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Host-associated populations in the lettuce root aphid, *Pemphigus bursarius* (L.)

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Pemphigus bursarius is a host-alternating aphid in which annual rounds of sexual reproduction on its primary host, *Populus nigra*, are interspersed with parthenogenesis on a range of secondary hosts. Evidence was sought for the existence of genetically distinct populations, associated with different secondary hosts, in *P. bursarius*. Microsatellite markers revealed that genetically distinct populations were present on three different secondary host species. Microsatellites were also used, in conjunction with mitochondrial DNA sequence variation, to investigate the relationships between aphids on *Populus*, following sexual reproduction, and those on the secondary hosts. Evidence was found for a distinct, cyclically parthenogenetic population that exploited *Lactuca sativa* as its secondary host. In contrast, populations associated with *Matricaria inodora* appeared to be largely composed of obligate parthenogens or may even have been another species of *Pemphigus*. Populations on *Lapsana communis* appeared to be a mixture of cyclical and obligate parthenogens and were more genetically heterogeneous than those on other secondary hosts, possibly due to founder effects. Experiments to measure the performance of *P. bursarius* clones on different secondary hosts were inconclusive, failing to demonstrate either the presence or absence of adaptations to secondary hosts.
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Introduction

Herbivorous insects, especially aphids, often form close ecological relationships with the plants upon which they feed. One consequence of this is that many species are structured into distinct populations associated with different host plant species within the overall host range. In a few cases there is evidence that these populations are host races that originally diverged in sympatry.

Studies of host races have revealed some of the key mechanisms that reduce gene flow between populations on different hosts. Perhaps the most important of these is host fidelity, a consistent tendency to feed and reproduce on a particular host, leading to assortative mating. Fitness tradeoffs between hosts can also promote reproductive isolation at the post-zygotic level as the hybrid offspring of host-adapted races will be maladapted to both parental hosts (Via, 2001; Drès and Mallet, 2002).

A recent study of microsatellite variation in the host-alternating aphid *Pemphigus bursarius* (L.) demonstrated substantial allele frequency differences between samples taken from its primary host, *Populus nigra*, in the spring and on *Lactuca sativa*, one of the secondary hosts, in summer at the same location (Miller *et al*, 2003). This divergence may be explained by the population on *Lactuca* being composed largely of obligate parthenogens that do not complete the sexual part of the life cycle on *Populus*. Alternatively, *P. bursarius* may be subdivided into genetically differentiated populations, one of which feeds on *Lactuca* but which co-occurs on *Populus* with other host-associated populations.

The host-alternating life cycle of *P. bursarius* is complex, involving an annual switch between sexual reproduction on the primary host and parthenogenesis on a range of secondary hosts (Dunn, 1959). Sexual reproduction takes place on the bark of poplar trees (*Populus nigra*, L.) in autumn and the resulting eggs remain in diapause through the winter. In spring, a single stem mother hatches from each egg and begins feeding on a new petiole, inducing the formation of a gall. After reaching maturity in the gall, the stem mother reproduces by viviparous parthenogenesis. The offspring of the stem mother are winged at maturity and migrate from *Populus* to a range of secondary hosts in the Compositae, including lettuce, *Lactuca sativa* (L.) (Dunn, 1959; Alleyne and Morrison, 1977), where they reproduce parthenogenetically and their offspring colonise the roots. Following several generations of wingless parthenogenetic aphids on the roots, a second winged morph emerges in the autumn. These migrate back to *Populus* where they give rise to the sexual forms. This part of the life cycle, by providing a shared breeding ground and thus increasing gene flow, will tend to prevent genetically divergent populations associated with different secondary hosts. In addition to the sexual cycle, *P. bursarius* can also pass through the winter parthenogenetically via a specialised cold-tolerant morph that remains in the soil (Phillips *et al*, 2000).

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The study reported here aimed to investigate whether the previously observed genetic divergence between *P. bursarius* on *Populus* and *Lactuca* was due to the existence of secondary host-specific populations. This was done by studying microsatellite and mitochondrial DNA variation between *P. bursarius* populations sampled from a range of secondary hosts. In the absence of population structuring by host plant, populations on the various secondary hosts were expected to show similar levels of genetic differentiation, regardless of their host species of origin. Under the alternative hypothesis that populations are structured by host plant, it was expected that lower genetic differentiation would be observed between populations on the same host species than between those on different host species. The absolute level of genetic differentiation could not be predicted in advance as this will be influenced by founder effects of unknown magnitude occurring during colonisation of secondary hosts. Microsatellite and mtDNA variation was also examined in populations on *Populus* with the aim of identifying groups of individuals that were genetically similar to populations sampled from the secondary hosts. The identification of such groups would indicate that any host-associated populations present in *P. bursarius* manage to retain a degree of reproductive isolation from each other despite sharing a common primary host.

Although not a defining characteristic, it is likely that populations associated with different secondary hosts will be adapted to them. Consequently, a host-switching experiment was performed with *P. bursarius* clones isolated from different secondary hosts to compare their performance on 'home' and 'away' hosts.

