Temporal habitat variability and the maintenance of sex in host populations of the pea aphid

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The evolutionary maintenance of sex, despite competition from asexual reproduction, has long intrigued the evolutionary biologists owing to its numerous apparent short-term costs. In aphids, winter climate is expected to determine the maintenance of sexual lineages in the high latitude zones owing to their exclusive ability to produce frost-resistant eggs. However, diverse reproductive modes may coexist at a local scale where climatic influence is counteracted by microgeographical factors. In this study, we tested the influence of local habitat characteristics on regional coexistence of reproductive modes in the pea aphid, *Acyrtosiphon pisum*. In the laboratory, the induction of sexual morph production of many pea aphid genotypes from the local fields of annual (pea and faba bean) and perennial (alfalfa and red clover) crops in Western France indicated that *A. pisum* lineages from annual crops had a significantly higher investment in sexual reproduction than *A. pisum* lineages from the perennial hosts. We propose that temporal habitat variability exerts a selective pressure to maintain the sexual reproduction in *A. pisum*. The ecological and evolutionary consequences of the association between the mode of reproduction and the host population on gene flow restriction and on ecological specialization are discussed.

Keywords: host races; temporal heterogeneity; sex maintenance; geographical parthenogenesis; sympatric speciation; ecological specialization

1. INTRODUCTION

The extreme apparent short-term costs of sex have long made the evolutionary persistence of sexual reproduction in the face of competition from asexual modes very puzzling to evolutionary biologists (Maynard-Smith 1978). The costs of sexual reproduction typically include the production of males ('twofold cost of sex'; Maynard-Smith 1971), the breakdown of favourable gene combinations by recombination and the time and resource allocations to mating activity (Lewis 1987). Despite these costs, the prevalence of sexual reproduction is a long-standing and unresolved problem and many theories based on either mutational (Kondrashov 1988; Peck 1994) or environmental hypotheses (Van Valen 1973; Hamilton 1980; Doncaster *et al.* 2000) have been proposed for the 'paradox of sex'. However, most of these theories still await validation.

Species in which both sexual and asexual modes of reproduction coexist may hold the key to resolving the paradox of sex. In this respect, aphids have proved to be ideal biological systems and have stimulated extensive theoretical and empirical work on the benefits of sex (Rispe *et al.* 1998; Simon *et al.* 2002; Wilson *et al.* 2003). The typical aphid life cycle consists of cyclical parthenogenesis, where several generations of parthenogenetic females in spring and summer are followed by a single sexual generation, which occurs in autumn and involves males and sexual females. This life cycle is achieved by 'sexual' lineages that switch from parthenogenesis to sex annually. Alternatively, 'asexual' lineages reproduce by continuous obligate parthenogenesis, although many of

them retain the possibility to produce some males in autumn (Simon *et al.* 2002). The distribution of these reproductive modes is the opposite of the pattern generally observed for geographical parthenogenesis: sexual lineages occupy higher latitudes, while asexual lineages predominate at lower latitudes (Moran 1992; Rispe *et al.* 1998; Dedryver *et al.* 2001). This pattern results from sexual lineages being favoured in regions with regular harsh winters because they produce cold-resistant eggs, while asexual lineages do not resist frost but take advantage of their faster multiplication rates in mild-winter areas (Rispe & Pierre 1998; Rispe *et al.* 1998; Simon *et al.* 2002). However, in temperate regions where the winter climate allows the survival of asexual lineages, additional factors could play a role in the maintenance of sex. In particular, environmental variability is expected to benefit sexual lineages owing to the genetic variation conferred by sexual reproduction (Peck *et al.* 1999), which allows sexual populations to develop over a broader range of ecological conditions (Pound *et al.* 2002). Hence, heterogeneous environments would select for high levels of investment in sexual reproduction.

