The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2011, **102**, 737–749. With 5 figures

Temporal genetic structuring of a specialist parasitoid, *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae) attacking a specialist aphid on tansy

FRANKLIN N. NYABUGA[1,2]*, HUGH D. LOXDALE FLS[1,2], DAVID G. HECKEL[2] and WOLFGANG W. WEISSER[1]

[1]*Institute of Ecology, Friedrich Schiller University, Dornburger Straße 159, D-07743 Jena, Germany*
[2]*Department of Entomology, Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, D-07745 Jena, Germany*

Received 13 August 2010; revised 29 November 2010; accepted for publication 29 November 2010

In insect species characterized by inbreeding, limited dispersal, and a metapopulation structure, high genetic differentiation and reduced genetic diversity within local populations are expected. Using the model system *Lysiphlebus hirticornis* Mackauer, a specialist parasitoid of the tansy aphid, *Metopeurum fuscoviride* Stroyan (Hemiptera: Aphididae), we examined within-site temporal population dynamics and genetics, including molecular variation at the tansy plant level. Aphid-parasitoid dynamics were surveyed and parasitoids sampled from 72 tansy plants at 11 sites in and around Jena, Germany, over one growing season. Thereafter, parasitoid samples were genotyped at 11 polymorphic microsatellite loci. Colonization, extinction, and recolonization events occurred during the season. Allele numbers and identities were highly variable over time. When samples from all sites were pooled, allele number over all loci showed a decreasing trend with time. At the level of sites, temporal changes in genetic diversity were more variable. Analysis of molecular variance revealed that samples at the plant level explained the highest variance compared to at site level. We conclude that the genetic structuring of this insect is very fine grained (i.e. at the tansy plant level) and the temporal genetic diversity is explained by a combination of extinction and recolonization events, as well as inbreeding. *Biological Journal of the Linnean Society*, 2011, **102**, 737–749.

ADDITIONAL KEYWORDS: alleles – diversity – gene flow – insects – metapopulation – *Metopeurum fuscoviride* – microsatellites – population dynamics – spatial genetics – *Tanacetum vulgare*.

INTRODUCTION

Genetic diversity is the material on which selection acts and its amount in a population at any given time results from a balance between the gain and loss of allelic variants (Amos & Harwood, 1998). An increase in genetic diversity can come from the creation of new alleles by mutation, through migration of individuals into the population bearing different alleles, and through sexual reproduction by mixing and recombination of the alleles present in a population. Loss of genetic diversity arises via natural selection, random genetic drift, and emigration (Amos & Harwood, 1998).

In structured populations characterized by low dispersal among patches, genetic variability in space and time is influenced by colonization and extinction cycles (Whitlock & McCauley, 1990; Husband & Barrett, 1996) and variations in population density (Lynch, Conery & Burger, 1995). Wright (1940) suggested that patterns of extinction and recolonization would enhance genetic differentiation of local populations because the number of individuals colonizing a patch is likely to be much smaller than the local carrying capacity. By contrast, Slatkin (1985) argued that such interdemic selection is inherently unlikely

*Corresponding author.
E-mail: franklin.nyabuga@uni-jena.de

because ongoing local extinction also implies ongoing recolonization, and such recolonization constitutes gene flow preventing local population differentiation. Wade & McCauley (1988) reported, however, that the outcome of extinction and recolonization depends on the mode of founding of new populations: thus, if colonizing propagules are large and contain individuals from many populations, turnover has homogenizing effects, whereas if propagules are small and homogenous, deriving from one or a few source populations, turnover of local populations enhanced their differentiation. Even so, neither Slatkin (1985), nor Wade & McCauley (1988) based their arguments on empirical data.

The seasonal decline of population size or density and founder events may reduce variability and create spatio-temporal genetic differentiation (Lynch *et al*., 1995). Such decline may also reduce variability and create differentiation between populations as a result of genetic drift resulting from bottlenecks and founder effects, although this reduction is strongly influenced by gene flow between populations.