Materials and methods

Aphid collection

Species reported to be good secondary hosts of *P. bursarius* (Alleyne and Morrison, 1977) were planted in three fields approximately 500 m–1.4 km apart (Figure 1) at Horticulture Research International (HRI), Wellesbourne, England in May 2002 and 2003. In each field, plots of 195 plants were laid out with 50 cm between plants and 10 m between plots. Two plots of each host species were planted in each field. Plots were positioned in different parts of the fields in the 2 years to avoid direct re-colonisation by aphids overwintering in the soil.

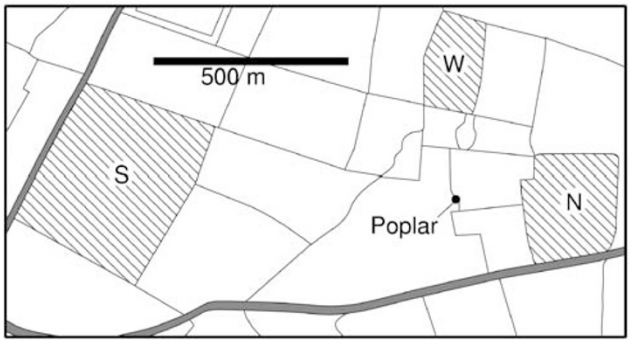

Figure 1 Arrangement of fields used to grow secondary hosts of *P. bursarius* and a stand of *P. nigra* (primary host) from which aphids were also sampled. The fields were identified as 'N', 'W' and 'S' (North, West and South) during the study.

Table 1 Numbers of *P. bursarius* sampled from plots of secondary hosts in 2002 and 2003

Host	*Year*					
	2002			*2003*		
	N	*S*	*W*	*N*	*S*	*W*
Lactuca	—	49, 49	62, 28	47, 51	48, 53	50, 54
Lapsana	—	36, 30	49, 34	36, 33	52, 46	—
Matricaria	31	51	39	47, 45	54, 50	49, 49

N, S and W represent different fields. A dash indicates that numbers were too low to sample.

Aphids were collected from the roots of the host plants between late August and early October, with each aphid being collected from a different plant to minimise the chance of sampling several clonally related individuals. Aphids taken from the same plot were regarded as a single sample. Sample sizes ranged from 28 to 62 aphids per plot (Table 1). Some plots were not sampled due to low levels of colonisation.

In 2002 the secondary host species planted were *L. sativa*, *Lapsana communis* L., *Taraxacum officinale* Weber and *Sonchus asper* (L.). Aphids were not found on the roots of *Taraxacum* or *Sonchus*, but were found on the roots of *Matricaria inodora* L. growing naturally in the fields. Consequently, samples were collected from *Matricaria* and in 2003 the set of secondary hosts was changed to *Lactuca*, *Matricaria* and *Lapsana*. This sampling strategy was designed to discriminate between the absence or presence of populations associated with different secondary hosts. If such populations do not occur in *P. bursarius*, any genetic differentiation would be due to founder effects during colonisation, either by new clones migrating from *Populus* or existing clones overwintering in the soil or both. Under these circumstances, there might be either no or equal differentiation between samples or a hierarchical pattern with increasing differentiation between samples taken from different plots in the same field and year, those from different fields in the same year and those from different years. Critically, in all the three cases, the genetic differentiation between samples is unrelated to their host plant of origin. Conversely, if *P. bursarius* is subdivided into secondary host-associated populations, samples taken from the same host plant species would be less differentiated than those taken from different host species.

In addition to the samples from secondary hosts, samples (551 and 448 individuals) of *P. bursarius* stem mothers were collected from a stand of *Populus* located between the three fields. Only this stand of trees was sampled since an earlier study did not find allele frequency differences between populations of *P. bursarius* on different stands of trees in the local area (Miller *et al*, 2003). Samples were taken in June 2002 and 2003 and therefore represented potential source populations for the aphids sampled from secondary hosts later in the year. All aphids were stored in 70% ethanol at −20°C.

Six individuals, two isolated from *Lactuca*, two from *Lapsana* and two from *Matricaria* were used to establish

six continuous parthenogenetic cultures on their home host plants for use in performance experiments. The microsatellite genotypes of these six individuals were obtained from their parthenogenetic descendants and verified that the six cultures were genetically distinct and, therefore, genuinely different clones.

PCR template preparation

Individual aphids were placed in the wells of a 96-well PCR plate containing 3 µl TE buffer and 2 µl proteinase K (Sigma) solution (25 mg ml^{-1}). Each aphid was crushed with a 200 µl pipette tip and 100–200 µl 5% Chelex 100 (Sigma) suspension was added. The mixture was incubated overnight at 56°C, then at 95°C for 15 min. The homogenate was stored at −20°C until required as template for PCR reactions.