Three host races have been reported for the pea aphid, *Acyrtosiphon pisum* (Harris): one on pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.), a second on alfalfa (*Medicago sativa* L.), and a third on red clover (*Trifolium pratense* L.; Caillaud & Via 2000; Hawthorne & Via 2001; Carré & Bournoville 2003; Simon *et al.* 2003; Frantz *et al.* 2006). These host races live in habitats with highly different levels of temporal stability owing to the impact of agricultural practices. Populations of *A. pisum* living on pea and faba bean regularly experience strong bottlenecks and obligate dispersal when these crops are

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harvested in summer, while the populations of *A. pisum* living on perennial alfalfa and clover crops, cultivated over several years, can remain on their host throughout the year and do not necessarily endure host shifts. In this study, we tested the prediction that sympatric pea aphid populations living on annual or perennial crops show different levels of investment in sexual reproduction in response to contrasted temporal stability of their habitats. Such an association between specialization on annual versus perennial habitat and reproductive mode would directly allow the coexistence of sexual and asexual reproductive modes in sympatry and would further restrict the gene flow between pea aphid host races, enhancing their phenotypic and genotypic differentiation.

2. MATERIAL AND METHODS

(a) *Biological material*

To compare the variation in reproductive mode between pea aphid populations on annual and perennial hosts, we used a collection of clonal lineages previously characterized for their microsatellite profile (see Frantz *et al.* (2006) for details on aphid collection and microsatellite scoring). This included 137 clonal lineages, each bearing a unique genotype at several microsatellite loci. Most of these genotypes (125) were collected in mid-June of 2002 and 2003 near Rennes (Western France) in 20 fields separated from each other by less than 30 km and planted with four crops, two annual (pea and faba bean) and two perennial (alfalfa and red clover). The remaining genotypes (12) were collected near Poitiers (Central France) on pea, alfalfa and red clover (table 1). Each of these 137 pea aphid genotypes was kept isolated as a clonal lineage in the laboratory and reared on a single faba bean plant at 18 °C and 16L : 8D (light/dark regime).

(b) *Characterization of reproductive modes*

Aphids from each lineage were placed under conditions inducing sexual reproduction to characterize their reproductive mode. For each lineage, a third instar larva (generation G_0) was isolated from the stock culture and placed on a faba bean plant at 12L : 12D (light/dark regime) and 18 °C (MacKay 1987) where it developed into a wingless adult. The first-born larva (generation G_1) was in turn isolated and reared under the same conditions; it then developed into a wingless parthenogenetic female that started offspring production (generation G_2). To avoid overcrowding, once it had produced about 20 G_2 larvae, the G_1 female was removed and placed on a new plant, where it continued offspring production. This procedure was repeated until the G_1 female died. Once the G_2 larvae developed into adults, they were counted and their morph and sex (parthenogenetic female, sexual female, or male) were determined using classical morphological criteria (Lamb & Pointing 1972). Sexual inductions were repeated three times for each lineage to get a reliable photoperiodic response at the clonal level. Lineages were considered 'sexual' while producing both sexual females and males at least in one of the three replicates and 'asexual' while producing males and parthenogenetic females or only the parthenogenetic females. All lineages were tested for their reproductive mode on faba bean, which is a favourable in-laboratory host for pea aphids irrespective of their plant origin (Losey & Eubanks 2000).

Table 1. Number of genotypes used for the characterization of reproductive modes among French populations of *A. pisum* according to crop and geographical origins.

crop origin	geographical origin		total
	Rennes (Western France)	Poitiers (Central France)	
pea	18	4	22
faba bean	28	0	28
alfalfa	46	5	51
red clover	33	3	36
total	125	12	137

Table 2. Results of the model fitted for the analysis of the effects of crop origin, year of sampling and their interaction on the proportion of 'sexual' lineages among pea aphid genotypes (d.f., degrees of freedom).

effect	d.f.	residual d.f.	deviance	χ^2	<i>p</i>
null	—	19	30.97	—	—
crop	3	16	13.25	17.73	0.0005***
year	1	15	10.38	2.87	0.0904 ^{NS}
crop × year	3	12	5.78	4.60	0.2032 ^{NS}

NS, non-significant at $p=0.05$.

