were scored a third time. Markers were named according to the primer pair combinations used in the selective amplification step: 'EXX-MYYYN', where 'E' refers to the *Eco*RI primer with two selective nucleotides 'XX', 'M' refers to the *Mse*I primer with the selective nucleotides 'YYY' and N is the marker number for that primer pair combination.

Linkage analysis and mapping of the api locus

We used the following protocol to select presumptive X-linked markers. Males are haploid for the X chromosome, and the segregation pattern of X-linked markers in an F$_2$ intercross is identical to the segregation pattern in a backcross. Thus, X-linked AFLP markers in male F$_2$ progeny are expected to segregate in a 1:1 ratio and autosomal AFLP markers that are inherited in a dominant fashion in a 3:1 ratio. Note here that we have no reason to suspect segregation distortion for these markers because we did not find evidence of segregation distortion at the *api* locus (since induced parthenogenetic females always produce winged and wingless males in a 1:1 ratio) (Caillaud *et al*, 2002; this study). We performed χ^2-tests for all AFLP polymorphisms to determine whether the segregation ratios of band presence/absence in the F$_2$ populations were significantly different from the expected 1:1 and 3:1 ratios. Significance levels were corrected for multiple comparisons (α/N) to give an experimentwise $\alpha = 0.05$.

We performed maximum-likelihood linkage analysis using MAPMAKER/Exp Version 3.0b (Lander *et al*, 1987) to calculate marker order and position of the target locus on the X chromosome. Map distances in centimorgans (cM) were derived from recombination frequencies using the Kosambi mapping function (Kosambi, 1944). The marker data were analysed according to Mapmaker's F$_2$ backcross (A-H) scheme, which correctly estimates the

recombination fractions and LOD scores for loci in haploid individuals. Marker alleles of each male were scored as 'A' (inherited from parent LSR1) or 'B' (inherited from parent PBR8), and missing or ambiguous samples were scored as 'M' (missing). We confirmed linkage of selected AFLP markers to a single linkage group at a LOD score of ≥ 4.0 and a maximum recombination fraction of 0.33 (40 cM) using the 'group' command. Map order of AFLP markers and position of the target locus were determined using the 'compare' and 'try' commands. Marker orders were verified using the 'ripple' command. MAPMAKER's 'error detection' command was used to identify candidate genotyping errors (Lincoln and Lander, 1992).The resulting linkage map was plotted using the software program Mapchart 2.1 (Voorrips, 2002).

We also determined the number of presumptive autosomal linkage groups using Mapmaker's F_2 inter-cross (A-B-C-D) scheme. For markers segregating 3:1 (banded: unbanded) among the male F_2 progeny, band absence was coded as A for homozygous for the parental allele a and band presence as C for nonhomozygous for parental allele a, when the band was absent in parent A (LSR1). Band absence and presence in parent B were coded correspondingly as B and D for parent B (PBR8).

Analysis of previously identified X-linked markers

Hawthorne and Via (2001) constructed an AFLP linkage map of the pea aphid and cloned two markers (SX380 = Codom380, Pag-Egc277 = Codom277) on the putative X chromosome (D Hawthorne, personal communication). We screened the two markers for size polymorphisms between the parental strains. SX380 showed no length polymorphisms. Marker Pag-Egc277 was polymorphic in one parent (LSR1) and mono-morphic in the other (PBR8). Only one of the F_1 clones ($F_{1-P \times L}$) was polymorphic for Pag-Egc277 and we scored the male F_2 progeny that inherited their X chromosome from this F_1 clone ($N = 24$) for their genotype at this locus and used the resulting data for linkage analysis. Marker amplifications were carried out in 15 µl reaction mixtures consisting of 0.2 U of Taq polymerase, $1 \times$ reaction buffer with MgCl₂, 200 µM dNTPs, 0.2 mM of each primer (5'-CAATATGCCTGTGAGGTTGG-3', 5'-GTGAATTTTA CAAGTTTTTGCTAG-3') and 1 µl of DNA (5 ng/µl). PCR reactions were amplified using a regime of initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min and elongation at 72°C for 45 s. This was followed by a final extension step at 72°C for 4 min.