Microsatellite analysis

The *P. bursarius* microsatellites Pb 02, Pb 10, Pb 16 and Pb 23 (Miller *et al*, 2000) and two additional, unpublished microsatellites isolated from the same library; Pb 71 (CA repeat, GenBank accession AY521593) and Pb 86 (GGC repeat, GenBank accession AY521594) were used. New PCR primers (Table 2) were designed for all microsatellites to facilitate amplification in a single multiplex PCR reaction. All six microsatellites were amplified in a single 10 µl PCR reaction containing 2 µl aphid homogenate, 200 µM each dNTP, 0.25 U 'HotStarTaq' DNA polymerase (Qiagen), 1 × Qiagen PCR buffer and PCR primers at the concentrations given in Table 2. PCR was carried out on a GeneAmp 9600 PCR machine (Applied Biosystems) with a cycle program of 95°C for 15 min, then 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s, followed by incubations at 72°C for 7 min and 60°C for 30 min. The forward member of each PCR primer pair was labelled at the 5′ end with a fluorescent dye, allowing PCR products to be analysed on an ABI 3100 capillary sequencing machine (Applied Biosystems). Fragment sizes were estimated by comparison to LIZ-labelled GeneScan-500 size standards (Applied Biosystems).

Mitochondrial DNA sequencing

Approximately 600 bp of the cytochrome oxidase subunit I gene was amplified by PCR from 108 aphids sampled in 2002 and from a single *P. populi* collected from the stand of *P. nigra* at HRI. The aphids were isolated from all the host plants sampled with 29, 28, 27 and 24 individuals coming from *Populus*, *Lactuca*, *Matricaria* and *Lapsana*, respectively. PCR was carried out in a 55 µl reaction containing 5.5 µl aphid homogenate, PCR primers CI-J-1718 and CI-N-2329 (Simon *et al*, 1994) at 0.25 µM, each dNTP at 200 µM, 1.375 U 'HotStarTaq' DNA polymerase and 1 × Qiagen PCR buffer. Amplification was carried out on a GeneAmp 9600 PCR machine with a cycle program of 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 40°C for 45 s and 72°C for 2 min, then a final incubation at 72°C for 7 min. A 5 µl aliquot of the reaction mixture was examined by agarose gel electrophoresis to confirm the successful amplification of the desired product. The remainder of the PCR product was purified using Millipore PCR96 cleanup plates and eluted into 50 µl H_2O. PCR products were sequenced in both directions using the PCR primers and BigDye dye-terminator kits (Applied Biosystems) according to the manufacturer's instructions, except that the primer annealing temperature was reduced to 40°C. Sequencing reactions were analysed on an ABI 3100 capillary DNA sequencing machine.

Performance experiments

An experiment was carried out to measure the performance of each of the six clonal cultures on all three host plant species (*Lactuca*, *Matricaria* and *Lapsana*). Prior to the experiment, each clone was cultured for 9 weeks (approximately six generations) on its 'home' host and the two 'away' hosts at 18–21°C, 16:8 L:D. Despite repeated attempts, only a few clones established on their away hosts (Table 3).

Table 3 Successful transfers of cultured *P. bursarius* clones to different host plants

Clone	*Home host*	*Survived on:*		
		Lactuca	*Matricaria*	*Lapsana*
1	*Lactuca*	Yes	No	No
2	*Lactuca*	Yes	Yes	No
3	*Matricaria*	No	Yes	No
4	*Matricaria*	No	Yes	No
5	*Lapsana*	No	Yes	Yes
6	*Lapsana*	No	Yes	Yes

Table 2 Properties of six *P. bursarius* microsatellite loci

Locus	*Primer sequence (5′–3′)*	*Conc.*	N_a	H_O	H_E
Pb 02	F: AACGGTATCAGTGCCCGTAA	0.1	11	0.474	0.690
	R: GCAGTCCGTCTCGACTTGTT	0.1			
Pb10	F: CTCTCGGGAGGGATTTGAAC	0.1	8	0.266	0.598
	R: CTATTCGTATCGCGCGTTTT	0.1			
Pb 16	F: GGACTAGCTCACTCTGGTCGT	0.1	9	0.190	0.694
	R: AACGCTAACTCCTTTGTCCTACA	0.1			
Pb 23	F: GGGCGCGTATATAAAGAATGA	0.05	10	0.444	0.739
	R: CAACACCGTCACTCCATCAC	0.05			
Pb 71	F: CGCATCATCACAGTCTCTCC	0.1	22	0.503	0.823
	R: GCGTAGTCCAAGCACGATTC	0.1			
Pb 86	F: ACGGGCGCGTATATAAAGAA	0.05	9	0.446	0.739
	R: CAACACCGTCACTCCATCAC	0.05			

Conc. denotes the final primer concentration (µM) used in multiplex PCR, N_a the number of alleles observed in this study, H_O the overall observed heterozygosity and H_E the overall expected heterozygosity.

After acclimatisation, two aphids were inoculated onto each of 24 plants per treatment (clone × host combination), divided into four replicate, randomised blocks with six plants per treatment per block and maintained at 18–21°C, 16:8 L:D. One plant from each treatment in each block was removed at six, eight, 10, 13, 15 and 17 days after inoculation and the number of aphids on each plant recorded.

