Climate effects on life cycle variation and population genetic architecture of the black bean aphid, *Aphis fabae*

Christoph Sandrock^{1,2}, Jabraeil Razmjou³ and Christoph Vorburger⁴

¹Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

²present address: Swiss Bee Research Centre, Agroscope Liebefeld-Posieux ALP, Schwarzenburgstrasse 161, 3003 Bern, Switzerland

³Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran

⁴Institute of Integrative Biology, ETH Zürich, Switzerland, and EAWAG, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, 8600 Dübendorf, Switzerland

Key words: *Aphis fabae*, contagious parthenogenesis, crop pest, cyclical parthenogenesis, host associated differentiation

Running title: Life cycle variation in *Aphis fabae*

correspondence: Christoph Sandrock
Institute of Evolutionary Biology and Environmental Studies
University of Zurich
Winterthurerstrasse 190
8057 Zürich
Switzerland
fax: ++41 44 635 68 21
e-mail: christoph.sandrock@ieu.uzh.ch

This document is the accepted manuscript version of the following article:
Sandrock, C., Razmjou, J., & Vorburger, C. (2011). Climate effects on life cycle variation and population genetic architecture of the black bean aphid, *Aphis fabae*. *Molecular Ecology*, 20(19), 4165–4181. <http://doi.org/10.1111/j.1365-294X.2011.05242.x>

Abstract

Aphid species may exhibit different reproductive modes ranging from cyclical to obligate parthenogenesis. The distribution of life cycle variation in aphids is generally determined by ecological forces, mainly climate, because only sexually produced diapausing eggs can survive harsh winters or periods of absence of suitable host plants. Aphids are thus interesting models to investigate intrinsic and environmental factors shaping the competition among sexual and asexual lineages. We conducted a Europe-wide sampling of black bean aphids, *Aphis fabae*, and combined population genetic analyses based on microsatellite data with an experimental determination of life cycle strategies. Aphids were collected from broad beans (*Vicia faba*) as well as some Chenopodiaceae, but we detected no genetic differentiation between aphids from different host plants. Consistent with model predictions, life cycle variation was related to climate, with aphids from areas with cold winters investing more in sexual reproduction than aphids from areas with mild winters. Accordingly, only populations from mild areas exhibited a clear genetic signature of clonal reproduction. These differences arise despite substantial gene flow over large distances, which was evident from a very low geographic population structure and a lack of isolation-by-distance among 18 sites across distances of more than 1000 kilometres. There was virtually no genetic differentiation between aphids with different reproductive modes, suggesting that new asexual lineages are formed continuously. Indeed, a surprising number of *A. fabae* genotypes even from colder climates produced some parthenogenetic offspring under simulated winter conditions. From this we predict that a shift to predominantly asexual reproduction could take place rapidly under climate warming.

Introduction

To explain the maintenance of sexual reproduction remains one of the fundamental problems of evolutionary biology (Maynard Smith, 1978). Asexual organisms avoid the cost of producing males, they can retain favourable gene combinations in the absence of recombination, and they are able to colonise new habitats via single individuals (Williams, 1975). Despite these advantages of asexuality, sexual reproduction predominates in eukaryotes (Bell, 1982). Various theoretical attempts tried to address this paradox, focusing on genetic constraints such as mutational load (Kondrashov, 1988; Peck, 1994), or hampered capabilities of asexuals for adaptation to environmental change (Doncaster *et al.*, 2000; Hamilton, 1980). There is general consensus that both aspects provide advantages to sexual reproduction (Maynard Smith, 1988), yet these benefits might be achieved only in the long term. Thus, there is still need for empirical data to understand how sexual reproduction can resist displacement in the short term (Barton & Charlesworth, 1998). Systems with coexisting sexual and asexual congeners are especially promising to advance mechanistic explanations of the maintenance of sex. Interesting candidates in this respect are cyclical parthenogens like cladocerans and aphids (Rispe & Pierre, 1998; Simon *et al.*, 2002). In aphids, the ancestral life cycle, cyclical parthenogenesis, includes several generations of apomictic parthenogenesis by viviparous females followed by a single sexual generation consisting of males and egg-laying females (Dixon, 1998; Moran, 1992). However, transitions to obligate parthenogenesis (i.e. the loss of the sexual generation) are common, and both reproductive modes may coexist in the same species or even population (Dedryver *et al.*, 2001; Delmotte *et al.*, 2001; Simon *et al.*, 2003; Vorburger *et al.*, 2003). This offers ample opportunities to evaluate hypotheses on the costs and benefits of sex.

In many organisms with coexisting sexual and asexual lineages, the geographic distribution of reproductive modes shows striking differences. This phenomenon is referred to

as geographic parthenogenesis (Peck *et al.*, 1998; Vandel, 1928). It may either reflect historic colonization patterns (Paland *et al.*, 2005) or else indicate strong ecological differences between sexual and asexual lineages. In most known cases of geographic parthenogenesis, asexuals tend to occur at higher latitudes and altitudes or generally in more extreme habitats (Lynch, 1984; Peck *et al.*, 1998). Aphids represent an exception to this rule. Obligate asexuality is more common at lower latitudes in a number of species, including *Sitobion avenae* Fabricius (Dedryver *et al.*, 2001), *Rhopalosiphum padi* L. (Delmotte *et al.*, 2002), *Macrosiphum rosae* L. (Wöhrmann & Tomiuk, 1988), *Acyrtosiphum pisum* Harris (Kanbe & Akimoto, 2009) and *Myzus persicae* Sulzer (Blackman, 1974). This pattern appears to be primarily determined by climate (Rispe & Pierre, 1998; Rispe *et al.*, 1998). Only sexual females can produce cold-resistant, diapausing eggs, which is the sole life-stage capable of surviving harsh winters in temperate regions because nymphs or adults are either killed by freezing or suffer from a seasonal absence of suitable hosts (Simon *et al.*, 2002). Under a mild climate, in contrast, parthenogenetic forms are favoured because they forego the cost of producing males and avoid an unnecessary diapause when overwintering as nymphs or adults is possible (Simon *et al.*, 2002). According to model predictions, coexistence among obligate and cyclical parthenogens can occur in areas with pronounced fluctuation of winter severity (Rispe & Pierre, 1998; Rispe *et al.*, 1998). However, there are large differences among aphid species in the geographic clines described above. These differences may be attributed to host availability. Aphids without host alternation (monoecy) like the tansy aphid, *Macrosiphoniella tanacetaria* Kaltenbach, are rarely host-limited for sexual reproduction and seem to retain a sexual generation in their life-cycle throughout (Loxdale *et al.*, 2011; Massonnet *et al.*, 2002). Yet also in monoecious aphids, the evolution of reproductive modes may be influenced by variation in the seasonal availability of host plants, as shown for the pea aphid (Frantz *et al.*, 2006b). Aphids with host alternation between sexual and asexual phases (heteroecy) tend to be highly specialised on a specific primary host (generally a woody plant),

on which mating takes place and overwintering eggs are laid. Thus, cyclical parthenogens can occur only where this host is available, whereas obligate parthenogens of the same species are freed from this constraint. This can determine the geographic distribution of reproductive modes in addition to climatic variation, which has been documented most clearly in the green peach aphid, *M. persicae* (Fenton *et al.*, 1998; Vorburger *et al.*, 2003).

However, dividing aphid reproductive modes into cyclical parthenogenesis (holocycly) and obligate parthenogenesis (anholocycly) is a strong simplification. There are aphid species that clearly fall into these categories, but others exhibit ample variation. Obligately parthenogenetic lineages frequently retain the ability to produce some males (androcycly). These males are generally fertile. Because obligate parthenogenesis has a heritable basis (Blackman, 1972; Dedryver *et al.*, 1998; Delmotte *et al.*, 2001; Rispe *et al.*, 1999; Simon *et al.*, 1999), such males can spawn new asexual lineages by mating with females from cyclical parthenogens, thereby effecting gene flow between lineages with different reproductive modes. This process, termed ‘contagious parthenogenesis’ (Simon *et al.*, 2003), is found frequently in organisms with sexual and asexual reproduction (Innes & Hebert, 1988; Sandrock & Vorburger, 2011; Simon *et al.*, 2002; Tas & Van Dijk, 1999). Some aphid lineages also exhibit intermediate or ‘bet-hedging’ strategies (Slatkin, 1974), which exhibit an incomplete switch to sexual reproduction in autumn, i.e. they produce males and sexual females while continuing to produce parthenogenetic offspring (Simon *et al.*, 2003). Thus, depending on the frequencies of lineages with different reproductive modes, aphid populations can be characterised by their relative investment in sexual and asexual reproduction. This makes aphids an interesting test case for models that predict the population genetic signature of different levels of clonal reproduction in natural populations. For instance, such models predict lower genotypic but higher allelic diversity with increasing clonality, as well as a concomitant heterozygote excess (Balloux *et al.*, 2003; Birky, 1996; de Meeûs *et al.*, 2006; Halkett *et al.*, 2005b). Some of these predictions were indeed supported

by population genetic studies on aphids (Delmotte *et al.*, 2002; Halkett *et al.*, 2008; Papura *et al.*, 2003; Simon *et al.*, 1999; Vorburger *et al.*, 2003).

Here we address these issues in the black bean aphid, *Aphis fabae* Scopoli, a widespread and damaging pest of several crops, particularly in the northern hemisphere (Blackman & Eastop, 2000). For this we collected *A. fabae* from different populations across Europe, experimentally studied their reproductive modes in relation to climatic variation, and compared these data to the genetic population structure inferred from microsatellite analysis.

