

A sex-linked locus controls wing polymorphism in males of the pea aphid, *Acyrtosiphon pisum* (Harris)

MC Caillaud^{1,4}, M Boutin², C Braendle³ and J-C Simon²

¹Department of Entomology, Cornell University, Ithaca NY 14853, USA; ²UMR INRA/ENSAR "Biologie des organismes et des populations appliquee a la protection des plantes", B.P. 29, 35653 Le Rheu Cedex, France; ³Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA

Discrete variation in wing morphology is a very common phenomenon in insects and has been used extensively in the past 50 years as a model to study the ecology and evolution of dispersal. Wing morph determination can be purely genetic, purely environmental, or some combination of the two. The precise genetic determinants of genetically based wing morph variation are unknown. Here we explore the genetic basis of wing polymorphism in the pea aphid, which can produce either winged or wingless males. We confirm that three types of pea aphid clones coexist in natural populations, those producing winged males only, those producing wingless males only, and those producing a mixture of both. A Mendelian genetic analysis revealed that male wing poly-

morphism in pea aphids is determined by a single locus, two alleles system. Using microsatellite loci of known location, we show that this locus is on the X chromosome. The existence of a simple genetic determinism for wing polymorphism in a system in which genetic investigation is possible may help investigations on the physiological and molecular mechanisms of genetically-based wing morph variation. This locus could also be used in the search for genes involved in the wing polyphenism described in parthenogenetic females and to investigate the interplay between polymorphisms and polyphenisms.

Heredity (2002) **88**, 000–000. doi:10.1038/sj.hdy.6800146

Keywords: wing polymorphism; wing polyphenism; allelic switch; Mendelian genetics; microsatellites

Introduction

In a wide variety of taxa, two or more discrete morphs coexist with intermediate morphs rare or absent. Distinct phenotypes can result from expression of alternative alleles at specific loci. The resulting phenotypic variation is referred to as 'polymorphism'. Early reported examples include the single-locus polymorphism for color pattern in the snow goose *Lamprolaima caerulea* (Pough, 1951) and the ladybird *Harmonia axyridis* (Tan, 1946). Similar genetic polymorphisms have been described for features of anatomy, life-cycle and behavior. Alternatively, production of different morphologies can be facultative and depend primarily on environmental conditions experienced by each individual as it develops. The resulting phenotypic variation is referred to as 'polyphenism'. The caste differentiation of social insects, in which alternative castes may differ drastically in morphology even though they are genetically identical, is one of the most dramatic examples of polyphenic development (Wilson, 1971; Nijhout and Wheeler, 1982).

Discrete variation in wing morphology is a very common phenomenon in insects and has been used extensively in the past 50 years as a model to study the ecology and evolution of dispersal and life history traits

(Harrison, 1980; Roff, 1986; Zera and Denno, 1997). One of the alternative morphs is winged, displays migratory tendency and has low fecundity, whereas the other morph is short winged or wingless, sedentary and highly fecund. Wing morph determination can be purely genetic, purely environmental, or some combination of the two (Roff and Fairbairn, 1991; Zera and Denno, 1997). In species that have a genetically controlled wing length, two types of genetic determination are known: single locus systems, typically with the short winged (or wingless) condition dominant, and polygenic systems (Roff, 1986; Roff and Fairbairn, 1991; Fairbairn, 1994). In a 1991 survey, single locus systems were listed for 31% of the cases of wing dimorphism reported (Roff and Fairbairn, 1991). In species that have a strictly environmentally controlled wing length, the environmental signal appears to be mediated by the endocrine system, as with other polyphenisms. It is well known, for instance, that exogenous juvenile hormone (JH) can induce the wingless or short-winged morph in wing-polyphenic grasshoppers, crickets, and several species of Hemiptera (McCaffery and Page, 1978; Hardie and Lees, 1985; Dingle and Winchell, 1997; Zera and Denno, 1997). JH could also be the mediator in genetically controlled wing length polymorphisms. According to the threshold model, the segregating alleles could determine the titre of JH at some critical stages for wing morph determination (Roff, 1986; Roff and Fairbairn, 1991). If the JH titre is above a critical threshold, short winged or wingless individuals are formed. Interestingly, the pea aphid represents a rare case in which wing presence or absence is determined either

Correspondence: MC Caillaud

⁴Present address: Department of Biology, 161 CNS, Ithaca College, Ithaca, NY 14850-7278, USA. E-mail: Cmc27@cornell.edu

Received 3 January 2002; accepted 7 May 2002

genetically or environmentally, depending on the morph of the individual (male or parthenogenetic female, respectively).