Data analysis

The MICRO-CHECKER (Van Oosterhout *et al*, 2004) computer program was used to look for evidence of null alleles at the microsatellite loci and to estimate their frequencies. Since MICRO-CHECKER relies on particular patterns of deviations from Hardy–Weinberg genotypic proportions to detect null alleles, only the samples of stem mothers, collected from *Populus*, were examined in this manner.

Observed and expected heterozygosities at microsatellite loci were calculated using GENEPOP 3.1d (Raymond and Rousset, 1995). Since *P. bursarius* is a cyclical parthenogen, clonally related individuals with identical multilocus microsatellite genotypes could occur in the samples. Therefore, each aphid that was successfully genotyped at all six loci was assigned to a 'genotypic class', a specific combination of alleles across all loci.

Under the null hypothesis of no population structuring by host plant, it is expected that there will be no heterogeneity among samples from different secondary hosts over that seen among samples from the same host (ie due to spatio-temporal effects). This was tested by conducting a hierarchical *F*-statistic analysis (Yang, 1998) in which plots were grouped within fields, within years, within hosts using HIERFSTAT (Goudet, 2005). The significance of departures of the *F*-statistics from zero was tested using permutation procedures within HIERFSTAT. This approach does not distinguish between the case of heterogeneity among all secondary hosts and that of one divergent population. Neither does it allow populations on the secondary hosts to be related to those on *Populus*. Consequently, the hierarchical analysis was supplemented by calculating F_{ST} values for all samples within each host (without hierarchical structuring) and pairwise F_{ST} values for each pair of hosts with samples pooled within hosts.

To investigate the possibility that genetically differentiated, secondary host-associated populations were present on the same stand of *Populus*, a clustering method was used. POPULATIONS (Langella, 2002) was used to produce UPGMA trees based on an allele-sharing distance between individuals sampled from *Populus* in the same year. The rationale for this approach was that if several genetically distinct populations were present in the samples from *Populus*, their members would tend to cluster together. Clusters of similar genotypes were identified from the trees and then treated as separate samples. Clusters were identified by eye rather than by a predefined level of similarity. If such clusters did genuinely represent distinct populations, the allele frequencies of the clusters would be expected to resemble those found in samples taken from the corresponding secondary host. The similarity in allele frequencies between samples from the secondary hosts and the clusters from *Populus* was visualised by producing an UPGMA tree based on Nei's standard genetic distance with POPULATIONS.

Mitochondrial DNA sequences were aligned using PILEUP (Accelrys), which also allowed different haplotypes to be identified. PHYLIP (Felsenstein, 2002) was used to calculate distances between haplotypes, using the Kimura two-parameter correction. The relationships between haplotypes were visualised by producing a minimum spanning tree with the aid of ARLEQUIN (Schneider *et al*, 2000).

The performance of aphid clones on different hosts was analysed using GENSTAT (Lawes Agricultural Trust) to fit generalised linear models, assuming a Poisson distribution and a log-link function, to the relationship between the $\log_{(10)}$ number of aphids on each plant and the time since inoculation. In all cases the model assumed a common intercept (equivalent to starting with an identical number of inoculating aphids). Each model provided predicted values for the number of aphids per plant as time after inoculation increased. As not all clones could be cultured on all host species, only the following comparisons could be made: the performance (1) of clones isolated from *Lapsana* on *Lapsana*, compared to their performance on *Matricaria*; (2) of one clone isolated from *Lactuca* on *Lactuca* compared to its performance on *Matricaria*; (3) on *Matricaria*, of two clones isolated from *Lapsana* and one from *Lactuca* compared to the performance of two clones isolated from *Matricaria*.

Results

Null alleles

Evidence for the presence of null alleles at the microsatellite loci was rather inconsistent between the two samples of stem mothers from *Populus*. In the 2002 sample, all loci exhibited patterns of excess homozygosity compatible with the presence of null alleles. The highest estimated null allele frequency, for locus Pb 16, was 0.09. However, this pattern of excess homozygosity is also explicable by the presence of inbred individuals. Inbreeding due to mating between sexual morphs from the same clone (effectively self-fertilisation) has previously been identified as a contributing factor to excess homozygosity in *P. bursarius* populations (Miller *et al*, 2003). In the 2003 sample, only two loci, Pb 16 and Pb 71, exhibited evidence of null alleles. The estimated null allele frequencies were 0.04 and 0.06 for Pb 16 and Pb 71, respectively.

Microsatellite allelic and genotypic diversity

Between nine and 22 alleles were observed at the microsatellite loci (Table 2). For the entire set of samples, overall observed heterozygosities were between 0.19 and 0.503. Expected heterozygosities were always greater than the observed and ranged from 0.598 to 0.823.