Material and methods

Study species

Aphis fabae is a host-alternating species. Viviparous spring fundatrices hatch from diapausing eggs on the primary host, mainly the European spindle tree (*Euonymus europaeus* L.) or, to a lesser extent, the snowball tree, *Viburnum opulus* L., and the Sweet Mock-orange, *Philadelphus coronarius* L. (Dixon, 1998; Iglisch, 1968; Müller, 1982). Their winged parthenogenetic descendants disperse to various herbaceous secondary host plants (see Dixon, 1998; Thieme, 1987), where numerous parthenogenetic generations take place throughout the growth season. In autumn, decreasing day lengths and temperatures induce the sexual phase of the life-cycle. Still on the secondary host, gynoparae and males are produced (Hardie & Vaz Nunes, 2001). These winged morphs migrate back to primary hosts, where gynoparae give birth to sexual females (oviparae), which mate with the males and deposit overwintering eggs (Dixon, 1998).

Based on diagnostic differential acceptance of secondary hosts and primary host use, five largely co-occurring taxa are currently distinguished within the *A. fabae* group (Blackman & Eastop, 2007; Iglisch, 1968; Müller, 1982; Raymond *et al.*, 2001; Thieme, 1988). Here we focused on *A. f. fabae*, the most abundant subspecies of the complex, which is characterised

as the only subspecies that is able feed on broad beans (*Vicia faba* L.) as well as several species of Chenopodiaceae such as sugar beet (*Beta vulgaris* L.) and goosefoot (*Chenopodium* sp.) as secondary hosts (Iglisch, 1968; Müller, 1982; Thieme, 1987). Broad beans, sugar beet and spinach (*Spinacia oleracea* L.) are economically important crops on which *A. f. fabae* causes considerable damage, either directly through feeding or by acting as a vector of multiple viral plant diseases (Blackman & Eastop, 2000).

Sample collection

We collected *A. f. fabae* from 18 sites across Europe (Tab. 1, Fig. 1). At each site, aphids were sampled within an area of approx. 10 km² surrounding the location indicated in Table 1. All of these sampling locations lie well within the distribution range of the European spindle tree, *A. fabae*'s main primary host plant (Meusel *et al.*, 1978). Thus, the potential for sexual reproduction of *A. fabae* was fulfilled at all locations, but possibly to a different extent. Although spindle trees were observed at all locations (C. Sandrock and C. Vorbürger, personal observations), we did not quantify their abundance. For collecting we focussed on two groups of secondary host plants, namely broad beans (*V. faba*, 50.1%) and chenopods (Chenopodiaceae), including *Chenopodium album* (45.7 %) and some cultivated forms such as spinach and beets (4 %). Two samples came from *Rumex* sp. Samples from both major groups of hosts could be obtained at thirteen locations. Samples were taken by clipping infested leaves or shoots. We collected only reproducing aphids to avoid including individuals that just stopped and probed on our target plants, but would be unable to develop on these hosts (e.g. other subspecies of *A. fabae*). To minimise the collection of clonal offspring from the same individual, colonies feeding on different plants and separated by at least five metres were sampled. Single individuals from haphazard subsamples of our collections were transferred to seedlings of *V. faba* var Scirocco in the laboratory to establish isofemale clonal lines. These cultures were kept in programme controlled plant growth chambers simulating

long day conditions (16 h light : 8 h dark at 20°C, hereafter LD). These conditions ensured continuous parthenogenetic reproduction until we determined the lines' reproductive mode experimentally (see below). Specimens of all field samples and laboratory lines were stored in absolute ethanol at -20° C prior to genetic analyses.

Microsatellite genotyping

We used Chelex (Biorad) to prepare genomic DNA (Walsh *et al.*, 1991) in a volume of 170 µl of 5% Chelex solution per aphid. A multiplex PCR assay was developed to genotype aphids at eight microsatellite loci (Coeur d'Acier *et al.*, 2004) in a single PCR reaction. The forward primer for each locus was labelled with a fluorescent dye such that loci with the same dye had non-overlapping allele size ranges: AF-48 (VIC), AF-50 (PET), AF-82 (NED), AF-85 (FAM), AF-86 (VIC), AF-181 (FAM), AF-beta (NED), AF-F (PET). PCR was carried out in 4.5 µl volumes using a Techne TC-512 thermocycler and the QIAGEN Multiplex PCR kit. Each reaction contained 1x QIAGEN Multiplex PCR MasterMix, including PCR buffer (3mM MgCl₂), a dNTP mix and HotStar *Taq* DNA polymerase, 1 µl of genomic DNA and 0.2 µM of every locus-specific primer (including 0.02 µM 5' labelled forward primer). Cycling conditions were as follows: after an initial denaturation of 15 min at 95 °C, 27 cycles were performed, each consisting of 30 s at 94 °C, 90 s at 60 °C and 60 s at 72°C, followed by a final extension step at 60 °C for 30 min. Fragment sizes were determined on an ABI 3730 sequencer and allele scoring was performed using the GeneMapper software version 3.7.

Genetic data analyses

Clonal richness. We used GenClone v.2.0 (Arnaud-Haond & Belkhir, 2007) to calculate clonal diversity, R , defined as $(G-1)/(N-1)$, where G is the number of different multilocus genotypes (MLG) and N is the number of individuals in a sample. We are aware

that treating individuals with the same MLG as members of the same clone relies on sufficient resolution provided by the markers (number and variability of the loci used) and ignores potential variation in other regions of the genome (Loxdale & Lushai, 2003). Therefore, we also calculated p_{sex} , the probability of replicate MLGs to be produced by independent sexual events, using GenClone v. 2.0 (Arnaud-Haond & Belkhir, 2007; Arnaud-Haond *et al.*, 2007).

Standard population genetics and population differentiation. Clonal amplification inevitably leads to violation of genetic equilibria (Halkett *et al.*, 2005b; Sunnucks *et al.*, 1997), thus only single copies of each MLG per sampling location were included in these analyses. We tested for linkage disequilibrium between locus pairs using a likelihood-ratio method executed in ARLEQUIN v3.11 (Excoffier *et al.*, 2005) and G-statistics provided by FSTAT (Goudet, 2001) for within populations and the total sample, respectively. Hardy-Weinberg equilibrium (HWE) for single loci within populations was tested as implemented in ARLEQUIN. Population specific multilocus F_{IS} (Weir & Cockerham, 1984) and single locus F_{IS} across populations were estimated and tested based on a randomisation approach using FSTAT. We used F_{ST} (Weir, Cockerham, 1984) to infer population genetic structure. Multilocus population pairwise F_{ST} , overall locus specific F_{ST} and global F_{ST} were computed with FSTAT. Significance levels of multiple non-independent statistical tests were adjusted using the sequential Bonferroni correction (Rice, 1989). Mantel tests as implemented in ARLEQUIN were conducted (based on 20,000 permutations) to test for isolation by distance (IBD), i.e. correlations between $F_{\text{ST}}/(1 - F_{\text{ST}})$ and the natural logarithm of geographic distance in kilometres (Rousset, 1997). Further, as recommended by de Meeûs *et al.* (2006, 2007), we performed a factorial correspondence analysis (FCA), implemented in GENETIX v4.03 (Belkhir *et al.*, 1996-2004) to visualise genetic structure.

Bayesian clustering analyses. We performed Bayesian clustering analyses using STRUCTURE (Pritchard *et al.*, 2000) to estimate (i) the most likely number of genetic clusters, K , in our sample of black bean aphids and (ii) q_i the vector of the proportions of the

individual i 's genome that is derived from each cluster. Both parameters can be inferred separately from the posterior probability distribution of allele frequencies, yet, random mating and independence of markers is assumed. Although these premises are violated in aphid systems harbouring asexually propagating lineages, this approach is robust to some deviations (Falush *et al.*, 2003) and has recently been applied successfully in comparable aphids systems (Frantz *et al.*, 2006a; Gilabert *et al.*, 2009; Halkett *et al.*, 2008). Simulations using default settings were based on 5×10^4 burn-in runs followed by 2×10^5 iterations using single copies per MLG per population. Twenty independent simulations were performed to test for consistency of the results, each with K set from 1 to 18. To detect the most reliable number of clusters in the dataset we quantified ΔK following Evanno *et al.* (2005).

Host-related molecular variance. A hierarchical analysis of molecular variance, AMOVA, was performed in ARLEQUIN to infer the amount of genetic variance attributable to geographic origin and to the host plant aphids were collected from (i.e. *V. faba* versus Chenopodiaceae). However, nesting host within location ignores the fact that the same two host plants are compared within each site. Therefore, we also calculated pairwise F_{ST} between aphids from *V. faba* and Chenopodiaceae and associated P -values for each site at which both hosts were sampled and used Fisher's method of combining P -values (Sokal & Rohlf, 1995) for an overall test of host-associated differentiation.

Characterization of reproductive modes

We conducted a life-cycle experiment to determine the reproductive modes of the isofemale clonal lines we had established from different European sites. For this we exposed them to conditions of short day length and reduced temperature (11h light : 13h dark at 15 °C, hereafter SD), which reliably induce the sexual phase in *A. fabae* if a clone is able to do so (Hardie & Vaz Nunes, 2001; Vaz Nunes & Hardie, 2000). Like other aphids, *A. fabae* may show some latitudinal variation around the critical night length that induces sexual

reproduction (Loxdale *et al.*, 1993; Smith & Mackay, 1990). Yet the night length chosen here was well beyond this range and should thus have been sufficient to induce the formation of sexual morphs in *A. fabae* from all our collection sites (Vaz Nunes & Hardie, 2000). We determined the life cycles of 136 lines from 15 sampling locations (Table 1), picking lines with different MLGs to maximise the variation assessed. Only three MLGs were represented twice in the experiment. In these three cases, lines with the same MLGs were collected from two different locations.