(c) *Statistical analyses*

Statistical analyses determined the effects of crop origin, year of collection and their interaction on the proportion of sexual lineages (generalized linear model; SAS Genmod procedure) and on the proportion of sexual morphs produced (generalized linear mixed model; SAS Glimmix macro). A binomial distribution with a logit link was assumed for both analyses. In the mixed model, the variable 'field' was treated as a random effect and replicates of the same lineage as repeated measurements in a covariance matrix of 'exchangeable' type. All statistical analyses were executed with SAS v. 8.1. Pea aphid lineages collected in Central France were not included in the statistical analyses owing to their low number, but corresponding proportions were used as illustrative data.

Finally, pairwise comparisons between the proportions of sexual lineages and the proportions of sexual morphs according to crop were achieved with the 'CONTRAST' statement. In both cases, the proportions presented in the figures were determined with the 'LSMEANS' statement to correct the other effects included in the model.

3. RESULTS

The proportion of sexual versus asexual lineages among the 125 genotypes of *A. pisum* collected in Western France strongly depended on crop origin but not on the year of collection or on the crop × year interaction (table 2; figure 1). The complete model proved to accurately describe the data as shown by the low ratio of the residual deviance to the residual degrees of freedom (rd : d.f. = 0.48). A simplified model, in which non-significant effects were removed, also accurately described the data (rd : d.f. = 0.83) suggesting no overdispersion and hence no significant field effect. The proportion of sexual lineages did not significantly differ between genotypes

Figure 1. Proportions of 'sexual' lineages among pea aphid genotypes according to their crop origin. All pairs of bars differ significantly except those surmounted by a horizontal line (NS, $p > 0.05$).

from pea and faba bean ($p = 0.27$), nor between genotypes from alfalfa and red clover ($p = 0.93$). This proportion was significantly higher among genotypes from pea than from alfalfa ($p = 0.0006$) and red clover ($p = 0.0011$) and was also higher among genotypes from faba bean than from alfalfa ($p = 0.011$) and red clover ($p = 0.016$).

The proportion of sexual versus asexual morphs produced was strongly associated with the crop origin of the lineages but not with the year of collection or the crop $\times$ year interaction (table 3; figure 2). This proportion was similar between lineages from pea and faba bean ($p = 0.37$) and between lineages from alfalfa and red clover ($p = 0.59$) but was higher among lineages from pea and faba bean than among lineages from alfalfa ($p = 0.0007$ and 0.0081) and red clover ($p = 0.0005$ and 0.0048).

The same qualitative pattern was observed among the 12 pea aphid genotypes collected in Central France. The proportion of 'sexual' genotypes was high among lineages from pea (75%) and low among lineages from alfalfa (20%) and red clover (33%), while the proportion of sexual morphs produced was high among lineages from pea (67%) and low among lineages from alfalfa (5%) and red clover (13%).

4. DISCUSSION

In aphids, winter climate is generally considered to be the main selective factor determining the geographical distribution of sexual and asexual reproduction (Rispe & Pierre 1998; Rispe *et al.* 1998). Rather than an abrupt shift in reproductive modes, sexual and asexual lineages show a climatic cline in their geographical distribution owing to the high migratory abilities of aphids (Simon *et al.* 2002). Hence, various reproductive modes coexist locally in temperate regions owing to a fluctuating regime of cold and mild winters associated with strong dispersal (Rispe & Pierre 1998). This was observed in our study under the climatic conditions of Western France, where sexual and asexual lineages were distinguished among sympatric

Table 3. Results of the model fitted for the analysis of the effects of crop origin, year of sampling and their interaction on the proportion of sexual morphs produced among pea aphid genotypes (d.f., degrees of freedom).

effect	d.f.	F-value	p
crop	3	6.62	0.0002***
year	1	2.99	0.0848 ^{NS}
crop $\times$ year	3	1.81	0.1454 ^{NS}

NS, non-significant at $p = 0.05$.