Results

The segregation of api in the F_2 generation was consistent with the previously proposed X linkage of api (Smith and MacKay, 1989; Caillaud et al, 2002). F_2 clones produced either both types of males, only winged males or only wingless males, and the ratio of different F_2 clones derived from each cross was 1:1 (Table 1). Heterozygous F_2 clones ($N = 78$) produced winged and wingless males in equal proportions (Appendix A1, Table A1).

We screened AFLP fragments amplified by 38 different primer pair combinations (EcoRI + 2/MseI + 3). On aver-age, an AFLP reaction resulted in 44.2 ± 1.8 (mean $\pm$ SE) bands (range: $N = 26$–74). The size of amplified frag-

Table 1 Pattern of male morph production in F_2 clones ($N = 170$) generated by the four F_2 crosses[a]

F_2 cross	Clones producing winged males (api^w/api^w)	Clones producing wingless males (api^{wl}/api^{wl})	Clones producing both male morphs (api^w/api^{wl})
1	3	0	5
2	0	17	20
3	54	0	50
4	0	15	6

[a]Each cross between the F_1 parents resulted in equal proportions of F_2 clones with different api genotypes (homozygous vs homo-zygous) (χ^2-test, all $P > 0.05$).

ments ranged from approximately 70 to 1500 bp, with most bands ranging from 100 to 500 bp. Of a total of 1681 amplified fragments, 38 (2.3%) were polymorphic between parental females, monomorphic (banded) in the F_1 females and segregating among the F_2 males. Segregation ratio analyses resulted in the identification of nine putative X-linked markers (1:1) and 27 putative autosomal markers (3:1). Two markers showed signifi-cant deviation from the expected ratios and were excluded from analysis.

The nine X-linked AFLP markers grouped into a single linkage group at a LOD threshold of 4.0. A subset of seven AFLP markers could be ordered unambiguously (LOD ≥ 2.0) using the 'compare' command. The target locus api was placed on this map at a LOD (linkage) score of 21.33 and its map position was supported by a LOD score of 2.70 ('try' command). The final map consists of seven AFLP markers plus the target locus and spans a chromosomal region of 74.1 cM (Figure 2). We verified the map order of the seven AFLP markers plus the target locus using the 'compare' and 'ripple' commands. All marker positions were supported by a LOD ≥ 2.0. The remaining two AFLP markers and marker Pag-Egc277 did not have unique map positions (LOD < 2.0) (see Figure 2 for details).

Presumptive autosomal markers segregating in a 3:1 ratio in the male F_2 population grouped into three (parent PBR8, $N = 14$) and two linkage groups (parent B, $N = 13$). For parent PBR8, the three linkage groups comprised nine, three and two markers (one marker was unlinked). For parent LSR1, the two linkage groups comprised four and three markers (six markers were unlinked).