Pea aphids (*Acyrtosiphon pisum*) are non-host-alternating cyclical parthenogens with a single sexual generation in the fall and many successive parthenogenetic generations from early spring to late fall. Sexual morphs (sexual females and males) are born from parthenogenetic females in the fall by a combination of cold temperatures and decreased photoperiods. Sex determination is of the XX/X0 (female/male) type. Males are generated by an unusual oocyte division or 'mini-meiosis' in which only one X chromosome undergoes reduction while the other homologue is lost after failing to attach to the spindle on metaphase plate (Orlando, 1974). Recent work using molecular markers linked to sex chromosomes has demonstrated, in the aphid species *Sitobion* near *fragariae*, that the loss of one or the other X is equally probable (Wilson and Sunnucks, 1997). In the spring, diapausing eggs hatch into fundatrices representing the first parthenogenetic generation. Since no recombination occurs during parthenogenesis (Blackman, 1987), each parthenogenetic genotype essentially represents a clone. Sexual females are mostly wingless (with notable exceptions, Miyazaki, 1987) but winged and wingless individuals can be found among parthenogenetic females, and among males. The production of winged morphs in parthenogenetic females is triggered by environmental stimuli such as high density, short day length, low temperature and /or poor food quality (Hardie and Lees, 1985). The wing polyphenism of parthenogenetic females is thought to be controlled by the JH, although the evidence is equivocal (Hardie, 1980). In contrast, wing morph variation in males seems to have a clear genetic component as shown for the pea aphid by Smith and MacKay (1989). Clones of this aphid species collected in the field appeared to fall into three distinct categories. Some clones produced only wingless males, others produced only winged males, and a third category of clones produced both winged and wingless males in a 1:1 ratio.

The coexistence of alternative (ie, environmental vs genetic) control mechanisms within a single species makes the pea aphid a unique study organism for examining the interplay between polyphenisms and polymorphisms. Although they are determined by different cues, polyphenisms and polymorphisms may involve similar genetic and developmental architectures. Both could result from genetically based 'switches' which regulate the expression of morph-specific alternative sets of genes (Hazel *et al*, 1990; West-Eberhard, 1992; Roff, 1994a, b). Determination of which set of genes will be expressed (and therefore of which phenotype will be expressed) could be governed by alleles at other loci within the genome (in the case of a genetically determined variation), or by external cues correlated with a particular agent of selection (in the case of an environmentally-determined variation). Pea aphids thus may provide the opportunity to uncover these developmental switches. An important first step in this endeavour is the thorough characterization of the genetic basis of wing polymorphism in pea aphids. In this paper, we first replicate the experiments of Smith and MacKay (1989) with clones of the pea aphid collected in a different geographical area. Then, we explore the genetic basis of wing polymorphism in males of the pea aphid by conducting crosses between winged

and wingless male-producing clones. Lastly, we use microsatellite markers to determine whether the locus that controls alary polymorphism is X-linked.

Materials and methods

We designed three experiments to analyze the genetic basis of wing polymorphism in males of pea aphids. In a first experiment, we evaluated the ratio of winged vs wingless males in 16 field-collected clones. These genotypes were collected in the summer of 1998, in alfalfa fields located in the vicinity of Ithaca (NY, Tompkins county, USA), in an area of approximately 40 square miles, and on the same plant species (Alfalfa, *Medicago sativa*). To induce the production of sexuals, and for each of the 16 clones, five 3rd instar parthenogenetic nymphs were taken from a stock culture (20°C, 16h:8h, L:D) and placed on alfalfa in a growth chamber at 18°C and a photoperiod of 13.5:10.5 (L:D). The photoperiod was then decreased every 3 days by 15 min until it reached a photoperiod of 12.5:11.5 (L:D). The temperature was then lowered to 16°C. Six to 7 weeks after the beginning of the induction, we started collecting males which can be easily recognized by the presence of two black claspers close to the tip of the abdomen (Miyazaki, 1987). For each clone, two replicates of this experiment were performed.