In total, 2104 aphids were genotyped at all six loci and could be assigned to one of 1138 distinct genotypic classes, 846 of which contained a single individual. Individuals from nonunique genotypic classes (ie that shared their genotypic class with at least one other individual) made up 59.7% of the total sample. Nonunique genotypic classes were found on all host plants with 42.6, 57.3, 75.1 and 80.7% of individuals from

Populus, *Lactuca*, *Lapsana* and *Matricaria* coming from nonunique classes. The higher proportions of individuals in the samples from secondary hosts that had nonunique genotypic classes, especially those from *Lapsana* and *Matricaria*, strongly suggests that some of the individuals in these samples were members of the same clones. The 292 nonunique classes contained between two and 60 classes. In all, 239 nonunique classes were only found on one host. One class was found on three different hosts (*Matricaria*, *Lapsana* and *Populus*) and 52 classes were found on two hosts. Of these, 22 classes were shared by *Lapsana* and *Matricaria*, 17 by *Populus* and *Lactuca*, 12 by *Populus* and *Lapsana*, and one by *Lactuca* and *Matricaria*.

Previous studies of microsatellite variation in aphid populations (eg Sunnucks *et al*, 1997a; Simon *et al*, 1999) have assessed the impact of parthenogenesis on measures of population structure by analysing both the full and an edited data set in which duplicated genotypic classes are removed. In these studies the microsatellite markers used were sufficiently variable that individuals from the same genotypic class could be assumed to be from the same clone. This assumption cannot be made in the present case, as demonstrated by the existence of stem mothers on *Populus* with a shared genotypic class. Also, individuals were found on *Lactuca* that shared a genotypic class but differed in their mtDNA haplotype. Nevertheless, analyses with and without duplicated genotypic classes did not produce different results. Consequently, only the results of analysing the full data set are given.

Genetic heterogeneity among samples

Hierarchical *F*-statistics revealed mild but significant heterogeneity between plots within fields ($F_{PLOT/FIELD} = 0.069$, $P \leq 0.001$). No additional heterogeneity was found between fields within years ($F_{FIELD/YEAR} = -0.025$, $P = 0.46$) over that already found between plots. Additional moderate heterogeneity was found between years within hosts ($F_{YEAR/HOST} = 0.031$, $P \leq 0.001$). Substantial heterogeneity was found between hosts ($F_{HOST/TOTAL} = 0.488$, $P = 0.015$), demonstrating that host plant was by far the most important factor in structuring the population.

Pairwise estimates of F_{ST} between hosts and estimates for all samples within the same host are shown in Table 4. All estimates were significantly greater than zero ($P \leq 0.01$). Pairwise comparisons all revealed substantial heterogeneity between hosts, especially for comparisons involving *Lactuca* and *Matricaria*. Heterogeneity between samples from the same host was low except in the case of *Lapsana*.

Clusters of similar genotypes from *Populus*

Clustering individual aphids from the samples from *Populus* identified five clusters of similar genotypes in 2002 and four clusters in 2003. Clusters 1–5 from 2002 contained 42, 95, 133, 169 and 109 individuals. Clusters 1–4 from 2003 contained 39, 80, 60 and 200 individuals. Three individuals from 2002 were not assigned to any cluster and 49 individuals from 2003 were not assigned.

The majority of the clusters were most similar to each other and to some of the samples collected from *Lapsana*. However, cluster 1 from the 2002 sample (42 individuals) bore a strong similarity to the samples collected from *Lactuca* (Figure 2).

Table 4 Estimates of F_{ST} for all samples from the same host (diagonal) and comparisons between hosts (below diagonal)

Populus	0.003			
Lactuca	0.453	0.004		
Lapsana	0.150	0.341	0.155	
Matricaria	0.489	0.653	0.410	0.015
	Populus	*Lactuca*	*Lapsana*	*Matricaria*

Mitochondrial DNA sequencing

A total of 505 base pairs of reliable, homologous DNA sequence was obtained for all 108 individuals and *Pemphigus populi*. In all, 10 distinct haplotypes were identified, all of which had greater sequence similarity to each other than to *P. populi*. Sequence divergence among the 10 haplotypes was between 0.20 and 4.55%, while divergence between the 10 haplotypes and the *P. populi* sequence was between 9.7 and 10.7%. The 10 haplotypes formed two distinct clades (Figure 3), one containing haplotypes 6, 7 and 10, which were predominantly found in aphids feeding on *Matricaria*, and the other containing all other haplotypes.

The distribution of haplotypes differed substantially between aphids from different host plants (Figure 3). No haplotypes were shared between aphids from *Lactuca* and *Lapsana* or *Lactuca* and *Matricaria*. The most common haplotypes found among aphids from *Lactuca* and *Lapsana* were present in aphids from *Populus*, but the sample from *Populus* contained six (20%) individuals with haplotype 5, which was not seen on any secondary host. Haplotype 6 was found in aphids from both *Matricaria* and *Lapsana*, but was common on *Matricaria* (93%) and much rarer on *Lapsana* (4%). No haplotypes were shared by aphids on *Matricaria* and *Populus*.