In social animals, Chesser (1991a) noted that the mating system affects the distribution of genetic variation on a local scale and that female philopatry plays an important role in the disparity of variance within lineages relative to random individuals within the population. Polygynous groups or local subpopulations consisting of mating pairs prevent the complete admixture of genes amongst groups (Sugg *et al*., 1996). In primary hymenopteran wasp parasitoids, especially gregarious species as well as the solitary species attacking gregarious hosts, local mate competition as a result of mating between siblings and brothers competing for mates at natal/ emergence patches is known to occur (Godfray, 1994). In solitary parasitoids attacking colony forming hosts, quasi-gregarious broods of parasitoids are produced, which favours sib-mating or inbreeding on the natal patch (Mackauer & Völkl, 2002). Inbreeding enhances reduced levels of genetic diversity and increases the probability of extinction of local populations (Saccheri *et al*., 1998). Inbreeding may also lead to fine-scale genetic structure or the nonrandom spatial distribution of individuals with respect to relatedness or allele frequencies (Chesser, 1991b).

To date, few studies of insects combining ecological and molecular techniques have examined genetic changes over the course of several seasons (aphids: Loxdale & Brookes, 1990; damselfly: Abbott *et al*., 2008), even more rarely, within a single season (rose aphids: Rhomberg, Joseph & Singh, 1985; fire ants: Goodisman, Sankovich & Kovacs, 2007; cotton aphid: Brevault *et al*., 2008) and none on hymenopterous primary parasitoids that we are aware of. In the present study, we explore population genetic diversity and structure as a function of time (i.e. over a single annual season), in a host–parasitoid system comprising the specialist primary wasp parasitoid, *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae: Aphidiinae) attacking the specialist aphid, *Metopeurum fuscoviride* Stroyan (Hemiptera: Aphididae) on tansy, *Tanacetum vulgare* L. (Family Asteraceae). A tansy plant (= genet) usually comprises several up to approximately 100 shoots (= ramets), each of which could host an aphid colony. This model system is characterized by a metapopulation structure (i.e. limited dispersal and colonization and extinction events) (Hanski, 1999; Weisser, 2000; Zheng *et al*., 2009). The life cycle of the parasitoid involves multiple overlapping generations in one year (i.e. it is multivoltine with approximately 14–17 generations per annum). Female parasitoids have been observed to oviposit large number of eggs (40–70), and often to forage until all available hosts are parasitized, independent of aphid colony size on a tansy ramet (Mackauer & Völkl, 2002). Extinctions of the aphid, and, consequently, the parasitoid as a result of predation at the level of tansy genets and ramets, are common in a single season (Weisser, 2000; Weisser & Härri, 2005).

Ecological studies, as well as inferences from microsatellites, have revealed low dispersal rates, inbreeding and partial sib-mating, population substructuring, and philopatry within demes (colonies), and also have confirmed the existence of a metapopulation in the *L. hirticornis–M. fuscoviride* model system (Mackauer & Völkl, 2002; Rauch & Weisser, 2007; Nyabuga *et al*., 2010). In light of these earlier results, in the present study, we further describe the spatio-temporal dynamics within parasitoid population demes as well as determine the extent of fine-scale spatial structuring. Specifically, we aimed to address two main questions: (1) how does *L. hirticornis* molecular variance at the tansy genet level compare with that at the site level (i.e. a spatial unit with many tansy genets?) and (2) how does the population genetic diversity of the parasitoid fluctuate over time?

MATERIAL AND METHODS

Field sites

The population dynamics of *M. fuscoviride* and its parasitoid, *L. hirticornis*, were surveyed and parasitoids sampled from 11 sites in and around Jena, Germany (50°54′N, 11°35′E) between June and October 2007. Sites with tansy plants were found mainly along the course of the River Saale, on the verge of railway tracks, and on ruderal habitats separated by human development, agricultural land, and

forests, with the closest being Jena Nord-1 and Jena Nord-2, which were approximately 217 m apart. Details on site description are provided elsewhere (Nyabuga *et al*., 2010: Appendix 1 and fig. 1).