In a second experiment, we performed reciprocal crosses between a clone producing only winged males (PBR8) and a clone producing only wingless males (LSR1). Sexual morphs for clones LSR1 and PBR8 were induced as described above. Six weeks after the beginning of the induction, we isolated sexual females and males from these stock cultures. In order to obtain virgin sexual females of known age, sexual females were isolated as nymphs from the stock culture and newly emerged adults were collected every day. Sexual females can be easily recognized by their thick hind tibia. Crosses were performed as described in Via (1992). Three replicates of two males and three females for each direction of the cross were established. 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 about 100 days of this cold treatment, eggs were removed from the incubator and the hatchling progeny were reared in Petri-dishes containing alfalfa foliage. Fifteen F₁ hybrid clones were obtained. Three months after these hybrid clones were initiated, thus after about six parthenogenetic generations, we chose two of them and crossed them to generate an F₂ generation: winged males from one F₁ hybrid clone Fem(PBR8)*Male(LSR1) were mated to sexual females from another F₁ clone Fem(LSR1)*Male(PBR8). All resulting F₂ clones were maintained under long-day conditions for 3 months, which corresponds to approximately five to eight parthenogenetic generations. We analyzed the proportion of winged versus wingless males in eight F₁ hybrid clones and 97 F₂ hybrid clones. For each clone, we established two replicates containing three to four third-instar-nymphs, which were exposed to short-day conditions to induce the production of the sexual morphs as described above. Over the course of the following 2 months, we screened all F₁ and F₂ clones for the occurrence of winged and wingless males. Once a week, all adult males were counted, removed and their phenotype noted. For each

clone that produced both winged and wingless males, numbers of the two morphs were tested for departure from a 1:1 ratio using a heterogeneity chi-Square test (Zar, 1999).

In a third experiment, we genotyped eight field-collected clones at 10 microsatellite loci, including three that were X-linked. Microsatellites Sm10 and Sm11 were cloned from *Sitobion miscanthi* (Sunnucks *et al*, 1997) and were shown to exhibit cross-amplification for *S. avenae* by Simon *et al* (1999). Microsatellites S10, S16b, S17b, S23, S25, S30, S35 were also isolated from *S. miscanthi* (Wilson and Sunnucks submitted) while Sa5L was cloned from *Sitobion avenae* (JC Simon *et al*, unpublished). DNA from individual aphids was extracted using the 'salting-out' protocol described by Sunnucks and Hales (1996). The DNA was then resuspended in 20 to 40 μ l of TE buffer (10 mM Tris pH 7.5, 0.1 mM EDTA) depending on the aphid size. The amount of DNA obtained was roughly quantified by gel electrophoresis. Dilutions were then performed in order to get a DNA concentration of about 5 to 7 ng/ μ l. Microsatellite amplifications were carried out in 15 μ l reaction mixtures consisting of 0.2 unit of Taq polymerase (Promega), 1 \times MgCl₂-free reaction buffer, 2 mM MgCl₂, 200 μ M dNTP, 0.2 mM of each primer and 1 μ l of the diluted aphid DNA. Amplifications were made in a PTC-100 programmable thermal controller (MJ Research) 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 for 1 min with temperature depending on locus and elongation at 72°C for 45 seconds. This was followed by an extend 72°C for 4 min. For loci S10, Sm10, Sm11, Sa5L, annealing took place at 56°C, for S23 at 58°C, S24, S25, S30, and S35 at 62°C and S17b at 68°C. A 1.8% agarose gel electrophoresis was used to check the quality of the amplification and the concentration of the PCR products. These were then diluted by half with a 4 \times -loading buffer before electrophoresis. For each sample, about 2.5 μ l (according to concentration) of the diluted PCR product was run on a 6% polyacrylamide gel (with urea present) for 2h30 to 3 h at 1000 V and 75 W in 0.5 \times TBE buffer. The gel was silver stained as described in Budowle *et al* (1991). The size of the alleles of each locus was estimated using a sequencing ladder (sequence of pGEM[®]-3Zf(+) vector PROMEGA). An X-OMATRA photograph of the gel was taken as a permanent document.

Results

Experiment 1

Three classes of clones were present among the 16 clones surveyed for male wing polymorphism (Table 1): seven clones produced only wingless males, two clones produced only winged males and seven clones produced winged and wingless males. In clones producing both male morphs, the number of wingless and winged males produced did not differ significantly from 1:1 in any of the clones (chi-square test, $P > 0.05$).