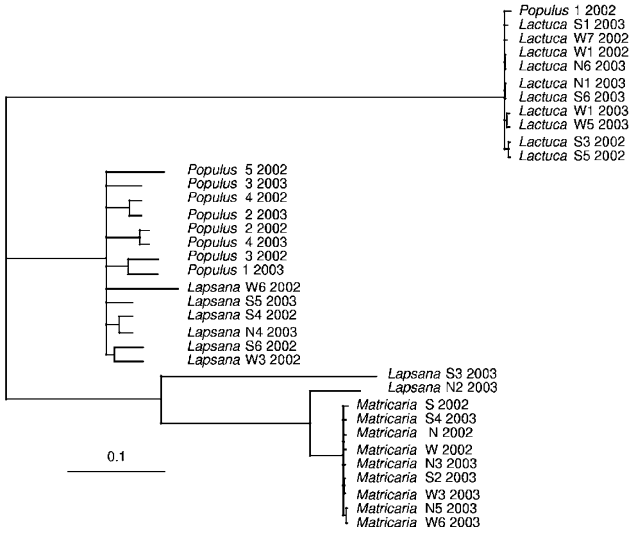

Figure 2 UPGMA dendrogram based on Nei's standard genetic distance illustrating the degree of similarity in microsatellite allele frequencies between samples taken from secondary hosts and clusters of genotypes identified within samples from *Populus*. Individual samples and clusters are identified by the host plant, a code denoting the cluster (*Populus*) or field and plot (secondary hosts) and the year of sampling.

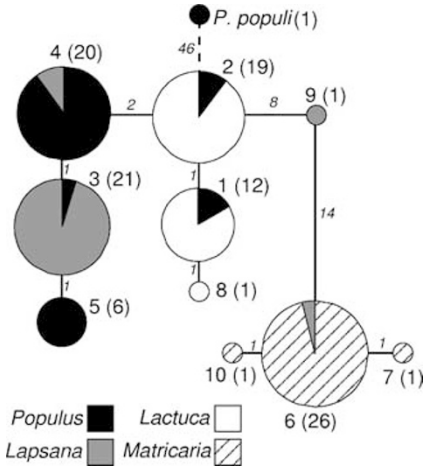

Figure 3 Minimum spanning tree of *P. bursarius* and *P. populi* COI haplotypes. Branch labels (italics) indicate the number of changes between haplotypes. The area of each node and the figures in parentheses indicate the number of individuals found with each haplotype. Segments indicate the proportions of individuals on different hosts.

Aphid performance experiment

Clones isolated from *Lapsana* performed significantly better on *Matricaria* than on *Lapsana* (deviance ratio (effect of plant species) = 28.4, 1 and 95 d.f., $P < 0.001$). Similarly, clone 2, isolated from *Lactuca*, performed better on *Matricaria* than on *Lactuca* (deviance ratio = 423.96, 5 and 42 d.f., $P < 0.001$). Clones isolated from different host plants performed equally well on *Matricaria* (deviance ratio (effect of source plant) = 1.28, 2 and 119 d.f., $P = 0.281$).

Discussion

Aphids with shared multilocus microsatellite genotypes were found on all three secondary host species. This was unsurprising given that aphids were sampled from secondary hosts following a period of parthenogenetic reproduction. However, identical genotypes were also found among stem mothers sampled from *Populus*. This was probably due to matings between sexual morphs from the same clone (effectively self-fertilisation), population structuring, or a combination of the two (Miller *et al*, 2003) and possibly the presence of null alleles at some loci. Null alleles will not only cause an excess in observed homozygosity, but will also distort measures of genetic divergence between populations since they will cause estimated allele frequencies to be distorted. However, even if null alleles were present at some of the loci used in this study, their estimated frequencies were moderate to low. Consequently, their influence on measures of population differentiation (eg F_{ST}) will also be low.

Hierarchical *F*-statistics, Nei's standard genetic distance and the distribution of mtDNA haplotypes all showed that the populations sampled from secondary hosts were strongly structured by host species.

Although significantly greater than zero, F_{ST} among samples taken from *Lactuca* was extremely low. Mitochondrial COI haplotypes that were found in aphids on *Lactuca* were also found in stem mothers on *Populus*. A cluster of microsatellite genotypes was identified among stem mothers sampled from *Populus* in 2002 that had allele frequencies similar to samples of *P. bursarius* taken from *Lactuca*. These observations indicate that there is a distinct, *Lactuca*-specific population within *P. bursarius* that maintains a degree of reproductive isolation despite carrying out its sexual cycle on *Populus* where individuals from other secondary hosts are also breeding.