The experiment was initiated by transferring three to four young adult females (generation G0) of each line from LD to SD conditions, under which they could reproduce for 3 days. Four randomly chosen offspring (generation G1) per line were reared to adulthood and allowed to larviposit for 20 days under SD. These G1 females were transferred to new plants every five days. The transfers were required to avoid crowding, which induces wing formation in virginoparae (Moran, 1992) that would then be morphologically indistinguishable from gynoparae (Hardie, 1980). Crowding might also reduce a G1 female's lifetime and thus cause biases because males are produced later during the reproductive period (Vaz Nunes & Hardie, 2000). The different batches of G2 offspring produced by the G1 females over consecutive 5-day intervals were labelled G2₍₁₋₅₎, G2₍₆₋₁₀₎, G2₍₁₁₋₁₅₎ and G2₍₁₆₋₂₀₎. They were reared separately under the experimental SD regime until adult ecdysis, when we counted them and determined their morphs. Three morphs were distinguished: wingless parthenogenetic females (apterae), winged females (including winged virginoparae as well as gynoparae, i.e. winged females that later give birth to oviparae, the egg-laying sexual females), and males. Because we avoided crowding, we assume that a substantial proportion of winged females were gynoparae, but we could not distinguish them from winged virginoparae, which may also be produced under the experimental conditions. To determine their exact proportions would have required rearing the offspring of all winged females

produced in G2, which was an impossible task at the scale of this experiment. Thus, we just refer to this mixed category as winged females.

The production of males and gynoparae in G2 characterise the switch to sexual reproduction (cyclical parthenogenesis), whereas the production of unwinged or winged parthenogenetic females in G2 is indicative of continued parthenogenetic reproduction (obligate parthenogenesis). However, aphid clones are frequently capable of producing all types of morphs under SD conditions (Simon *et al.*, 2003). The near-continuous variation of their investment in sexual reproduction is therefore best quantified by the relative frequencies of the different morphs, although the uncertainty about the true nature of winged females prevented us from obtaining absolute estimates of each line's investment in sexual reproduction. Nevertheless, the data still allowed comparisons of the relative investment in sexual and asexual reproduction, because this can be based solely on the proportion of males and unwinged females, of which we know they represent investment in sexual and asexual reproduction, respectively. The proportion of winged females is then already determined by one minus the proportions of the other two morphs.

To visualise the genetic relationships among lines with different reproductive modes, we used the software POPULATIONS v1.2.30 (Langella, 1999) to produce a neighbour-joining (NJ) tree based on the matrix of pairwise allele-shared distances, D_{AS} (Chakraborty & Jin, 1993), on which we superimposed the information about the production of males and winged females as a rough proxy of the investment in sexual morph production under SD conditions. As an attempt to formally test for an association between genotypic variation and variation in reproductive mode we used a Mantel test implemented in the software ZT (Bonnet & van de Peer, 2002) to compare the matrix of pairwise D_{AS} with a matrix of pairwise differences in the investment in males and winged females of the same lines (absolute values of the difference in the production of these morphs under SD conditions).

Analyses of variance in life cycle strategies

We performed multivariate and univariate analyses of variance to investigate variation in LCSs. We used proportions of G2 morphs (arcsine square root transformed) as dependent variables, testing for the effects of collection site, host plant (*Ch. album* or *V. faba*), and the site $\times$ host interaction. Four lines were excluded from this analysis because they had been collected from different host species. The total number of G2 offspring was included as a covariate to account for the possibility that longer-lived G1 mothers (producing more G2 offspring) might have produced more males (see above). Because the proportions of the three morphs add up to 1 and are therefore correlated, we first carried out a MANOVA on the proportions of males and winged females only (the proportion of wingless parthenogenetic females being uniquely determined by the other two responses), followed by univariate analyses to test for differences in the production of each morph separately. Additionally, population averages of G2 morph proportions were correlated (*i*) with genetic indicators expected to be influenced by asexual reproduction (e.g. genotypic diversity and F_{IS}), and (*ii*) with climatic variables that are expected to affect the potential for continuous parthenogenetic reproduction, essentially winter severity. Climate data were taken from the nearest measurement station (for all sample sites located at very similar altitudes within a radius of $\leq$ 65 km), available from the European Climate Assessment & Dataset (<http://eca.knmi.nl/>). We focussed on the average yearly number of frost days (FD, days with $T < 0^{\circ}\text{C}$) (Tank *et al.*, 2002), as an estimate for winter severity, and we considered the average over the last 50 years prior to sampling as well as the average over the last decade. Statistics were performed using SPSS 17.0.

Results

Genetic and clonal diversity

The number of alleles ranged from 4 to 27 per locus, with 104 alleles identified across all 8 loci (Tab. 2). Among 655 aphids, 587 unique MLGs were discriminated. Within-population estimates of p_{sex} ranged from 6.83×10^{-25} to 0.009 and suggested that replicate MLGs were not assembled independently by sexual reproduction but were members of the same clone. We detected 29 MLGs more than once, mostly within sampling locations. Six MLGs occurred at two locations. One was found at two Swiss sites (*Val & Zu2*), all others were shared between sites in Southern Europe (one in *Tos & Rom*, two in *Cam & Cot* and two in *Tos & Cot*). Nevertheless, finding them at sites that are up to 300 km apart suggest that certain clones can achieve very wide distributions.

Clonal diversity, R , equaled 1 in most sampling locations, and was never lower than 0.75 in sites from central and northwestern Europe (Table 1). For the four Southern European sites along the Mediterranean coast, much lower values between 0.46 and 0.62 were observed.

Geographic variation in life-cycle strategies

The variation in the production of different morphs under SD conditions was very large and nearly continuous (Fig. 2, Table S1), yet the MANOVA revealed significant variation among populations in the relative investment in apterae, winged females and males (Wilk's Lambda = 0.45, $F_{28, 210} = 3.68$, $P < 0.001$). However, there was no significant effect of the host plant clones were collected from (*V. faba*, $N = 54$; *Ch. album*, $N = 78$; Wilk's Lambda = 0.98, $F_{2, 105} = 1.15$, $P = 0.320$), nor was there a significant host plant $\times$ population interaction (Wilk's Lambda = 0.94, $F_{18, 210} = 0.40$, $P = 0.987$). The total number of G2 offspring produced during the life-cycle experiment (mean value = 93.6) did not have a significant effect on the response (Wilk's Lambda = 0.96, $F_{2, 105} = 2.40$, $P = 0.094$). Univariate analyses showed that the population effect was significant or nearly significant for the proportions of all three morphs produced in G2 (males: $F_{14, 106} = 1.78$, $P = 0.051$; winged

354 females: $F_{14, 106} = 6.78$, $P < 0.001$; wingless parthenogenetic females: $F_{14, 106} = 6.96$, $P <$
 355 0.001).

356 The average proportions of the three G2 morph categories and the geographic origins
 357 of all tested populations are shown in Figure 1. The most obvious pattern is that *A. fabae* from
 358 the four southernmost populations on the Mediterranean coast produced much lower
 359 proportions of males and winged females under SD conditions. This pattern is also evident
 360 when morph production is related to climatic variation, that is winter severity estimated as the
 361 mean number of frost days (FD) per year (Fig. 3a). The mean proportion of males and winged
 362 females increased while the proportion of wingless parthenogenetic females decreased
 363 significantly with higher average numbers of FD over the previous 10 years (Pearson's r ;
 364 wingless parthenogenetic females: $r = -0.781$, $P = 0.001$; males: $r = 0.696$, $P = 0.004$; winged
 365 females: $r = 0.782$, $P = 0.001$; Fig 3a). These correlations were also significant with the mean
 366 number of FDs over the previous 50 years, albeit somewhat weaker (not shown). Across all
 367 sites, the average yearly number of FDs over the previous decade was 16.3 % lower than the
 368 average over the previous 50 years, consistent with a warming climate.

369 The higher investment in sexual reproduction observed in populations inhabiting
 370 colder regions translated into a higher clonal diversity at such sites. We observed significant
 371 relationships between the proportions of all three morphs produced under SD and population-
 372 specific clonal diversity (Pearson's r ; wingless parthenogenetic females: $r = -0.781$, $P =$
 373 0.001; males: $r = 0.849$, $P < 0.001$; winged females: $r = 0.784$, $P = 0.001$; Fig. 3b).
 374 Accordingly, R significantly increased with winter severity over the previous 10 years
 375 (Pearson's $r = 0.624$, $P = 0.013$). This correlation was somewhat weaker when winter severity
 376 of the last 50 years was considered (Pearson's $r = 0.586$, $P = 0.022$).

377

378 *Population genetic characteristics*

After discarding replicate MLGs within populations, only 10 out of 504 comparisons of pairs of loci (28 per population) displayed significant linkage disequilibrium in a total of seven populations. No locus pair was in significant linkage disequilibrium in more than one population. This overall suggests that the loci studied here are not effectively linked. Even across pooled populations, only three locus pairs out of 28 comparisons exhibited marginally significant linkage disequilibrium.

Mean observed heterozygosities ranged from 0.32 to 0.64 within populations, and averaged 0.55 over all loci. Each locus in each population did not deviate significantly from Hardy-Weinberg expectations, even in populations which obviously contained obligate asexuals. Averaged across loci, HWE was violated only in a single population, *SGa*, which displayed a significant overall homozygote excess ($P < 0.001$). Averaged across populations, significant homozygote excess was detected for loci AF86 and AF181 ($P < 0.001$ and $P = 0.003$, respectively). Null alleles might cause such patterns, yet, as we did not detect null allele homozygotes at any locus, we believe that null alleles at AF86 and AF181 are at least not present at high frequencies. Instead, pooling of partially differentiated populations might have created a Wahlund effect at these loci, which readily produces significant deviations at such large sample sizes (Halkett *et al.*, 2005b). Both effects would have contributed to a globally (across loci and populations) significant F_{IS} of 0.042 ($P < 0.001$, Tab. 2).