Experiment 2

F₁ hybrids between a clone producing only wingless males and a clone producing only winged males produced wingless and winged males in a 1:1 ratio (chi-Square test, $P > 0.05$, Table 2). The direction of the cross

Table 1 Pattern of male morph production for 16 clones (two replicates per clone). A chi-square test shows that, within a replicate, the proportions of wingless and winged males are not different ($P > 0.05$)

Clones	replicate	No. of wingless males	No. of winged males	Chi-square test for 1:1 ratio
PBG7	1	40	–	
	2	36	–	
LSR3	1	78	70	NS
	2	82	95	NS
FVG3	1	24	27	NS
	2	25	28	NS
LSG1	1	32	26	NS
	2	61	38	NS
PBR8	1	–	37	
	2	–	44	
LSG2	1	104	–	
	2	101	–	
PBG3	1	19	32	NS
	2	26	31	NS
PBR2	1	–	21	
	2	–	20	
LSR1	1	64	–	
	2	67	–	
LSG3	1	30	–	
	2	35	–	
PBG4	1	42	–	
	2	68	–	
FVR1	1	45	–	
	2	29	–	
PBG6	1	36	–	
	2	51	–	
PBR7	1	41	32	NS
	2	55	51	NS
LSR2	1	71	59	NS
	2	54	61	NS
PBR1	1	29	21	NS
	2	16	29	NS

Table 2 Pattern of male morph production for eight F₁ hybrid clones between a clone producing only wingless males (LSR1) and a clone producing only winged males (PBR8). A chi-square test shows that, within a replicate, the proportions of wingless and winged males are not different ($P > 0.05$)

Cross	F1 clone no.	No. of wingless males	No. of winged males	Chi-square test for 1:1 ratio
Fem(PBR8)* male(LSR1)	1	72	54	NS
	2	101	69	NS
	3	52	65	NS
	4	75	70	NS
Fem(LSR1)* male (PBR8)	1	164	159	NS
	2	141	126	NS
	3	75	86	NS
	4	71	76	NS

Table 3 Pattern of male morph production for 47 F₂ hybrid clones generated by mating a winged male from an F₁ hybrid clone to a female of another F₁ hybrid clone. A heterogeneity chi-square test shows that, overall, the proportions of wingless and winged males are not different ($P < 0.05$)

F2 Clone no.	No. of wingless males	No. of winged males	F2 Clone no.	No. of wingless males	No. of winged males
8	37	27	106	47	38
22	4	6	107	11	11
24	1	3	109	3	3
25	34	26	113	20	13
26	5	4	117	15	12
32	6	9	125	12	9
35	5	5	132	48	58
38	2	3	136	13	21
49	18	13	137	25	12
56	14	18	139	10	5
60	4	6	143	12	4
61	16	8	157	11	9
63	14	10	159	24	20
67	5	2	168	13	24
70	19	18	176	15	16
73	9	13	189	20	29
76	6	7	191	27	29
79	15	19	192	6	2
82	15	22	193	5	5
84	16	13	196	19	8
93	4	7	204	10	12
94	4	1	210	10	11
97	22	12			
98	23	17			
104	14	10			

did not influence this ratio. Furthermore, 50 clones out of 97 F₂ hybrid clones (~50%) produced only winged males while 47 clones (~50%) produced both winged and wingless males. In those 47 heterozygous clones, the number of wingless and winged males did not differ significantly from 1:1 (Heterogeneity chi-Square test, $P = 0.41$, Table 3).

Experiment 3

Every aphid clone has a unique multilocus genotype (Table 4). We determined whether the 10 microsatellite loci were autosomal or X-linked by comparing the genotype of sexual females and males for each locus. Let us consider a microsatellite locus with two alleles, a and b. If this locus is located on the X-chromosome, then clones

that have heterozygous sexual females (X^aX^b) should also have males that display only one of the two alleles (they are either X^a0 or X^b0). Table 5 shows that three of the 10 microsatellites considered were X-linked. For instance, sexual females of clone LSR2 are heterozygous for locus S17b (they have alleles 216 and 218) while the males have either allele 216 or allele 218. Last, we examined the genotype of winged and wingless males for X-linked microsatellite loci S17b, S10 and Sm11 (Table 6). In clones that produce both wingless and winged males, we found that each morph inherited one of the two allele types carried by the females. Let us examine the case of LSR3 for instance. Sexual females (XX') show two alleles for marker S10 while males ($X0$ or $X'0$), as expected, show only one allele type. However, the distribution of allele