Discussion

The segregation pattern of api in the F_2 population supports the previously proposed model that this trait is controlled by a single X-linked locus (aphicarus) with two alternative alleles causing the winged and wingless male phenotype (Smith and MacKay, 1989; Caillaud et al, 2002). Using a subset of 78 F_2 males, we generated nine X-linked AFLP markers, seven of which were used to determine the map position of api. We identified two AFLP markers confining api to a map interval of 10 cM. One marker maps 1.5 cM from api and another marker maps 8.4 cM on the other side of the locus. The fact that we did not find any AFLP markers that segregated perfectly with api suggests that the gene is not positioned
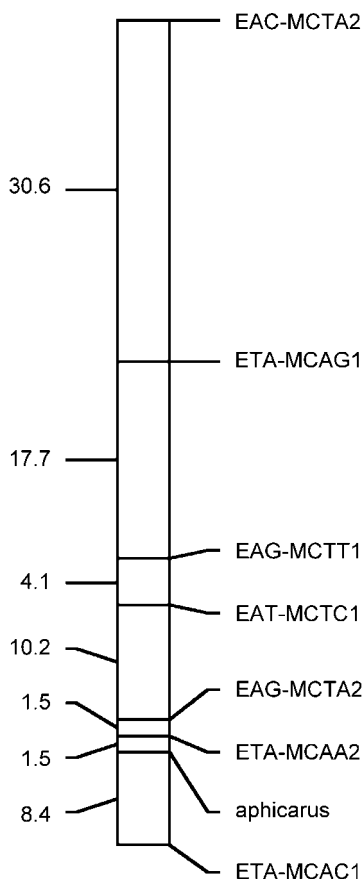
Figure 2 Genetic map of the X chromosome region containing *aphicarus*. The linkage map consists of seven AFLP markers plus the target locus, covering 74.1 cM with a mean intermarker distance of 10.6±3.67 cM. The map interval containing *api* spans 9.9 cM, delimited by markers ETA-MCAC1 (8.4 cM) and ETA-MCAA2 (1.5 cM). For two X-linked AFLP markers, no unique map position could be determined: ETC-MCAC1 with a LOD (linkage) score of 17.91 falls into marker interval EAG-MCTT1–ETA-MCAG1 (LOD = 0.00) or EAG-MCTT1–EAT-MCTC1 (LOD = 0.15) (the next best position of the marker is less likely at a LOD score of 1.86). Marker EAT-MCTC with a LOD (linkage) score of 6.19 falls into marker interval EAG-MCTA2–EAT-MCTC1 (LOD = 0.00) or EAG-MCTT1–ETA-MCAG1 (LOD = 0.27) (the next best position of the marker is less likely at a LOD score of 1.44). The previously identified X-linked marker Pag-Egc277 (Hawthorne and Via, 2001) with a LOD (linkage) score of 7.22 falls in the marker interval ETA-MCAA2–EAG-MCTA2 (LOD = 0.00) or EAG-MCTA2–EAT-MCTC1 (LOD = 0.00), with the next best map position being less likely at a LOD score of 2.79.

within a large inversion and that we should be able to localize it further with standard meiotic mapping.

In our AFLP assay, we found that approximately 2% of amplified fragments were polymorphic between parents and suitable for marker analysis, which corresponds to about one marker per primer pair combination. This marker recovery rate is low compared to AFLP studies on other insects using divergent parental lines (eg Hawthorne, 2001; Parsons and Shaw, 2002). However, in our study, the parental clones were derived from the same population (and the same species of host plant). In addition, genetic differentiation (inferred from mtDNA and allozyme analysis) between pea aphid clones may be low generally, even when clones are specialized for

different host plants (Simon *et al*, 1982; Dalmasso and Bournoville, 1983; Boulding, 1998; Birkle and Douglas, 1999). Despite little genetic divergence between the parental clones used in our experiment, we were able to gain sufficient information to place the target locus within an interval of 10 cM. Our mapping experiment was facilitated by the fact that aphid males are haploid for the X chromosome, resulting in complete haplotype information for X-linked AFLP markers. In addition, using *api* as a marker, we were able to select an F_2 mapping population of informative males and avoided screening males with paternally derived, nonrecombinant X chromosomes.

The pea aphid genome has a haploid set of four chromosomes (Sun and Robinson, 1966). In addition to the linkage group corresponding to the X chromosome, we found that presumptive autosomal markers grouped into three and two linkage groups for the parents PBR8 and LSR1. The recovery of only two autosomal linkage groups for parent LSR1 may have resulted from the small number of markers available.

Hawthorne and Via (2001) generated a whole-genome AFLP linkage map of *A. pisum* using a female F_2 population. The two parental maps corresponding to the X chromosomes were approximately 30 cM (five markers) and 100 cM (12 markers). In this study, we used seven AFLP markers (plus the target locus) to construct a map that covered a distance of 74 cM. Therefore, it seems likely that these differences in map length stem from differences in the numbers of markers used to generate these maps. Due to the small number of markers, this region is unlikely to span the entire X chromosome. The map length of 100 cM by Hawthorne and Via (2001) may be the most reliable estimate of the genetic length of the pea aphid X chromosome as this estimate is based on the largest number of markers.