The populations' F_{IS} values increased slightly with decreasing clonal diversity, yet, this relationship was not significant (Pearson's $r = -0.122$, $P = 0.664$), nor was the relationship between F_{IS} and the number of FDs over the previous decade as an indicator of winter severity ($r = 0.199$, $P = 0.430$).

Genetic population structure

The genetic relationships among 18 European populations of *A. f. fabae* based on pairwise F_{ST} are detailed in Table 3. F_{ST} values revealed little genetic structure, with only 11

out of 153 pairwise comparisons exceeding 0.1 (all affecting the populations of *Cam* and *Zu2*), while 110 were below 0.05 (Table 3). Although generally weak, population differentiation was significant for each locus and overall (global $F_{ST} = 0.05$; $P < 0.001$, Tab. 2). The degree of population differentiation was not simply a function of geographic separation, because IBD was not significant (Mantel test, $P = 0.23$). IBD remained non-significant when the four Southern European populations with major contributions of asexuals were excluded ($P = 0.322$; overall $F_{ST} = 0.047$), and when only the 2006 samples were analyzed separately ($P = 0.15$; overall $F_{ST} = 0.029$). Clustering of the Southern European populations is indicated, yet differentiation among them is relatively large compared to the more homogenous group including most Northern and Central European populations (Table 3). Swiss populations also exhibit apparent genetic similarity, yet it is intriguing that the three populations collected around Zurich, which are in closest physical proximity, exhibit rather strong differentiation. Their differentiation might have a temporal component, however, because they were not all sampled in the same year (Table 1).

FCA, as an alternative way to illustrate population structure, placed the two populations from Southern France, which invest very little in sexual reproduction, separate from a rather dense cluster comprising most other populations (Fig. 4). However, this could not be attributed to private alleles that would be indicative of mutation accumulation, because these populations together harbored only a single private allele. In contrast, the Dutch population, which was not assessed in the life cycle experiment, possessed several private alleles in a comparatively small sample, which is why it was also projected distant from the main cluster by FCA (Fig. 4).

Relationship among life cycle strategies

Superimposing the information on the aphid lines' production of males and winged females under SD conditions as a rough proxy of investment in sexual reproduction on a NJ

tree based on microsatellite allele shared distance (D_{AS}) of their genotypes revealed no clear pattern (Fig. 5). Genotypes with zero or very low investment in sexual reproduction under simulated autumn conditions (obligate asexuals) were scattered throughout the tree. Accordingly, there was no evidence that lines with similar genotypes showed similar investment in the production of sexual morphs (Mantel test, $r = -0.011$, $P = 0.328$). Together this suggests the recent and recurrent emergence of obligate asexuals and frequent gene flow among different LCSs that appears to be mediated by intermediate lineages, thus contributing to the genotypic diversity of obligate asexuals.

Host associated genetic differentiation

The AMOVA revealed that geographic origin explained 4.56 % of the variance in allele frequencies, while host (*V. faba* vs Chenopodiaceae) nested within site only explained 0.64 %, which was not significant (site: VC = 0.109, df = 17, $P < 0.001$; host: VC = 0.015, df = 13, $P = 0.110$, within host: VC = 2.260, df = 1155, $P < 0.001$). Pairwise F_{ST} between samples from *V. faba* and chenopods at the same site were generally low and non-significant except for site *Tic* ($F_{ST} = 0.021$, $P = 0.018$). An overall test of significance using Fisher's method of combining probabilities also provided no evidence for host associated differentiation ($\chi^2 = 34.63$; df = 26, $P = 0.12$).

Bayesian cluster analyses

The distribution of the log-likelihoods for the number of genetic clusters (K) estimated by STRUCTURE was very shallow. Values were higher at the lower end ($K < 7$) and decreased towards $K = 18$. Estimation of ΔK clearly supported $K = 2$ as the most likely number of genetic clusters, but note that the support for $K = 1$ cannot be assessed because estimates of ΔK for $K = 1$ are not possible (Evanno *et al.*, 2005). However, under $K = 2$, the relative contribution of both clusters to individual genomes, q , was not bimodal. Taking an

arbitrary threshold of $q = 0.9$, only 6 % of all genotypes could be confidently assigned to one of the two clusters. The most parsimonious interpretation of these analyses is thus that there is no clear genetic subdivision in European *A. f. fabae*.

Discussion

Geographic variation in life cycles and genotypic diversity

Here we reported the first large-scale assessment of geographic variation in the life-cycle of *A. f. fabae*. This variation is substantial and it is related to climatic conditions. Under simulated autumn conditions, aphids from areas with severe winters invested more in sexual reproduction than aphids from areas with mild winters, which mostly continued to reproduce by parthenogenesis. This is consistent with model predictions (Rispe & Pierre, 1998; Rispe *et al.*, 1998) and parallels findings from other aphid species such as *R. padi* (Delmotte *et al.*, 2002; Gilabert *et al.*, 2009), *S. avenae* (Dedryver *et al.*, 2001; Papura *et al.*, 2003), *M. persicae* (Guillemaud *et al.*, 2003) or *A. pisum* (Kanbe & Akimoto, 2009). These kinds of studies are very valuable in conjunction with the development of genomic tools to investigate the genetic basis of life-cycle differences (Le Trionnaire *et al.*, 2008), which will allow to infer whether similar genetic changes underly these transitions in different species. However, we cannot exclude that the availability of primary host plants also influenced the distribution of life-cycle variation in *A. f. fabae*. Spindle trees occurred at all sampling locations, but the Mediterranean sites were closer to the southern edge of the tree's distribution, which reaches the southern Pyrenees, southern Italy and the southern Balkans (Meusel *et al.*, 1978). If the abundance of spindle trees was lower at our Mediterranean sampling locations, this could also have favored a reduced investment in sexual reproduction. Note that also under this mechanism, the geographic distribution of reproductive modes may respond to climatic changes, if these result in altered distributions of primary hosts. In *A. fabae*, the geographic

differences in life-cycles occur in a population that is characterised by very weak genetic structure (global $F_{ST} = 0.05$), suggesting that selection for or against parthenogenetic overwintering can be sufficiently strong to counteract substantial gene flow.

The geographic life-cycle variation in *A. f. fabae* also translated into a gradient of genotypic diversity, which was lower in sites where aphids tend to overwinter parthenogenetically. However, compared to other aphid species, *A. f. fabae* seems to exhibit high rates of sexual reproduction. Apart from the populations along the Mediterranean coast, the genetic composition of most populations was essentially indistinguishable from that of purely sexual populations (Halkett *et al.*, 2005b). This is different in other species such as *M. persicae*, *S. avenae* or *R. padi*, which show a clear genetic signature of long-term clonal reproduction (low genotypic diversity, widespread distribution of common clones) even in areas characterised by a generally mild oceanic climate like Scotland or northern France (Delmotte *et al.*, 2002; Fenton *et al.*, 1998; Papura *et al.*, 2003). It remains to be investigated whether this difference is a result of (i) the ubiquitous availability of *A. fabae*'s main primary host, spindle, (ii) a lack of suitable secondary host plants that persist through winter, which may thus constrain long-term persistence of asexual lineages (see Frantz *et al.*, 2006b), or (iii) a lower tolerance of cold temperatures compared to other aphid species. Possibly, our results were also influenced by the fact that in the winter preceding our sampling (2005/2006), higher numbers of frost days occurred in several regions compared to the last decade's average (Dedryver *et al.*, 2008; Gilabert *et al.*, 2009). Interannual variation in winter temperatures was shown to have detectable effects on genotypic composition the grain aphid, *Sitobion avenae* (Llewellyn *et al.*, 2003), suggesting that climate-driven gradients in clonal diversity can build up or decay relatively rapidly.

We were surprised on the other hand, that even in areas characterised by a continental climate with cold winters, e.g. Budweis in the Czech Republic, a substantial fraction of genotypes exhibited an intermediate strategy and continued to produce parthenogenetic

females under SD conditions. Thus, although the genetic structure indicates predominantly sexual reproduction in autumn, such populations retain the potential to overwinter parthenogenetically and could thus respond rapidly to a change in climatic conditions. We have no evidence at present that descendants of overwintering parthenogens contribute to the summer populations at such sites (only unique genotypes were detected in locations with samples from more than one year). Nevertheless, the experimental data suggest that this may occur once a mild winter permits parthenogenetic overwintering. The large proportions of intermediates in *A. f. fabae* in continental areas contrast with other aphid species, for which an abundance of intermediates is found under mild and unpredictable climate, where this allows periodical persistence through winter (Delmotte *et al.*, 2001; Halkett *et al.*, 2006, 2008; Llewellyn *et al.*, 2003; Rispe *et al.*, 1998), but apparently not in continental populations (Dedryver *et al.*, 2001; Papura *et al.*, 2003).

Genetic relationships among life cycle strategies.

Conspecific populations with different life cycle strategies like obligate and cyclical parthenogenesis may show clear genetic differentiation as observed in French *R. padi* (Delmotte *et al.*, 2001), or they may be genetically similar as observed in Australian *M. persicae* (Vorburger *et al.*, 2003). The difference depends on the mechanisms of transition to obligate parthenogenesis. Differentiation is expected if obligately parthenogenetic lineages are descendants of an ancient transition to asexuality, or if the loss of sex is a consequence of hybridization. Both mechanisms have been inferred to occur in *R. padi* (Delmotte *et al.*, 2001, 2003). No or very limited differentiation is expected under the recurrent formation of parthenogenetic lineages that are derived from the sexual population. This is a well-known phenomenon in aphids, and it occurs because long-term parthenogens frequently retain the ability to produce sexual morphs (see Introduction). Matings between males produced by long-term parthenogens and females of cyclical parthenogens give rise to new genetically

unique asexual lines. This mechanism has been inferred as the main source of asexuals in *M. persicae* (Vorburger *et al.*, 2003) and *S. avenae* (Simon *et al.*, 1999), and our data here suggest that the same is true for *A. f. fabae*. Genotypes with different life-cycles were closely related and wildly interspersed on the microsatellite NJ tree (Fig. 5). This pattern is expected if transitions were recent and frequent, as would be the case under contagious parthenogenesis.