Samples of aphids taken from *Matricaria* were also much more similar to each other than to samples from other secondary hosts. However, no mtDNA haplotypes were shared between the samples from *Matricaria* and those from *Populus*. Furthermore, the mtDNA haplotypes typical of aphids feeding on *Matricaria* were rather different from those found among aphids feeding on other hosts. Given that *Matricaria* has not been recorded as being an important secondary host of *P. bursarius* (Dunn, 1959; Alleyne and Morrison, 1977), this might indicate that the aphids on *Matricaria* were members of some other species of *Pemphigus*. However, the levels of nucleotide sequence divergence among the 10 (presumed) *P. bursarius* haplotypes were comparable to those observed among cytochrome oxidase I sequences from the different biotypes of the aphid *Schizaphis graminum* (Shufran *et al*, 2000). Furthermore, *Pemphigus* on the roots of *Matricaria* have previously been found to be morphologically indistinguishable from *P. bursarius* (Furk and Prior, 1975). Also, a microsatellite genotypic class was shared between aphids from *Matricaria* and *Populus*, the latter of which can be unambiguously identified as *P. bursarius*. Although the microsatellites used in this study were insufficiently variable to discriminate reliably among *P. bursarius* clones, it is unlikely that individuals from different species would share identical multilocus genotypes. Thus, it appears that at least some of the aphids sampled from *Matricaria* were genuinely *P. bursarius*. This does not exclude the possibility that the aphids sampled from *Matricaria* were a mixture of *P. bursarius* and another species, but the sequence divergence between mtDNA haplotypes found in aphids on *Matricaria* was low (maximum 0.4%), as was microsatellite allele frequency heterogeneity among samples from *Matricaria*. Both these observations suggest that the majority of aphids on *Matricaria* were the same species.

Although the possibility that the *Matricaria* feeding aphids were not *P. bursarius* cannot be entirely discounted, an alternative scenario is that these samples were largely composed of obligately parthenogenetic *P. bursarius*. This interpretation is suggested by the high proportion of nonunique genotypic classes found in these samples. Presumably, obligately asexual *P. bursarius* clones are able to survive winter conditions by virtue of the specialised, cold-tolerant morph known to occur in this species (Phillips *et al*, 2000). The coexistence of cyclically and obligately parthenogenetic lineages within an aphid species is not uncommon (for recent reviews, see Simon *et al*, 2002; Wilson *et al*, 2003). Genetic divergence between cyclically and obligately parthenogenetic aphids has been observed in allozymes (Simon *et al*, 1996a; Simon and Le Gallic, 1998), microsatellites (Simon *et al*, 1999; Delmotte *et al*, 2002) and maternally inherited mitochondrial and endosymbiont plasmid DNA (Martinez Torres *et al*, 1996, 1997; Simon *et al*,

1996b). However, the existence of a genotypic class shared between aphids on *Matricaria* and those on *Populus* suggests that some *Matricaria*-feeding aphids may reproduce sexually.

A high proportion of individuals sampled from *Lapsana* were also in nonunique genotypic classes. However, COI haplotypes found among aphids on *Lapsana* were also present in stem mothers on *Populus*. Furthermore, clusters of microsatellite genotypes identified within the samples from *Populus* usually bore some similarity to the samples from *Lapsana*. It may be the case that the population feeding on *Lapsana* is a mixture of cyclical and obligate parthenogens.

The samples from *Lapsana* were markedly more heterogeneous than those from other hosts. During the collection of samples, it was apparent that *Lapsana* plants were colonised at a rate much lower than *Matricaria* and especially *Lactuca*. The elevated heterogeneity among samples from *Lapsana* could be a consequence of a more pronounced founder effect during the migration from *Populus* to the secondary host.

One of the COI haplotypes (5) was found in *P. bursarius* stem mothers on *Populus* but not in aphids on any of the secondary hosts. Haplotype 5 was most similar to haplotype 3, which was shared between aphids on *Populus* and *Lapsana*. This may indicate the existence of an additional population, closely related to the aphids feeding on *Lapsana*, but exploiting some unknown secondary host. Heterogeneity, as measured by F_{ST}, between the two samples taken from *Populus* was low. This implies that if several genetically divergent populations were completing their sexual cycle on *Populus*, they were present in similar proportions each year.

The results of the experiment to measure the performance of *P. bursarius* clones on different secondary hosts were inconclusive. Attempts to transfer aphid cultures between different secondary host species were unsuccessful, with the exception of transfers from *Lactuca* and *Lapsana* onto *Matricaria*. This might be interpreted as indicating that the clones were so specialised as to be unable to survive on 'away' hosts, with the exception of *Matricaria*. However, it may also be an artefact of using wingless aphids that do not normally move between host plant species. Where transfers were successful and comparisons could be made, clones isolated from *Lactuca* and *Lapsana* actually performed better on *Matricaria* than on their home host. All clones that could be tested performed equally well on *Matricaria*, regardless of their original host. Clearly, this experiment did not provide evidence of adaptation to different secondary host species. This does not, however, prove that no such adaptation exists. Firstly, only two clones per host species were used in the experiment and only a small subset of the possible home host *vs* away host comparisons could be made. The results of this experiment may not, therefore, be representative of the *P. bursarius* population as a whole. Secondly, only a single trait, the reproductive output of the clones, was measured. It is always possible that some other, unconsidered, trait is adapted to different hosts. Clearly, further experimental elucidation is required.