We were unable to unambiguously map the X-linked marker Pag-Egc277 developed in the study of Hawthorne and Via (2001), but this marker grouped with our X chromosome linkage group at a high LOD score. Pag-Egc277 appears to map somewhere between markers ETA-MCAA2 and EAT-MCTC1, suggesting that it is located within 1.5–13.2 cM of *api* (Figure 2). Although we cannot unambiguously orient our map with the map of Hawthorne and Via (2001) based on this single marker, the relatively telomeric location of Pag-Egc277 (approximately 10 cM from the end of their map and 9.9–21.6 from the end of our map) suggests that our terminal marker ETA-MCAC1 is located on the same chromosome end as their terminal marker E2-600 and that *api* is located within approximately 10 cM of the end of both maps.

We currently estimate the pea aphid X chromosome to cover approximately 30% of the genome, based on several chromosome photographs (Mandrioli *et al*, 1999; Bizzaro *et al*, 2000, RL Blackman, unpublished data). The pea aphid genome is relatively small and estimated to be 300 Mb (Finston *et al*, 1995). The X chromosome thus contains approximately 90 Mb and the estimated relationship between physical and genetic distance is approximately 900 kb/cM. However, such recombination rate estimates may vary greatly within and between chromosomes and reliable estimates of recombination rates per physical distance are impossible to obtain in the absence of physical maps (eg Beye *et al*, 1999).

Currently, we have no information hinting at the function of *api*. Further characterization of *api* will therefore provide basic information on the molecular control mechanisms governing the expression of this wing polymorphism. With the identification of *api*, we may also gain insights into the molecular mechanisms underlying an environmentally sensitive expression of alternative phenotypes. The wing polyphenism in parthenogenetic pea aphids generates winged and wingless females that closely resemble the different phenotypes observed in the male wing polymorphism. Therefore, polymorphic and polyphenic expressions of these alternative phenotypes share some of their underlying developmental processes; yet, it is unclear whether and how *api* is involved in the divergence of environmentally induced alternatives. Initial results indicate that allelic variation at *api* may correlate with genetic differences in the propensity to produce winged females, indicating genetic linkage of factors controlling the wing polyphenism and wing polymorphism (Braendle *et al*, in press). Characterizing *api* and testing its functional relevance during the development of alternative phenotypes in the wing polyphenism will further address these questions. This, in turn, may shed light on the molecular genetic basis of adaptive phenotypic plasticity and the evolutionary transitions between polyphenism and polymorphism.

Acknowledgements

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Appendix A1

The pattern of male morph production in the F$_2$ hybrid clones used for mapping is shown in Table A1.

Table A1 Pattern of male morph production in the 78 F$_2$ hybrid clones used for mapping[a]

F$_2$ cross	No.	Winged males	Wingless males	F$_2$ cross	No.	Winged males	Wingless males
1	4	3	6	3	73	13	9
1	21	1	1	3	76	7	6
1	23	13	9	3	79	19	15
1	32	5	9	3	82	22	15
1	36	12	6	3	84	13	16
2	4	16	19	3	93	7	4
2	16	4	5	3	94	1	4
2	20	4	9	3	97	12	22
2	26	14	13	3	98	17	23
2	31	30	26	3	104	10	14
2	35	4	7	3	106	38	47
2	61	7	8	3	107	11	11
2	77	28	28	3	109	3	3
2	78	5	22	3	113	13	20
2	93	5	11	3	117	12	15
2	95	11	11	3	125	9	12
2	102	8	12	3	132	58	48
2	104	11	15	3	136	21	13
2	106	15	13	3	137	12	25
2	120	12	12	3	139	5	10
2	122	4	4	3	143	4	12
2	128	24	12	3	157	9	11
2	155	10	10	3	159	20	24
2	160	10	12	3	168	24	13
3	8	27	37	3	176	16	15
3	22	6	4	3	189	29	20
3	24	3	1	3	191	29	27
3	25	26	34	3	192	2	6
3	26	4	5	3	193	5	5
3	32	9	6	3	196	8	19
3	35	5	5	3	204	12	10
3	38	3	2	3	210	11	10
3	49	13	18	4	71	1	1
3	56	18	14	4	79	8	7
3	60	6	4	4	90	19	19
3	61	8	16	4	128	22	15
3	63	10	14	4	140	4	2
3	67	2	5	4	174	27	34
3	70	18	19				

[a]A heterogeneity χ^2 test (Zar, 1999) shows that, overall, the proportions of winged and wingless males are not different ($P > 0.05$).