Interestingly, a very high frequency of genotypes that were classified as having an intermediate life-cycle (Fig. 2), i.e. genotypes that produce asexual females, sexual females and males under SD conditions. This life-cycle type can be interpreted as a bet-hedging strategy (Slatkin, 1974) that offers many advantages under conditions of unpredictable winter severity. Although bet hedgers are known from several other aphids with mixed cyclical and obligate parthenogenesis, androcyclic genotypes tend to be more common, i.e. genotypes that produce only asexual females and males (Dedryver *et al.*, 1998; Halkett *et al.*, 2006, 2008; Vorburger *et al.*, 2003). Because gynoparae deriving from intermediate lineages have been shown to be generally fertile (Dedryver *et al.*, 1998; Hullé *et al.*, 1999), they are also expected to transmit parthenogenesis-inducing alleles to their offspring. Androcyclic or intermediate genotypes can contribute a substantial proportion of the sexual morphs at the end of the growing season (Halkett *et al.*, 2005a, 2006), as well as to the pool of sexually produced offspring (Halkett *et al.*, 2008). This ensures that alleles inducing parthenogenetic overwintering are present in the pool of overwintering eggs and are thus retained in the population even after severe winters, when most individuals overwintering parthenogenetically are killed without leaving any descendants. Model simulations indeed suggest that the coexistence among divergent life cycle strategies is possible across wide geographic ranges if occasionally produced sexual morphs of facultative asexuals contribute to sexual reproduction (Rispe & Pierre, 1998). Further, these models indicate that evolutionary consequences might differ, depending on whether facultative asexuals invest in

males only or in males and gynoparae. The latter scenario could offer even broader ranges of coexistence among life cycle strategies at the expense of holocyclic lineages with a complete switch to sexual reproduction (Rispe & Pierre, 1998), exactly what we observed for *A. f. fabae* compared to other aphid species. These models do not even take into account that females might be more efficient in establishing gene flow among reproductive modes than males (Britton & Mogie, 2001).

Although the reduced genotypic diversity provided evidence for parthenogenetic overwintering in the southernmost populations of our study, we did not detect any evidence for other population genetic consequences predicted for clonal reproduction, e.g. increased heterozygosity (Balloux *et al.*, 2003; de Meeûs *et al.*, 2006; Halkett *et al.*, 2005b), which has been observed in other aphid species (Delmotte *et al.*, 2002; Halkett *et al.*, 2008; Kanbe & Akimoto, 2009; Simon *et al.*, 1999; Vorburger *et al.*, 2003). This is not surprising, however, because such effects become evident only in populations that are almost completely clonal (Balloux *et al.*, 2003). For *A. f. fabae*, this does not seem to be the case in any of the sites we investigated.

Host plant associations

Gene flow in aphids may be restricted due to host specialization (Hales *et al.*, 1997), which appears to evolve readily in species without host alternation, as exemplified by host race formation in the pea aphid (Frantz *et al.*, 2006a; Via, 1999) or *S. avenae* (Vialatte *et al.*, 2005). In host-alternating species such as *A. fabae*, the evolution of specialization on different secondary host plants is expected to be hampered by interbreeding on the common primary host (*E. europaeus*). Despite that, host specialization has played an important role in the evolution of *A. fabae*. It resulted in the formation of races or ecotypes that use different secondary hosts (Iglisch, 1968; Müller, 1982), exhibit genetic differentiation at allozyme loci (Jörg & Lampel, 1996), and are therefore treated as subspecies in the current aphid taxonomy.

Only one of the described subspecies, *A. f. mordvilkoii*, also differs in primary host use in that it uses *Viburnum opulus* or *Philadelphus coronarius* rather than *E. europaeus* (Blackman & Eastop, 2007). How host specialisation is maintained despite the potential for gene flow on the shared primary host is still unclear. Possible explanations include assortative mating (Thieme & Dixon, 1996) or host-related inversion polymorphisms (Feder *et al.*, 2003). Given the obvious role played by host specialisation in the evolution of the *A. fabae* complex, it is feasible that host associated differentiation (HAD) might also be present between aphids of the same subspecies using different, unrelated secondary host plants. We tested this for the two main groups of secondary hosts of *A. f. fabae*, broad beans and chenopods, yet we detected no evidence for HAD, indicating that aphids from these plants belong to a single gene pool. This is supported by findings of Mackenzie (1996) who did not find differences in fecundity between clones from either broad beans or *Ch. album* cultured on broad beans, and our own observations that black bean aphids from chenopods readily establish on broad bean in the laboratory.

Assuming that *A. fabae* subspecies are genetically divergent (Jörg & Lampel, 1996), we expect that between-subspecies hybrids (Iglisch, 1968; Müller, 1982; Thieme, 1987) would have been readily detected by our genetic cluster analyses. The lack of evidence for admixture from other genetic clusters thus suggests that (i) hybridization among subspecies is strongly restricted (Raymond *et al.*, 2001; Thieme & Dixon, 1996) or (ii) that occasional hybrids are unfit. This inference is only valid, however, if the genetic differentiation among subspecies observed at allozyme loci (Jörg & Lampel, 1996) also pertains to the microsatellites. Because allozyme patterns in aphids show peculiar patterns and may be influenced by the presence of endosymbionts (Hales *et al.*, 1997), this is uncertain and remains to be tested (see Gauffre & Coeur d'Acier, 2006).

Conclusions

Aphid pests have recently been highlighted as models to assess the impact of global environmental changes due to their rapid response to climate variation (Gilabert *et al.*, 2009). Considering that climate change prognoses foresee a continued increase in temperatures and especially milder winters (Walther *et al.*, 2002), we predict an overall increase of proportions of obligate asexual lineages for *A. fabae* and a northward expansion of asexual genotypes in Europe. Although we conducted only a single monitoring, which does not account for spatial and temporal fluctuations known from comparable aphid systems (Llewellyn *et al.*, 2003), the large geographic range here investigated provides a sound basis to infer such trends. Our data indicate that asexuals will increase wherever winter climate becomes permissive of viviparous overwintering. This can have important consequences for agriculture because it will entail a shift in how natural and human-imposed selection acts. Under cyclical parthenogenesis, clonal genotypes are broken up by recombination once per year. Selection in the long term will thus act on the additive effect of alleles expressed across different genetic backgrounds. Under obligate parthenogenesis the clonal genotypes (also expressing non-additive genetic effects) become the units of selection. Clonal selection is a very powerful force. This is seen in aphid populations in the field in which clonal frequencies can undergo rapid changes (Loxdale *et al.*, 2011; Vorburger, 2006), or in experimental populations showing rapid erosion of clonal diversity under strong directional selection (Herzog *et al.*, 2007). Present agricultural land use practices do result in relatively monotonous and strongly selective environments (Gilabert *et al.*, 2009) and have been proposed to be a major driver in favoring asexuality in insect pests (Hoffmann *et al.*, 2008). Indeed, highly abundant ‘superclones’ are observed in a number of pest aphids and illustrate that genetic diversity might be dramatically reduced in clonal pests inhabiting human-altered landscapes (Brevault *et al.*, 2008; Charaabi *et al.*, 2008; Fenton *et al.*, 1998; Gilabert *et al.*, 2009; Haack *et al.*, 2000; Llewellyn *et al.*, 2004; Vorburger *et al.*, 2003). This may cause major challenges for pest control because it allows, for example, for the rapid spread of clones with multiple pesticide resistance (Carletto *et al.*, 2010; Fenton *et*

al., 2010). Also, we are largely unaware of the effects that reduced genotypic diversity in a numerically important food web component like the aphids would have on the entire ecosystem.

Acknowledgements

We thank Alexandre Gouskov, Søren Banke and Sandra Röthlisberger for laboratory and field assistance and Heinz-Ulrich Reyer for providing research facilities. Bernard Chaubet and Jean-Christophe Simon helped with field collections in France. A fellowship from the Iranian Ministry of Science funded J. Razmjou's research stay in C. Vorburger's lab. We are grateful to Thierry de Meeûs and Thomas Thieme for helpful suggestions. This work was supported by the Swiss National Science Foundation.

Supplementary online material

Table S1. Results of the experimental determination of reproductive modes.

Author information box

This study is part of the PhD thesis of C. Sandroock, which focused on the population genetics, the evolution of alternative reproductive modes and genetic interactions in an aphid-hymenopteran host-parasitoid system. This work was supervised by C. Vorburger, who is interested in the coevolution of aphid parasitoids with their hosts and the endosymbiotic defense mechanisms they employ. J. Razmjou is an agricultural entomologist working in the field of integrated pest management.