Table 5 Genotype of eight clones (sexual females and males) at three microsatellite loci (S17b, S10, Sm11). The presence of two allelic categories within the males of heterozygous clones shows that the locus is X-linked

clones	Locus S17b	Locus S10	Locus Sm11
LSR2			
Sexual females	216:218	105:107	142:144
males	216 or 218	105 or 107	142 or 144
LSR3			
Sexual females	216:218	113:118	142:142
males	216 or 218	113 or 118	142
LSG2			
Sexual females	218:218	118:118	142:149
males	218	118	142 or 149
LSG3			
Sexual females	218:218	105:105	142:142
Males	218	105	142
LSG1			
Sexual females	216:218	105:118	142:142
males	216 or 218	105 or 118	142
FVG3			
Sexual females	218:218	105:118	142:149
males	218	105 or 118	142 or 149
PBG7			
Sexual females	216:218	113:118	142:142
males	216 or 218	113 or 118	142
PBR8			
Sexual females	218:218	105:118	142:142
males	218	105 or 118	142

Table 4 Genotype of eight clones (parthenogenetic females) at 10 microsatellite loci. Some clones share the same genotype at a given microsatellite locus but each clone has a unique multilocus genotype

clones	Microsatellite loci									
	S17b	S23	S30	S5.L	Sm10	S25	S35	S24	S10	Sm11
LSR2	216:218	135:137	172:174	205:205	150:153	135:135	109:109	167:171	105:107	142:144
LSR3	216:218	133:137	172:174	205:205	150:153	135:139	109:109	167:171	113:118	142:142
LSG2	218:218	133:137	172:174	205:205	150:150	135:135	109:109	167:171	118:118	142:149
LSG3	218:218	133:137	172:174	205:205	150:153	135:135	109:109	167:171	105:105	142:142
LSG1	216:218	133:133	172:172	205:205	150:150	135:135	109:109	167:171	105:118	142:142
FVG3	218:218	133:137	172:174	205:205	150:150	135:139	109:109	167:171	105:118	142:149
PBG7	216:218	133:137	163:172	205:205	150:150	135:135	109:109	170:174	113:118	142:142
PBR8	218:218	132:133	172:174	205:207	150:153	135:135	109:111	167:171	105:118	142:142

Table 6 Genotype of sexual females, winged males and wingless males at an X-linked microsatellite locus. The male phenotype (winged or wingless) and the marker genotype cosegregate

Clones	Male phenotype	X-linked locus	Sexual females genotype	Winged male genotype	Wingless male genotype
LSR2	Both morphs	Sm11	142:144	144 (<i>n</i> = 5)	142 (<i>n</i> = 5)
LSR3	Both morphs	S10	113:118	118 (<i>n</i> = 13)	113 (<i>n</i> = 14)
	Both morphs	S17b	216:218	216 (<i>n</i> = 15)	218 (<i>n</i> = 15)
LSG1	Both morphs	S10	105:118	105 (<i>n</i> = 5)	118 (<i>n</i> = 4)
FVG3	Both morphs	Sm11	142:149	149 (<i>n</i> = 6)	142 (<i>n</i> = 5)
LSG2	100% wingless	Sm11	142:149	none	142 (<i>n</i> = 3) OR 149 (<i>n</i> = 7)
PBG7	100% wingless	S10	113:118	none	113 (<i>n</i> = 20) OR 118 (<i>n</i> = 8)
	100% wingless	S17b	216:218	none	216 (<i>n</i> = 6) OR 218 (<i>n</i> = 7)
PBG4	100% wingless	S10	105:107	none	105 (<i>n</i> = 5) OR 107 (<i>n</i> = 5)
PBR8	100% winged	S10	105:118	105 (<i>n</i> = 4) OR 118 (<i>n</i> = 6)	None

types among male morphs is not random. All winged males bear one type of allele (they are all X⁰) while all wingless males bear the alternate allele (they are all X'⁰). In other words, variation at the S10 marker co-segregates with phenotypic variation for the presence of wings in males of pea aphids.