Figure 2. Proportions of sexual morphs (males and sexual females) produced by pea aphid genotypes according to their crop origin. All pairs of bars differ significantly except those surmounted by a horizontal line (NS, $p > 0.05$).

populations of *A. pisum* based on their response to sexual induction in the laboratory. While the coexistence of sexual and asexual lineages in *A. pisum* can be attributable to the local climate, as proposed for two cereal aphid species collected at the same sites (i.e. in *Sitobion avenae* (Haack *et al.* 2000; Papura *et al.* 2003), and in *Rhopalosiphum padi* (Delmotte *et al.* 2002; Halkett *et al.* 2005)), this factor cannot account for the striking differences in reproductive modes among sympatric pea aphid host populations. This suggests that characteristics of the habitat other than the climatic ones interfere in the selection of pea aphid reproductive modes at a local scale.

In this study, the investment level in the sexual phase (i.e. the proportion of sexual lineages and the proportion of sexual morphs produced) was higher among host populations originating from pea and faba bean than among the host populations from alfalfa and red clover. Interestingly, sexual and asexual lineages did not constitute monophyletic clusters on genetic trees relating French *A. pisum* genotypes, but rather appeared evenly distributed within each host race (Frantz *et al.* 2006; data not shown). Therefore, rather than the result of a founder effect, this association between the reproductive mode and host populations is likely to be adaptive, raising questions about its evolutionary maintenance.

We hypothesized that this association may be owing to differences in selective pressures exerted by perennial and annual hosts. The fields of annual crops such as pea and faba bean are completely harvested in summer and therefore represent periodically destroyed habitats.

Aphid populations living on these crops are subjected to frequent bottlenecks and are forced to colonize other seasonally available hosts. The capacity to rapidly combine favourable mutations provides sexual lineages with better adaptability to the changing environments (Kondrashov 1988; Hadany & Becker 2003). Higher temporal habitat variability would then exert a selective pressure for the maintenance of sex in aphid populations living on annual crops, allowing them to face variable biotic and abiotic constraints on different hosts. Conversely, the relatively high temporal stability on perennial crops, cultivated over several years (such as red clover and alfalfa fields), would allow the maintenance of asexual lineages. An association between the degree of sexual investment and crop cultural mode among sympatric pea aphid populations is consistent with the hypothesis that temporal variability of the habitat is a local driving force for the maintenance of sex. However, this hypothesis remains to be adequately demonstrated by considering a wide range of potential hosts.

It has been shown recently that one facultative symbiont of the pea aphid (*Regiella insecticola*) could modify several life-history traits of its host including the production of sexual morphs (Leonardo & Mondor 2006). In particular, the effect of *Regiella* removal was to decrease the production of sexual offspring in two out of three manipulated Californian clones. *Regiella*-infected individuals of French pea aphid predominate in the clover race (Simon *et al.* 2003), where asexual lineages also predominate (this study), suggesting that *Regiella* does not have the same involvement (if any) on sexual morph production in France and in California. Whatever the possible influence of facultative symbionts on sexual morph production, the ultimate factor responsible for the maintenance of high levels of sexual reproduction would be ecological, possibly habitat temporal variability as proposed earlier.

The highly different levels of investment in the sexual phase observed under laboratory conditions among pea aphid lineages are also likely to occur in the wild. The reproductive mode characterized in laboratory experiments has previously been confirmed by following the sexual morph production of aphid lineages placed outdoors (Dedryver *et al.* 1998, 2001; Hullé *et al.* 1999; Papura *et al.* 2003). This association between the reproductive mode and the host race would have profound ecological and evolutionary consequences on ecological specialization in *A. pisum*. The very low proportion of sexual morphs on perennial crops will readily restrict the gene flow between populations on annual and perennial crops. On annual crops, both the high level of investment in the sexual phase and the frequent migrations to other hosts will promote genetic admixture and prevent pea aphid host specialization. On the contrary, aphid populations could specialize on red clover and alfalfa, respectively, because they can remain on their hosts while reproducing by parthenogenesis, which maintains coadapted gene complexes. This is consistent with both the lack of genetic differentiation between aphid populations on pea and faba bean, which are considered as a single host race (Frantz *et al.* 2006), and the genetic divergence between aphids collected on red clover and alfalfa (Caillaud & Via 2000; Hawthorne & Via 2001; Carré & Bournoville 2003; Simon *et al.* 2003; Frantz *et al.*

2006). According to the sympatric speciation scenario, it is assumed that specialization on a new host-plant species may result in strong ecological divergence leading to the formation of what is known as 'host races' (Drès & Mallet 2002). Here, this divergence affected the degree of sexual investment of aphid populations living on annual versus perennial crops. However, whether differences in reproductive modes among populations of the same species are a cause or a consequence of their ecological divergence remains to be elucidated.