References

- Arnaud-Haond S, Belkhir K (2007) GENCLONE: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes* **7**, 15-17.
- Arnaud-Haond S, Duarte CM, Alberto F, Serrao EA (2007) Standardizing methods to address clonality in population studies. *Molecular Ecology* **16**, 5115-5139.
- Balloux F, Lehmann L, de Meeûs T (2003) The population genetics of clonal and partially clonal diploids. *Genetics* **164**, 1635-1644.
- Barton NH, Charlesworth B (1998) Why sex and recombination? *Science* **281**, 1986-1990.
- Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996-2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations (Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France)).
- Bell G (1982) *The Masterpiece of Nature: the Evolution and Genetics of Sexuality* University of California Press, Berkeley.
- Birky CW (1996) Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. *Genetics* **144**, 427-437.
- Blackman RL (1972) The inheritance of life-cycle differences in *Myzus persicae* (Sulz.) (Hem., Aphididae). *Bulletin of Entomological Research* **62**, 281-294.
- Blackman RL (1974) Life-cycle variation of *Myzus persicae* (Sulz.) (Hem., Aphididae) in different parts of the world. *Bulletin of Entomological Research* **63**, 595-607.
- Blackman RL, Eastop VF (2000) *Aphids on the World's Crops: An Identification and Information Guide*, 2nd edn. John Wiley and Sons, Chichester.
- Blackman RL, Eastop VF (2007) Taxonomic issues. In: *Aphids as Crop Pests* eds. Van Emden H, Harrington R). CAB International, Wallingford UK.
- Bonnet P, van de Peer Y (2002) zt: a software tool for simple and partial Mantel tests. *Journal of Statistical Software* **7**, 1-12.
- Brevault T, Carletto J, Linderme D, Vanlerberghe-Masutti F (2008) Genetic diversity of the cotton aphid *Aphis gossypii* in the unstable environment of a cotton growing area. *Agricultural and Forest Entomology* **10**, 215-223.
- Britton NF, Mogie M (2001) Poor male function favours the coexistence of sexual and asexual relatives. *Ecology Letters* **4**, 116-121.
- Carletto J, Martin T, Vanlerberghe-Masutti F, Brevault T (2010) Insecticide resistance traits differ among and within host races in *Aphis gossypii*. *Pest Management Science* **66**, 301-307.
- Chakraborty R, Jin L (1993) A unified approach to the study of hypervariable polymorphisms: statistical considerations of determining relatedness and population distances. In: *DNA Fingerprinting: State of the Science* (eds. Pena SDJ, Chakraborty R, Epplen JT, Jeffreys AJ), pp. 153-175. Birkhäuser, Basel, Switzerland.
- Charaabi K, Carletto J, Chavigny P, et al. (2008) Genotypic diversity of the cotton-melon aphid *Aphis gossypii* (Glover) in Tunisia is structured by host plants. *Bulletin of Entomological Research* **98**, 333-341.
- Coeur d'Acier A, Sembene M, Audiot P, Rasplus JY (2004) Polymorphic microsatellites loci in the black Aphid, *Aphis fabae* Scopoli, 1763 (Hemiptera, Aphididae). *Molecular Ecology Notes* **4**, 306-308.
- de Meeûs T, Lehmann L, Balloux F (2006) Molecular epidemiology of clonal diploids: A quick overview and a short DIY (do it yourself) notice. *Infection Genetics and Evolution* **6**, 163-170.

- de Meeûs T, McCoy KD, Prugnolle F, *et al.* (2007) Population genetics and molecular epidemiology or how to "débusquer la bête". *Infection Genetics and Evolution* **7**, 308-332.
- Dedryver CA, Hullé M, Le Gallic JF, Caillaud MC, Simon JC (2001) Coexistence in space and time of sexual and asexual populations of the cereal aphid *Sitobion avenae*. *Oecologia* **128**, 379-388.
- Dedryver CA, Le Gallic JF, Gauthier JP, Simon JC (1998) Life cycle of the cereal aphid *Sitobion avenae* F.: polymorphism and comparison of life history traits associated with sexuality. *Ecological Entomology* **23**, 123-132.
- Dedryver CA, Le Gallic JF, Haack L, *et al.* (2008) Seasonal and annual genotypic variation and the effect of climate on population genetic structure of the cereal aphid *Sitobion avenae* in northern France. *Bulletin of Entomological Research* **98**, 159-168.
- Delmotte F, Leterme N, Bonhomme J, Rispe C, Simon JC (2001) Multiple routes to asexuality in an aphid species. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 2291-2299.
- Delmotte F, Leterme N, Gauthier JP, Rispe C, Simon JC (2002) Genetic architecture of sexual and asexual populations of the aphid *Rhopalosiphum padi* based on allozyme and microsatellite markers. *Molecular Ecology* **11**, 711-723.
- Delmotte F, Sabater B, Leterme N, *et al.* (2003) Phylogenetic evidence for hybrid origins of asexual lineages in an aphid species. *Evolution* **57**, 1291-1303.
- Dixon AFG (1998) *Aphid Ecology*, 2nd edn. Chapman & Hall, London.
- Doncaster CP, E. PG, Cox SJ (2000) The ecological cost of sex. *Nature* **404**, 281-285.
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611-2620.
- Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, 47-50.
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* **164**, 1567-1587.
- Feder JL, Berlocher SH, Roethele JB, *et al.* (2003) Allopatric genetic origins for sympatric host-plant shifts and race formation in Rhagoletis. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 10314-10319.
- Fenton B, Margaritopoulos JT, Malloch GL, Foster SP (2010) Micro-evolutionary change in relation to insecticide resistance in the peach-potato aphid, *Myzus persicae*. *Ecological Entomology* **35**, 131-146.
- Fenton B, Woodford JAT, Malloch G (1998) Analysis of clonal diversity of the peach-potato aphid, *Myzus persicae* (Sulzer), in Scotland, UK and evidence for the existence of a predominant clone. *Molecular Ecology* **7**, 1475-1487.
- Frantz A, Plantegenest M, Mieuze L, Simon JC (2006a) Ecological specialization correlates with genotypic differentiation in sympatric host-populations of the pea aphid. *Journal of Evolutionary Biology* **19**, 392-401.
- Frantz A, Plantegenest M, Simon JC (2006b) Temporal habitat variability and the maintenance of sex in host populations of the pea aphid. *Proceedings of the Royal Society B-Biological Sciences* **273**, 2887-2891.
- Gauffre B, Coeur d'Acier A (2006) New polymorphic microsatellite loci, cross-species amplification and PCR multiplexing in the black aphid, *Aphis fabae* Scopoli. *Molecular Ecology Notes* **6**, 440-442.
- Gilabert A, Simon JC, Mieuze L, *et al.* (2009) Climate and agricultural context shape reproductive mode variation in an aphid crop pest. *Molecular Ecology* **18**, 3050-3061.
- Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from <http://www.unil.ch/izea/software/fstat.html>. Updated from Goudet (1995).

- Guillemaud T, Mieuze L, Simon JC (2003) Spatial and temporal genetic variability in French populations of the peach-potato aphid, *Myzus persicae*. *Heredity* **91**, 143-152.
- Haack L, Simon JC, Gauthier JP, Plantegenest M, Dedryver CA (2000) Evidence for predominant clones in a cyclically parthenogenetic organism provided by combined demographic and genetic analyses. *Molecular Ecology* **9**, 2055-2066.
- Hales DF, Tomiuk J, Wöhrmann K, Sunnucks P (1997) Evolutionary and genetic aspects of aphid biology: A review. *European Journal of Entomology* **94**, 1-55.
- Halkett F, Kindlmann P, Plantegenest M, Sunnucks P, Simon JC (2006) Temporal differentiation and spatial coexistence of sexual and facultative asexual lineages of an aphid species at mating sites. *Journal of Evolutionary Biology* **19**, 809-815.
- Halkett F, Plantegenest M, Bonhomme J, Simon JC (2008) Gene flow between sexual and facultatively asexual lineages of an aphid species and the maintenance of reproductive mode variation. *Molecular Ecology* **17**, 2998-3007.
- Halkett F, Plantegenest M, Prunier-Leterme N, *et al.* (2005a) Admixed sexual and facultatively asexual aphid lineages at mating sites. *Molecular Ecology* **14**, 325-336.
- Halkett F, Simon JC, Balloux F (2005b) Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology & Evolution* **20**, 194-201.
- Hamilton WD (1980) Sex versus non-sex versus parasite. *Oikos* **35**, 282-290.
- Hardie J (1980) Reproductive, Morphological and Behavioral Affinities between the Alate Gynopara and Virginopara of the Aphid, *Aphis fabae*. *Physiological Entomology* **5**, 385-396.
- Hardie J, Vaz Nunes M (2001) Aphid photoperiodic clocks. *Journal of Insect Physiology* **47**, 821-832.
- Herzog J, Müller CB, Vorburger C (2007) Strong parasitoid-mediated selection in experimental populations of aphids. *Biology Letters* **3**, 667-669.
- Hoffmann AA, Reynolds KT, Nash MA, Weeks AR (2008) A high incidence of parthenogenesis in agricultural pests. *Proceedings of the Royal Society B-Biological Sciences* **275**, 2473-2481.
- Hullé M, Maurice D, Rispe C, Simon JC (1999) Clonal variability in sequences of morph production during the transition from parthenogenetic to sexual reproduction in the aphid *Rhopalosiphum padi* (Sternorrhyncha : Aphididae). *European Journal of Entomology* **96**, 125-134.
- Iglisch I (1968) Über die Entstehung der Rassen der "Schwarzen Bohnenläuse" (*Aphis fabae* Scop. und verwandte Arten), über ihre phytopathologische Bedeutung und über die Aussichten für erfolgversprechende Bekämpfungsmassnahmen (Homoptera: Aphididae). *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* **131**, 1-34.
- Innes DJ, Hebert PDN (1988) The origin and genetic basis of obligate parthenogenesis in *Daphnia pulex*. *Evolution* **42**, 1024-1035.
- Jörg E, Lampel G (1996) Enzyme electrophoretic studies on the *Aphis fabae* group (Hom., Aphididae). *Journal of Applied Entomology*, 7-18.
- Kanbe T, Akimoto SI (2009) Allelic and genotypic diversity in long-term asexual populations of the pea aphid, *Acyrtosiphon pisum* in comparison with sexual populations. *Molecular Ecology* **18**, 801-816.
- Kondrashov AS (1988) Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**, 435-440.
- Langella O (1999) Populations 1.2.30. Available at <http://bioinformatics.org/~tryphon/populations/>.
- Le Trionnaire G, Hardie J, Jaubert-Possamai S, Simon JC, Tagu D (2008) Shifting from clonal to sexual reproduction in aphids: physiological and developmental aspects. *Biology of the Cell* **100**, 441-451.