Discussion

The results of this study are most simply explained if male wing morph is determined by alternative alleles at a locus on the X-chromosome. If Wg and Wl represent alleles for wingedness and winglessness respectively, then sexuparae (parthenogenetic females precursors of males and sexual females) can be X^{Wg}X^{Wg} and produce 100% of X^{Wg}0 males (winged), X^{Wl}X^{Wl} and produce 100% of X^{Wl}0 males (wingless), or X^{Wg}X^{Wl} and produce 50% of X^{Wg}0 (winged) and 50% of X^{Wl}0 (wingless) males. If a clone that is X^{Wg}X^{Wg} (such as PBR8) mates with a clone that is X^{Wl}X^{Wl} (such as LSR1), then all F₁ hybrid clones are X^{Wg}X^{Wl} and produce an equal proportion of winged and wingless males (Table 2). If a winged male from a F₁ hybrid clone X^{Wg}X^{Wl}, thus carrying only X^{Wg} since males are X⁰, mates with a female of another F₁ hybrid clone X^{Wg}X^{Wl}, then 50% of the progeny will be X^{Wg}X^{Wl} while the rest will be X^{Wg}X^{Wg}. This genetic interpretation was first proposed by Smith and MacKay (1989) but it was not supported by genetic data. Our results show that wing dimorphism in male pea aphids is determined by a single sex-linked locus, hereafter called 'WgMa'. This locus appears to be sex limited in expression since sexual females do not show phenotypic variation for wing morphology, whatever their genotype at the locus WgMa is.

The existence of a simple genetic determinism for wing polymorphism in a system in which genetic investigation is possible may help investigations on the physiological mechanisms of genetically-based wing morph variation. Current knowledge of the precise determinants of polymorphism in general is very rudimentary (Nijhout, 1994, 1999). Wing polymorphism has been used for over a decade as a model for elucidating its physiological determinants (Zera and Denno, 1997; Roff *et al*, 1997). The most widely discussed hypothesis focuses on JH as the key regulator of wing morph variation. The juvenile hormone-wing hypothesis posits that an elevated JH titre during critical stages of development inhibits the full growth and differentiation of wings and/or flight muscles resulting in short-winged or wingless morphs

(Southwood, 1961; Wigglesworth, 1961). Despite considerable research effort, this hypothesis has been supported so far mostly by correlational data rather than functional data (Fairbairn and Roff, 1999; Zera, 1999). Knowledge of the causal relationship between endocrine physiology and the production of alternative wing morphs could be greatly enhanced using molecular tools because it would help identifying candidate genes responsible for the expression of alternative phenotypes. The demonstration that male alary polymorphism in *A. pisum* depends on a single locus-2 alleles system located on the X chromosome is a first step toward the molecular characterisation of a locus controlling wing morph variation.

Further analysis of the genetic basis of wing polymorphism in male pea aphids may also help elucidate the proximate control of the wing polyphenism described in parthenogenetic females. To date, most of the work on these proximate mechanisms has focused on JH, but the evidence for the involvement of this hormone is equivocal. Some studies do not support the hypothesis that JH is involved in aphid wing polyphenisms (reviewed in Applebaum and Heifetz, 1999). Other studies reported that application of anti-JH agents induced the production of winged individuals (Mackauer *et al*, 1979; Delisle and Cloutier, 1980). In one case, application of JH caused the winged precursors of sexual females to develop into wingless individuals but a similar experiment carried out on regular parthenogenetic females showed no effect of JH application (Hardie, 1980). Location and characterization of the gene controlling male wing polymorphism in pea aphids would provide a candidate gene to test JH function during polyphenic development. Suppression of this gene could cause asexual females to produce only one of the phenotypes regardless of the presence or absence of relevant environmental stimuli. Furthermore, variation at the locus controlling the male wing polymorphism could account for variation in the propensity to produce winged offspring of parthenogenetic females. Pea aphid clones show considerable variation in the production of winged offspring when exposed to the same environmental stimulus, suggesting that there is heritable variation in the sensitivity to the environmental cue governing morph production (Lamb and MacKay, 1979, 1983; Weisser and Braendle, 2001). This could be tested by analyzing genetic variation in the production of winged offspring and molecular variation in the vicinity

of (and eventually at) the WgMa locus, for a set of field-collected clones.