The results of the performance experiment differ from findings from a number of other aphid species in which negative fitness correlations have been observed between alternative host plants (Weber, 1985; Via, 1991; Mackenzie, 1996; Sunnucks *et al*, 1997b, 1998). The most comprehensively studied example of fitness tradeoffs in an aphid species is that of the host races in the pea aphid, *Acyrthosiphon pisum* Harris on alfalfa and red clover (for a review, see Via, 2001). In *A. pisum*, negative fitness correlations contribute to post-zygotic reproductive isolation since hybrids between host races are maladapted to both hosts (Via *et al*, 2000). Unlike *P. bursarius*, *A. pisum* does not host alternate. Sexual reproduction in the autumn takes place on the same herbaceous hosts that support parthenogenetic reproduction in the summer (Blackman and Eastop, 2000). Winged *A. pisum* exhibit a strong preference for their natal host type, which also contributes to reproductive isolation between host races (Via, 1999). The combination of fitness tradeoffs and host choice as a mechanism for the reproductive isolation of host races is not confined to autoecious (non-host alternating) aphids. *Cryptomyzus galeopsidis* (Kaltenbach) is divided into two host races that reproduce sexually on different primary hosts (*Ribes rubrum* L. and *Ribes nigrum* L.) but have the same secondary host. These are maintained by a combination of fitness tradeoffs and a preference among autumn migrants returning to the primary host for the natal host species of the stem mother of their clone (Guldemond, 1990b, c).

Clearly, host fidelity cannot prevent gene flow between secondary host-associated populations in *P. bursarius* because of the common primary host. Furthermore, this study did not find evidence of adaptation to different hosts. It should be noted that it remains unclear whether or not the host-associated populations in *P. bursarius* are true host races that arose in sympatry. Although *L. sativa*, *L. communis* and *M. inodora* currently have similar distributions, their past distributions are unknown. It is therefore quite plausible that the different host-associated populations originally diverged in allopatry and subsequently extended their ranges. Nevertheless, *Lactuca*- and *Lapsana*-feeding aphids, at least, appeared to carry out their sexual cycle on the same primary host, so mechanisms to reduce gene flow between them must exist.

One possible mechanism is the timing of the autumn migration to *Populus* from different secondary hosts. It has been reported that autumn migrants from *Lapsana* mature later than those from *Lactuca* (Dunn, 1959). Photoperiod has been shown to have a role in the triggering of the production of autumn migrants (Phillips *et al*, 1999) possibly indirectly via the host plant. Hence, perception of photoperiod and the consequent development of autumn migrants may be modified by different host plant physiologies. This mechanism of reproductive isolation could be essentially passive on the part of the aphid.

Active mechanisms to reduce gene flow between host races might also exist, as seen in the aphids *Cryptomyzus galeopsidis* and *C. maudamanti* Guldemond. Although the two are now recognised as sibling species, *C. maudamanti* was previously regarded as a host race of *C. galeopsidis*. The two species are sympatric and host alternating with a common primary host (*Ribes rubrum*) but different secondary hosts (Guldemond, 1990a). When presented with a choice, *C. galeopsidis* males prefer the sex pheromones of conspecific females (Guldemond *et al*, 1993; Guldemond and Dixon, 1994). This is reinforced by the females of the two species releasing pheromones at

different times of the day, when conspecific males are most active (Guldemond and Dixon, 1994; Guldemond *et al*, 1994b). Hybridisation is further reduced by lower insemination rates for interspecific, compared to intraspecific, mating (Guldemond *et al*, 1994a).

Microsatellite allele frequency heterogeneity among samples of *P. bursarius* samples taken from *Lactuca* in particular over 2 years was low. This indicates that the *Lactuca*-specific population re-established its association with its host following the intervening winter. It seems probable that the mechanism that maintains this association in *P. bursarius* is also associated with the active mechanisms of locating and accepting their preferred secondary host plants. These mechanisms are complex and are linked to the host-plant chemistry. For example, *Aphis fabae* Scop. has been shown to take off from suitable host plants if presented with non-host odours immediately after landing (Storer *et al*, 1996). Similarly, Caillaud and Via (2000) have demonstrated in *A. pisum* that the behavioural acceptance of a plant is based on chemical cues perceived prior to feeding. The precise mechanisms in *P. bursarius* remain to be elucidated. Samples were taken from the secondary hosts after several parthenogenetic generations had already occurred. It is therefore not possible to determine the extent to which the observed association with *Lactuca* was a result of spring migrants choosing between host plants or selection acting after colonisation. Both processes are known to contribute to the separation of *C. galeopsidis* and *C. maudamanti* onto their respective secondary hosts (Guldemond, 1991).

In conclusion, the genetic data presented in this study indicate the existence of genetically divergent populations within *P. bursarius* that exploit different secondary host plants. At least two of these populations appear to be either partly or wholly cyclically parthenogenetic, yet maintain a degree of reproductive isolation despite mating on a common primary host. The processes that are responsible for this isolation are unknown. Until they are elucidated, it is not possible to judge whether the populations could have diverged in sympatry or would have required an initial period of geographical isolation.

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