- 815 Llewellyn KS, Loxdale HD, Harrington R, *et al.* (2003) Migration and genetic structure of the
 816 grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as
 817 revealed using microsatellites. *Molecular Ecology* **12**, 21-34.
- 818 Llewellyn KS, Loxdale HD, Harrington R, Clark SJ, Sunnucks P (2004) Evidence for gene
 819 flow and local clonal selection in field populations of the grain aphid (*Sitobion*
 820 *avenae*) in Britain revealed using microsatellites. *Heredity* **93**, 143-153.
- 821 Loxdale HD, Hardie J, Halbert S, *et al.* (1993) The relative importance of short-range and
 822 long-range movement of flying aphids. *Biological Reviews of the Cambridge*
 823 *Philosophical Society* **68**, 291-311.
- 824 Loxdale HD, Lushai G (2003) Rapid changes in clonal lines: the death of a 'sacred cow'.
 825 *Biological Journal of the Linnean Society* **79**, 3-16.
- 826 Loxdale HD, Massonnet B, Schöfl G, Weisser W (2011) Evidence for a quiet revolution:
 827 seasonal variation in colonies of the specialist tansy aphid, *Macrosiphonella*
 828 *tanacetaria* (Kaltenbach) (Homoptera: Aphididae) studied using microsatellite
 829 markers. *Bull. Entomol. Res.* **101**, 221-239.
- 830 Lynch M (1984) Destabilizing hybridization, general-purpose genotypes, and geographic
 831 parthenogenesis. *Quarterly Review of Biology* **59**, 257-290.
- 832 Mackenzie A (1996) A trade-off for host plant utilization in the black bean aphid, *Aphis*
 833 *fabae*. *Evolution* **50**, 155-162.
- 834 Massonnet B, Simon JC, Weisser WW (2002) Metapopulation structure of the specialized
 835 herbivore *Macrosiphoniella tanacetaria* (Homoptera, Aphididae). *Molecular Ecology*
 836 **11**, 2511-2521.
- 837 Maynard Smith J (1978) *The Evolution of Sex* Cambridge University Press, Cambridge.
- 838 Maynard Smith J (1988) The evolution of recombination. In: *The Evolution of Sex: an*
 839 *Examination of Current Ideas* (eds. Michod RE, Levin BR), pp. 106-125. Sinauer,
 840 Sunderland, MA.
- 841 Meusel H, Jäger E, Rauschert S, Weinert E (1978) Vergleichende Chronologie der
 842 zentraleuropäischen Flora. Karten-Band II. Fischer Verlag, Jena.
- 843 Moran NA (1992) The evolution of aphid life cycles. *Annual Review of Entomology* **37**, 321-
 844 348.
- 845 Müller FP (1982) Das Problem *Aphis fabae*. *Zeitschrift für angewandte Entomologie* **94**, 432-
 846 446.
- 847 Paland S, Colbourne JK, Lynch M (2005) Evolutionary history of contagious asexuality in
 848 *Daphnia pulex*. *Evolution* **59**, 800-813.
- 849 Papura D, Simon JC, Halkett F, *et al.* (2003) Predominance of sexual reproduction in,
 850 Romanian populations of the aphid *Sitobion avenae* inferred from phenotypic and
 851 genetic structure. *Heredity* **90**, 397-404.
- 852 Peck JR (1994) A ruby in the rubbish - beneficial mutations, deleterious mutations and the
 853 evolution of sex. *Genetics* **137**, 597-606.
- 854 Peck JR, Yearsley JM, Waxman D (1998) Explaining the geographic distributions of sexual
 855 and asexual populations. *Nature* **391**, 889-892.
- 856 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using
 857 multilocus genotype data. *Genetics* **155**, 945-959.
- 858 Raymond B, Searle JB, Douglas AE (2001) On the processes shaping reproductive isolation
 859 in aphids of the *Aphis fabae* (Scop.) complex (Aphididae : Homoptera). *Biological*
 860 *Journal of the Linnean Society* **74**, 205-215.
- 861 Rice WR (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223-225.
- 862 Rispe C, Bonhomme J, Simon JC (1999) Extreme life-cycle and sex ratio variation among
 863 sexually produced clones of the aphid *Rhopalosiphum padi* (Homoptera : Aphididae).
 864 *Oikos* **86**, 254-264.

- 865 Rispe C, Pierre JS (1998) Coexistence between cyclical parthenogens, obligate parthenogens,
866 and intermediates in a fluctuating environment. *Journal of Theoretical Biology* **195**,
867 97-110.
- 868 Rispe C, Pierre JS, Simon JC, Gouyon PH (1998) Models of sexual and asexual coexistence
869 in aphids based on constraints. *Journal of Evolutionary Biology* **11**, 685-701.
- 870 Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under
871 isolation by distance. *Genetics* **145**, 1219-1228.
- 872 Sandrock C, Vorburger C (2011) Single-locus recessive inheritance of asexual reproduction in
873 a parasitoid wasp. *Current Biology* **21**, 433-437.
- 874 Simon JC, Baumann S, Sunnucks P, *et al.* (1999) Reproductive mode and population genetic
875 structure of the cereal aphid *Sitobion avenae* studied using phenotypic and
876 microsatellite markers. *Molecular Ecology* **8**, 531-545.
- 877 Simon JC, Delmotte F, Rispe C, Crease T (2003) Phylogenetic relationships between
878 parthenogens and their sexual relatives: the possible routes to parthenogenesis in
879 animals. *Biological Journal of the Linnean Society* **79**, 151-163.
- 880 Simon JC, Rispe C, Sunnucks P (2002) Ecology and evolution of sex in aphids. *Trends in*
881 *Ecology & Evolution* **17**, 34-39.
- 882 Slatkin M (1974) Hedging Ones Evolutionary Bets. *Nature* **250**, 704-705.
- 883 Smith MAH, Mackay PA (1990) Latitudinal variation in the photoperiodic responses of
884 populations of pea aphid (Homoptera, Aphididae). *Environmental Entomology* **19**,
885 618-624.
- 886 Sokal RR, Rohlf FJ (1995) *Biometry*, 3 edn. W.H. Freeman and Co, San Francisco USA.
- 887 Sunnucks P, De Barro PJ, Lushai G, Maclean N, Hales DF (1997) Genetic structure of an
888 aphid studied using microsatellites: cyclic parthenogenesis, differentiated lineages and
889 host specialization. *Molecular Ecology* **6**, 1059-1073.
- 890 Tank A, Wijngaard JB, Konnen GP, *et al.* (2002) Daily dataset of 20th-century surface air
891 temperature and precipitation series for the European Climate Assessment.
892 *International Journal of Climatology* **22**, 1441-1453.
- 893 Tas ICQ, Van Dijk PJ (1999) Crosses between sexual and apomictic dandelions (*Taraxacum*).
894 I. The inheritance of apomixis. *Heredity* **83**, 707-714.
- 895 Thieme T (1987) Members of the complex of *Aphis fabae* Scop. and their host plants. In:
896 *Population Structure, Genetics and Taxonomy of Aphids and Thysanoptera* eds.
897 Holmann J, Pelikan J, Dixon AFG, Weisman L), pp. 314-323. SPB Academic
898 Publishing, The Hague.
- 899 Thieme T (1988) On the Biology of *Aphis fabae mordwilkwowi* Börner and Janisch, 1922
900 (Hom, Aphididae). *Journal of Applied Entomology-Zeitschrift für angewandte*
901 *Entomologie* **105**, 510-515.
- 902 Thieme T, Dixon AFG (1996) Mate recognition in the *Aphis fabae* complex: Daily rhythm of
903 release and specificity of sex pheromones. *Entomologia Experimentalis Et Applicata*
904 **79**, 85-89.
- 905 Vandel A (1928) La parthénogenèse géographique. Contribution à l'étude biologique et
906 cytologique de la parthénogenèse naturelle. *Bulletin Biologique de la France et de la*
907 *Belgique* **62**, 164-281.
- 908 Vaz Nunes M, Hardie J (2000) On critical night lengths and temperature compensation in the
909 photoperiodic response of two geographical clones of the black bean aphid, *Aphis*
910 *fabae*. *Physiological Entomology* **25**, 303-308.
- 911 Via S (1999) Reproductive isolation between sympatric races of pea aphids. I. Gene flow
912 restriction and habitat choice. *Evolution* **53**, 1446-1457.
- 913 Vialatte A, Dedryver CA, Simon JC, Galman M, Plantegenest M (2005) Limited genetic
914 exchanges between populations of an insect pest living on uncultivated and related

- 915 cultivated host plants. *Proceedings of the Royal Society B-Biological Sciences* **272**,
916 1075-1082.
- 917 Vorburger C (2006) Temporal dynamics of genotypic diversity reveal strong clonal selection
918 in the aphid *Myzus persicae*. *Journal of Evolutionary Biology* **19**, 97-107.
- 919 Vorburger C, Lancaster M, Sunnucks P (2003) Environmentally related patterns of
920 reproductive modes in the aphid *Myzus persicae* and the predominance of two
921 'superclones' in Victoria, Australia. *Molecular Ecology* **12**, 3493-3504.
- 922 Walsh PS, Metzgar DA, Higuchi R (1991) ChelexE 100 as a medium for the simple
923 extraction of DNA for PCR based typing from forensic material. *Biotechniques*, 91-
924 98.
- 925 Walther GR, Post E, Convey P, *et al.* (2002) Ecological responses to recent climate change.
926 *Nature* **416**, 389-395.
- 927 Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population
928 structure. *Evolution* **38**, 1358-1370.
- 929 Williams GC (1975) *Sex and Evolution* Princeton University Press, Princeton.
- 930 Wöhrmann K, Tomiuk J (1988) Life-cycle strategies and genotypic variability in populations
931 of aphids. *Journal of Genetics* **67**, 43-52.
- 932
- 933