If the locus controlling male wing polymorphism in the pea aphid is also involved in the wing polyphenism during the asexual generations, this may help understanding the interplay between polymorphisms and polyphenisms. Several lines of evidence suggest that they involve similar genetic and developmental architectures. First, phenotypes developed under environmental or genetic control are sometimes strikingly similar. In the case of variation in wing morphology in aphids for instance, genetically-induced winged individuals and environmentally-induced individuals are similar in many morphological features including the structure of the sensory system (eg, Kring, 1977). Second, in many instances a similar alternative phenotype is expressed as an environmentally controlled polyphenism in some species and as a genetically controlled polymorphism in another (closely related) species (Nijhout, 1999). Third, a similar phenotype can be determined by either the environment or variation at a single locus. In the buckeye butterfly, *Precis coenia*, the autumn morph is most of the time induced by low temperature and short days, but there is also a gene (*rosa*) whose recessive allele produces the autumn phenotype when homozygous (Rountree and Nijhout, 1995). It appears likely that there is a physiological/functional link between polyphenisms and polymorphisms, but it remains unclear whether a genetically controlled polymorphism could evolve from an environmentally controlled polyphenism (or *vice versa*). As discussed by Nijhout (1999), once a polyphenism has been established, it is possible for one or the other morph to become fixed in a population. This is seen as advantageous if the population enters a habitat that is not favorable for induction of the polyphenic switch (West-Eberhard, 1989). A polymorphism-to-polyphenism transition could also be achieved. If the genetic effect was originally mediated by the endocrine system, there are many ways in which hormone secretion, metabolism, thresholds, or sensitive periods, could be made responsive to environmental variation (Nijhout, 1999). At present, insufficient genetic, endocrine, or developmental information is available to examine the evolutionary transition between polymorphism and polyphenism. The coexistence of both a polyphenism and a polymorphism creating the same alternative phenotypes in a single organism, and a single genotype, gives the pea aphid the potential to become a key model in the study of this transition.

Acknowledgements

This work was supported by a Hatch grant (Cornell University) to MCC. CB was supported by the Boehringer Ingelheim Fonds, the Roche Research Foundation, and the Janggen-Poehn-Stiftung. Bryan Danforth (Cornell University, USA) and Manuel Plantegenest (Ecole Nationale Supérieure Agronomique de Rennes, France) read an early version of this manuscript.

References

- Applebaum SW, Heifetz Y (1999). Density-dependent physiological phase in insects. *Annu Rev Entomol* **44**: 317–344.
Blackman RL (1987). Reproduction, cytogenetics and development. In: Minks AK, Harrewijn P (eds) *Aphids, Their Biology,*

- Natural Enemies and Control*, vol. 2A, Elsevier: Amsterdam. pp 163–196.
Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC (1991). Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. *Am J Hum Genet* **48**: 137–144.
Delisle J, Cloutier CF (1980). A study of morph determination in the potato aphid using precocene, a compound with anti-juvenile hormone activity. Abstracts of the XVI International Congress of Entomology, Kyoto, Japan.
Dingle H, Winchell R (1997). Juvenile hormone as a mediator of plasticity in insect life histories. *Arch Insect Biochem Physiol* **35**: 359–373.
Fairbairn DJ (1994). Wing dimorphism and the migratory syndrome: correlated traits for migratory tendency in wing dimorphic insects. *Res Popul Ecol* **36**: 157–163.
Fairbairn DJ, Roff DA (1999). The endocrine genetics of wing polymorphism in *Gryllus*: a response to Zera. *Evolution* **53**: 977–979.
Hardie J (1980). Juvenile hormone mimics the photoperiodic apterization of the alate gynopara of aphid, *Aphis fabae*. *Nature* **286**: 602–604.
Hardie J, Lees AD (1985). Endocrine control of polymorphism and polyphenism. In: Kerkutand GA, Gilbert LI (eds) *Comprehensive Insect Physiology, Biochemistry and Pharmacology*: vol. 8, Pergamon Press: New York. pp 441–449.
Harrison RG (1980). Dispersal polymorphism in insects. *Ann Rev Ecol Syst* **11**: 95–118.
Hazel WN, Mock RS, Johnson MD (1990). A polygenic model for the evolution and maintenance of conditional strategies. *Proc R Soc Lond B Biol Sci* **242**: 181–187.
Kring JB (1977). Structure of the eyes of the pea aphid, *Acyrtosiphon pisum*. *Ann Ent Soc Am* **70**: 855–860.
Lamb RJ, Mackay PA (1979). Variability in migratory tendency within and among natural populations of the pea aphid, *Acyrtosiphon pisum*. *Oecologia* **39**: 289–299.
Lamb RJ, Mackay PA (1983). Micro-evolution of the migratory tendency, photoperiodic response and developmental threshold of the pea aphid, *Acyrtosiphon pisum*. In: Brown VK, Hodek I (eds) *Diapause and Life Cycle Strategies in Insects*. Dr W Junk Publishers: The Hague. pp 209–217.
Mackauer M, Nair KK, Unnithan GC (1979). Effects of precocene II on alate production in the pea aphid, *Acyrtosiphon pisum*. *Can J of Zool* **57**: 856–859.
McCaffery AR, Page WW (1978). Factors influencing the production of long-winged *Zonocerus variegatus*. *J Insect Physiol* **24**: 465–472.
Miyazaki M (1987). Morphs and morphs of aphids. In: Minks AK, Harrewijn P (eds) *Aphids, Their Biology, Natural Enemies and Control*, vol. 2A, Elsevier: Amsterdam. pp 27–50.
Nijhout HF (1994) *Insect Hormones*. Princeton University Press: Princeton.
Nijhout HF (1999). Control mechanisms of polyphenic development in insects. *Bioscience* **49**: 181–192.
Nijhout HF, Wheeler DE (1982). Juvenile hormone and the physiological basis of insect polymorphisms. *Q Rev Biol* **57**: 109–133.
Orlando E (1974). Sex determination in *Megoura viciae*: Homoptera: Aphididae. *Monit Zool Ital* **8**: 61–70.
Roff DA (1986). The evolution of wing dimorphisms in insects. *Evolution* **40**: 1009–1020.
Roff DA (1994a). The evolution of dimorphic traits: predicting the genetic correlation between environments. *Genetics* **136**: 395–401.
Roff DA (1994b). The evolution of dimorphic traits: effects of directional selection on heritability. *Heredity* **71**: 36–41.
Roff DA, Fairbairn DJ (1991). Wing dimorphisms and the evolution of migratory polymorphisms among the insects. *Am Zool* **31**: 243–251.
Roff DA, Stirlings G, Fairbairn DJ (1997). The evolution of threshold traits: a quantitative genetic analysis of the physiological