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REFERENCES

- Caillaud, M. C. & Via, S. 2000 Specialized feeding behavior influences both ecological specialization and assortative mating in sympatric host races of pea aphids. *Am. Nat.* **156**, 606–621. (doi:10.1086/316991)
- Carré, S. & Bournoville, R. 2003 Specialization of spring sympatric populations of *Acyrtosiphon pisum* (Hemiptera: Aphididae) according to Fabaceae. *Ann. Soc. Entomol.* **39**, 391–397.
- Dedryver, C. A., Le Gallic, J. F., Gauthier, J. P. & Simon, J. C. 1998 Life cycle of the cereal aphid *Sitobion avenae* F.: polymorphism and comparison of life history traits associated with sexuality. *Ecol. Entomol.* **23**, 123–132. (doi:10.1046/j.1365-2311.1998.00113.x)
- Dedryver, C. A., Hullé, M., Le Gallic, J. F., Caillaud, M. C. & Simon, J. C. 2001 Coexisting in space and time of sexual and asexual populations of the cereal aphid *Sitobion avenae*. *Oecologia* **128**, 379–388. (doi:10.1007/s004420100674)
- Delmotte, F., Leterme, N., Gauthier, J. P., Risper, C. & Simon, J. C. 2002 Genetic architecture of sexual and asexual populations of the aphid *Rhopalosiphum padi* based on allozyme and microsatellite markers. *Mol. Ecol.* **11**, 711–723. (doi:10.1046/j.1365-294X.2002.01478.x)
- Doncaster, C. P., Pound, G. E. & Cox, S. J. 2000 The ecological cost of sex. *Nature* **404**, 281–285. (doi:10.1038/35005078)
- Drès, M. & Mallet, J. 2002 Host races in plant-feeding insects and their importance in sympatric speciation. *Phil. Trans. R. Soc. B* **357**, 471–492. (doi:10.1098/rstb.2002.1059)
- Frantz, A., Plantegenest, M., Mieuze, L. & Simon, J. C. 2006 Ecological specialization correlates with genotypic differentiation in sympatric host-populations of the pea aphid. *J. Evol. Biol.* **19**, 392–401. (doi:10.1111/j.1420-9101.2005.01025.x)
- Haack, L., Simon, J. C., Gauthier, J. P., Plantegenest, M. & Dedryver, C. A. 2000 Predominance of generalist clones in a cyclically parthenogenetic organism evidenced by combined demographic and genetic analyses. *Mol. Ecol.* **9**, 2055–2066. (doi:10.1046/j.1365-294X.2000.01108.x)
- Hadany, L. & Becker, T. 2003 On the evolutionary advantage of fitness-associated recombination. *Genetics* **165**, 2167–2179.
- Halkett, F., Plantegenest, M., Prunier-Leterme, N., Mieuze, L., Delmotte, F. & Simon, J. C. 2005 Admixed sexual and facultatively asexual aphid lineages at mating sites. *Mol. Ecol.* **14**, 325–336. (doi:10.1111/j.1365-294X.2004.02358.x)