934 **Data accessibility**

935

936 Sampling details and microsatellite data: DRYAD entry: doi: 10.5061/dryad.2d1b8

937 **Table 1** Summarised sampling details for 18 European populations of *Aphis fabae fabae*. Population-specific indices address clonal diversity (R),
 938 inbreeding coefficient (F_{IS}) and the sampling area's mean yearly number of frost days over the last decade (FD). The asterisk indicates a significant
 939 difference from zero. Life cycle strategies (LCS) were experimentally assayed in a random subsample of clones from 15 populations (total numbers
 940 are indicated). ^a Two additional aphid samples collected from *Rumex sp.*

Sample site (region and country)	Population tag	Coordinates	Collection date	Samples from <i>V. faba</i>	Samples from chenopods	Total sample	Unique genotypes	R	F_{IS}	FD	LCS
Sarzana (Toscana, I)	<i>Tos</i>	44°06' N, 9°55' E	08.05.2006	23	-	25 ^a	14	0.54	0.130	13.2	12
Cesena (Emilia-Romagna, I)	<i>Rom</i>	44°07' N, 12°15' E	08.05.2006	18	4	22	14	0.62	-0.019	29.6	12
Lunel (Camargue, F)	<i>Cam</i>	43°40' N, 4°08' E	16./17.05.2006	-	25	25	12	0.46	0.060	21.4	3
Grimaud (Côte d'Azur, F)	<i>Cot</i>	43°16' N, 6°31' E	17./18.05.2006	9	15	24	13	0.52	-0.009	1.1	7
Martigny (Valais, CH)	<i>Val</i>	46°08' N, 7°06' E	30.05.2006	10	26	36	36	1.00	0.010	89.1	12
Sant'Antonino (Ticino, CH)	<i>Tic</i>	46°08' N, 8°56' E	07.06.2006	16	16	32	32	1.00	-0.062	26.6	11
Bülach (Zurich-northern, CH)	<i>Zu1</i>	47°32' N, 8°32' E	10.06.2006 & 19.07.2007	-	90	90	90	1.00	0.087	70.0	6
Altstetten (Zurich-City, CH)	<i>Zu2</i>	47°22' N, 8°29' E	13./29.05.2008	93	16	109	109	1.00	0.015	70.0	-
Wildberg (Zurich-eastern, CH)	<i>Zu3</i>	47°25' N, 8°49' E	24.05.2007 & 12.06.2008	24	2	26	26	1.00	0.019	70.0	-
Rennes (Brittany, F)	<i>Bri</i>	48°07' N, 1°45' E	15.06.2006	32	4	36	29	0.80	-0.001	28.6	12
Chur (Grisons, CH)	<i>Gri</i>	46°52' N, 9°32' E	12.06.2006	3	13	16	16	1.00	0.068	65.1	6
Budweis (Budweis-area, CZ)	<i>Bud</i>	48°54' N, 14°29' E	20./21.06.2006	23	30	53	48	0.91	0.072	129.0	14
Basel (Basel-area, CH)	<i>Bas</i>	47°29' N, 7°37' E	27.06.2006	6	3	9	9	1.00	0.048	64.1	6
Gelnhausen (Hesse, D)	<i>Hes</i>	50°10' N, 9°09' E	02.07.2006 & 16.05.2008	51	18	69	69	1.00	0.048	70.0	12
Brunsbüttel (Dithmarschen, D)	<i>Dit</i>	53°55' N, 9°09' E	12.07.2006	3	34	37	32	0.87	-0.006	70.0	12
St. Margrethen (St. Gallen, CH)	<i>SGa</i>	47°27' N, 9°38' E	01.07.2006	-	9	9	7	0.75	0.408*	71.1	5
Cambridge (Cambridgeshire, UK)	<i>CUK</i>	52°13' N, 0°02' E	11.07.2006	17	8	25	25	1.00	0.068	43.6	6
Steenwijk (Holland, NL)	<i>Hol</i>	52°46' N, 6°08' E	27.04.2008	-	12	12	12	1.00	0.098	53.1	-

Table 2 Allelic diversity and *F*-statistics for eight microsatellite loci in a pooled sample of European *Aphis fabae fabae* (18 populations, one copy per multi locus genotype each). Significant values are highlighted in bold.

	Microsatellite								global
	AF-48	AF-50	AF-82	AF-85	AF-86	AF-181	AF-beta	AF-F	
Alleles	11	8	27	7	4	11	21	15	104
<i>F</i> _{IS}	0.028	-0.005	0.020	0.044	0.113	0.066	0.030	0.047	0.042
<i>F</i> _{ST}	0.045	0.054	0.034	0.098	0.029	0.045	0.023	0.067	0.050

945 **Table 3** Pairwise genetic differentiation among 18 European populations of *Aphis fabae fabae*. F_{ST} estimates are based on eight microsatellite
 946 markers (see Table 2) and single copies per multi locus genotype of each population. Population labels and sample sizes are given in Table 1.
 947 Significant estimates at a table-wide level of $\alpha = 0.05$ following sequential Bonferroni correction are highlighted in bold.

	<i>Rom</i>	<i>Cam</i>	<i>Cot</i>	<i>Val</i>	<i>Tic</i>	<i>Zu1</i>	<i>Zu2</i>	<i>Zu3</i>	<i>Bri</i>	<i>Gri</i>	<i>Bud</i>	<i>Bas</i>	<i>Hes</i>	<i>Dit</i>	<i>SGa</i>	<i>CUK</i>	<i>Hol</i>
<i>Tos</i>	0.006	0.066	-0.001	0.027	0.014	0.028	0.067	0.029	0.017	0.018	0.033	0.012	0.031	0.032	-0.001	0.024	0.021
<i>Rom</i>		0.099	0.035	0.092	0.052	0.023	0.092	0.039	0.019	0.048	0.037	0.005	0.030	0.027	-0.005	0.028	0.036
<i>Cam</i>			0.036	0.158	0.120	0.073	0.216	0.130	0.037	0.111	0.071	0.078	0.080	0.084	0.122	0.057	0.039
<i>Cot</i>				0.089	0.049	0.034	0.125	0.070	0.021	0.053	0.040	0.026	0.050	0.051	0.054	0.036	0.044
<i>Val</i>					0.018	0.061	0.069	0.042	0.079	0.015	0.059	0.052	0.055	0.074	0.027	0.070	0.061
<i>Tic</i>						0.033	0.067	0.045	0.046	-0.002	0.031	0.019	0.036	0.037	0.021	0.040	0.037
<i>Zu1</i>							0.092	0.030	0.007	0.018	0.006	0.004	0.004	0.006	0.005	0.004	0.021
<i>Zu2</i>								0.027	0.119	0.072	0.128	0.032	0.091	0.112	0.021	0.139	0.097
<i>Zu3</i>									0.046	0.030	0.048	0.007	0.019	0.042	-0.013	0.051	0.040
<i>Bri</i>										0.040	0.012	0.016	0.008	0.014	0.029	0.000	0.031
<i>Gri</i>											0.024	0.007	0.023	0.019	-0.002	0.032	0.025
<i>Bud</i>												0.024	0.004	0.007	0.022	-0.005	0.028
<i>Bas</i>													0.013	0.024	-0.028	0.030	0.001
<i>Hes</i>														0.007	0.003	0.004	0.029
<i>Dit</i>															0.004	0.002	0.025
<i>SGa</i>																0.024	0.014
<i>CUK</i>																	0.029

Figure captions

Fig. 1 European map depicting locations of sampled populations of *Aphis fabae fabae*. Corresponding pie charts represent population averages of experimentally determined morph production under simulated autumn conditions; Males (black), winged females (white), wingless parthenogenetic females (grey). Open circles indicate populations which have not been experimentally assayed. For population labels see Table 1.

Fig. 2 Simplex of experimental life cycle responses of 136 genotypes of *Aphis fabae fabae* from 15 European populations. Summits of the triangle account for 100 % production of corresponding morphs under experimental short day conditions. Open circles represent lineages from the four Mediterranean populations (*Rom*, *Tos*, *Cot* and *Cam*), filled circles show those clones from all other, more temperate regions.

Fig 3 Correlations of average proportions (arcsine square root transformed) of experimental life cycle responses of 15 European populations of *Aphis fabae fabae* with corresponding mean numbers of the last decade's frost days (a) and of observed clonal diversity (b). Filled circles & dotted line, wingless parthenogenetic females; open circles & dashed line, winged females; male symbols & solid line, males.

Fig. 4 Projection of the 'centers of gravity' of 18 European populations of *Aphis fabae fabae*, based on eight microsatellite loci, in a three dimensional space according to factorial correspondence analysis. The percentage of total inertia explained by the three first axes is shown. Replicate multi locus genotypes have been excluded in each population. For population labels see Table 1.

974 **Fig. 5** Neighbour-joining dendrogram based on allele shared distance (D_{AS} , eight
975 microsatellite loci) among 136 lineages of *Aphis fabae fabae* from 15 European populations
976 assayed in a life cycle experiment. The shading represents the lines' production of males and
977 winged females under SD conditions as a rough proxy for their investment in sexual
978 reproduction. The shading ranges from black (0-10% males and winged females) to white (90
979 - 100%) in increments of 20%. Note that in the three cases where two lines with the same
980 MLG were included in the life-cycle experiment (double symbols), the two symbols do not
981 always share the same shading but consistently indicate a low investment in sexual
982 reproduction. Bootstrap values were all below 70% and are thus not shown.

Molecular Ecology

Wingless parthenogenetic females

Molecular Ecology