- and life-history correlates of wing dimorphism in the sand cricket. *Evolution* **51**: 1910–1919.
- Rountree DB, Nijhout HF (1995). Genetic control of a seasonal morph in *Precis coenia* (Lepidoptera: Nymphalidae). *J Insect Physiol* **41**: 1141–1145
- Simon JC, Baumann S, Sunnucks P, Hebert PDN, Pierre JS, LeGallic JF, Dedryver CA (1999). Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. *Mol Ecol* **8**: 531–545
- Smith MAH, Mackay PA (1989). Genetic variation in male alary polymorphism of pea aphid, *Acyrtosiphon pisum*. *Entomol Exp Appl* **51**: 125–132.
- Southwood TRE (1961). A hormonal theory of the mechanisms of wing polymorphism in Heteroptera. *Proc Roy Entomol Soc Lond (A)* **36**: 63–66.
- Sunnucks P, Hales D (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Mol Biol Evol* **13**: 510–524.
- Sunnucks P, De-Barro PJ, Lushai G, MacLean N, Hales D (1997). Genetic structure of an aphid studied using microsatellites: cyclic parthenogenesis, differentiated clones and host specialization. *Mol Ecol* **6**: 1059–1073.
- Tan CC (1946). Mosaic dominance in the inheritance of color patterns in the lady-bird beetle, *Harmonia axyridis*. *Genetics* **31**: 195–210.
- Via S (1992). Inducing the sexual morphs and hatching the eggs of pea aphids. *Entomol Exp Appl* **65**: 119–127.
- Weisser WW, Braendle C (2001). Body colour and genetic variation in winged morph production in the pea aphid. *Ent Exp Appl* **99**: 217–223.
- West-Eberhard MJ (1989). Phenotypic plasticity and the origins of diversity. *Ann Rev Syst Ecol* **20**: 249–278.
- West-Eberhard MJ (1992). Behavior and evolution. In: Grant PR, Grant HS (eds) *Growing Points in Evolutionary Biology. Molds, Molecules and Metazoa*, Princeton University Press: Princeton. pp 57–75.
- Wigglesworth VB (1961). Insect polymorphism—a tentative synthesis. *Symp Roy Entomol Soc Lond* **1**: 103–113.
- Wilson EO (1971). *The Insect Societies*. Belknap Press: Cambridge.
- Wilson ACC, Sunnucks P, Hales DH (1997). Random loss of X chromosome at male determination in an aphid, *Sitobion near fragariae*: detected using and X-linked polymorphic microsatellite marker. *Genet Res* **233–236**.
- Zar JH (1999). *Biostatistical Analysis*. Prentice-Hall. Englewood Cliffs, NJ.
- Zera AJ (1999). The endocrine genetics of wing polymorphism in *Gryllus*: critique of recent studies and state of the art. *Evolution* **53**: 973–976.
- Zera AJ, Denno RF (1997). Physiology and ecology of dispersal polymorphism in insects. *Annu Rev Entomol* **42**: 207–231