- Hamilton, W. D. 1980 Sex versus non-sex versus parasite. *Oikos* **35**, 292–290.
- Hawthorne, D. J. & Via, S. 2001 Genetic linkage of ecological specialization and reproductive isolation in pea aphids. *Nature* **412**, 904–907. (doi:10.1038/35091062)
- Hullé, M., Maurice, D., Rispe, C. & Simon, J. C. 1999 Clonal variability in sequences of morph production during the transition from parthenogenetic to sexual reproduction in the aphid *Rhopalosiphum padi* (Sternorrhyncha: Aphididae). *Eur. J. Entomol.* **96**, 125–134.
- Kondrashov, A. S. 1988 Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**, 435–440. (doi:10.1038/336435a0)
- Lamb, R. J. & Pointing, P. J. 1972 Sexual morph determination in the pea aphid, *Acyrtosiphon pisum*. *J. Insect Physiol.* **18**, 2029–2042. (doi:10.1016/0022-1910(72)90170-9)
- Leonardo, T. E. & Mondor, E. B. 2006 Symbiont modifies host life-history traits that affect gene flow. *Proc. R. Soc. B* **273**, 1079–1084. (doi:10.1098/rspb.2005.3408)
- Lewis, W. M. 1987 The cost of sex. In *The evolution of sex and its consequences* (ed. S. C. Stearns), pp. 33–57. Basel, Germany: Birkhäuser-Verlag.
- Losey, J. E. & Eubanks, M. D. 2000 Implications of pea aphid host-plant specialization for the potential colonization of vegetables following post-harvest emigration from forage crops. *Environ. Entomol.* **29**, 1283–1288.
- MacKay, P. A. 1987 Production of sexual and asexual morphs and changes in reproductive sequence associated with photoperiod in the pea aphid, *Acyrtosiphon pisum* (Harris). *Can. J. Zool.* **65**, 2602–2606.
- Maynard-Smith, J. 1971 The origin and maintenance of sex. In *Group selection* (ed. G. C. Williams), pp. 163–175. Chicago, IL: Aldine Atherton.
- Maynard-Smith, J. 1978 *The evolution of sex*. Cambridge, UK: Cambridge University Press.
- Moran, N. A. 1992 The evolution of aphid life cycles. *Annu. Rev. Entomol.* **37**, 321–348. (doi:10.1146/annurev.en.37.010192.001541)
- Papura, D., Simon, J. C., Halkett, F., Delmotte, F., Le Gallic, J. F. & Dedryver, C. A. 2003 Predominance of sexual reproduction in Romanian populations of the aphid *Sitobion avenae* inferred from phenotypic and genetic structure. *Heredity* **90**, 397–404. (doi:10.1038/sj.hdy.6800262)
- Peck, J. R. 1994 A ruby in the rubbish: beneficial mutations, deleterious mutations and the evolution of sex. *Genetics* **137**, 597–606.
- Peck, J. R., Yearsley, J. & Barreau, G. 1999 The maintenance of sexual reproduction in a structured population. *Proc. R. Soc. B* **266**, 1857–1863. (doi:10.1098/rspb.1999.0857)
- Pound, G. E., Doncaster, C. P. & Cox, S. J. 2002 A Lotka–Volterra model of coexistence between a sexual population and multiple asexual clones. *J. Theor. Biol.* **217**, 535–545. (doi:10.1006/jtbi.2002.3040)
- Rispe, C. & Pierre, J. S. 1998 Coexistence between cyclical parthenogens, obligate parthenogens, and intermediates in a fluctuating environment. *J. Theor. Biol.* **195**, 97–110. (doi:10.1006/jtbi.1998.0784)
- Rispe, C., Pierre, J. S., Simon, J. C. & Gouyon, P. H. 1998 Models of sexual and asexual coexistence in aphids based on constraints. *J. Evol. Biol.* **11**, 685–701. (doi:10.1007/s000360050113)
- Simon, J. C., Rispe, C. & Sunnucks, P. 2002 Ecology and evolution of sex in aphids. *Trends Ecol. Evol.* **17**, 34–39. (doi:10.1016/S0169-5347(01)02331-X)
- Simon, J. C., Carré, S., Boutin, M., Prunier-Leterme, N., Sabater-Muñoz, B., Latorre, A. & Bournoville, R. 2003 Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. *Proc. R. Soc. B* **270**, 1703–1712. (doi:10.1098/rspb.2003.2430)
- Van Valen, L. M. 1973 A new evolutionary law. *Evol. Theory* **1**, 1–30.
- Wilson, A. C. C., Sunnucks, P. & Hales, D. F. 2003 Heritable genetic variation and potential for adaptive evolution in asexual aphids (Aphidoidea). *Biol. J. Linn. Soc.* **79**, 115–135. (doi:10.1046/j.1095-8312.2003.00176.x)