Field surveys and population sampling

At all sites, tansy plants were visited and inspected for *M. fuscoviride* every 10 days during the growing season. If colonized by aphids, the tansy genet was labelled and its phenology noted, and the number of ramets per genet were counted, along with the number of ramets colonized by the aphid (Nyabuga *et al*., 2010). The number of aphids in a colony was counted and further inspected for *L. hirticornis* aphid mummies. The mummies provided reliable estimates of the presence or absence of developing *L. hirticornis* and were therefore used to determine parasitoid population dynamics (Eber & Brandl, 1994; Dempster, Atkinson & Cheesman, 1995; Weisser, 2000). The dark brownish coloration of *L. hirticornis* aphid mummies easily distinguished them from those of other parasitoid species attacking *M. fuscoviride*, in particular *Aphidius tanacetarius* Mackauer and *Ephedrus* spp. Haliday (Hymenoptera: Braconidae: Aphidiinae) (Weisser, 2000). Because the sampling of insects from plants was destructive in nature, a maximum of ten aphid mummies were sampled per genet to avoid seriously impacting parasitoid population densities and genetics. Mummies were collected in 1.5-mL Eppendorf tubes for transport back to the laboratory, where they were left until the adult winged wasps emerged; these adults were then sexed with the aid of a stereo binocular microscope. Male and female wasps from a single genet were stored separately in Eppendorf tubes in 100% ethanol at 4 °C until DNA extraction. During subsequent visits, the fate of marked genets with aphid colonies was followed and any new genet colonization was noted and marked, and variables measured and sampled as described above. The study was continued for the entire growing season, until the plant shoots senesced and the aphid colonies collapsed.

Microsatellite analysis

Results from only diploid female parasitoids are here reported even though both males and females were genotyped. This is because males are haploid, and only diploid females provided genotypes for Hardy–Weinberg equilibrium (HWE) analysis. Male parasitoids were used to check for the existence of null alleles (Nyabuga *et al*., 2009). DNA was extracted using the 'salting-out' method described by Sunnucks & Hales (1996) from 610 female individuals collected from the 11 sites (for the number of samples at each site, see Appendix S1). Eleven polymorphic microsatellite loci were used in genotyping; of these, nine (*Lhirt 01*, *Lhirt02*, *Lhirt03*, *Lhirt04*, *Lhirt06*, *Lhirt08*, *Lhirt10*, *Lhirt15*, and *Lhirt23*) were developed from *L. hirticornis*, and a further two loci derived from published primer sequences for other *Lysiphlebus* species, namely *Lysi08* from *Lysiphlebus fabarum* (Marshall), and *Lysi6b12* from *Lysiphlebus testaceipes* (Cresson) (Nyabuga *et al*., 2009).

Statistical analysis

Hierarchical partitioning of the genetic variance of parasitoid samples was performed in ARLEQUIN, version 3.1 (Excoffier, Laval & Schneider, 2005) using an analysis of molecular variance (AMOVA) framework (Weir & Cockerham, 1984; Excoffier, Smouse & Quattro, 1992). Pairwise and global F_{ST} were calculated *sensu* Weir & Cockerham (1984) in ARLEQUIN. Population genetic diversity was quantified as observed heterozygosity (H_O) and with the unbiased estimates of expected heterozygosity (H_E), calculated using ARLEQUIN. The number of alleles per locus (A) and allelic richness (R) was calculated in FSTAT, version 2.9.3.2 (Goudet, 2002). Within-sample deviation from HWE was tested in GENEPOP, version 4.0 (Raymond & Rousset, 1995), using the dual null hypotheses of both heterozygote excess and deficit at individual loci as well as over all loci.

Several analyses were conducted by subdividing the dataset into subsamples. First (grouping A), the 610 parasitoids genotyped from the 11 sites were pooled as if a single population, and microsatellite variability was determined. Second (grouping B), the 610 parasitoids were grouped into three according to time of sampling as: early summer (June to 15 July); mid-summer (16 July to end of August) and late summer (September to October). These three 'time groups' were analyzed using AMOVA, with time instead of space for the subgroup calculations to establish temporal variability. The rationale of grouping the samples into three (i.e. early summer, mid-summer, and late summer) rather than performing molecular analysis every 10 days according to the survey and sampling procedure was a result of insufficient female sample sizes attributed to the sparing sampling performed to avoid human impact on parasitoid population densities and eventual genetics, the number of females emerging from the mummies collected, and the stochastic insect population dynamics. Third (grouping C), from the 179 samples in early summer, 279 in mid-summer, and 152 in late summer, we randomly selected subsets of 100 samples for each time point using the statistical package R (R Development Core Team, 2008). Fifty subsets (with replacement) were obtained for each of these three time

points. For each of these 150 subsets obtained, means for alleles per locus, H_O, H_E, and F_{IS} were determined using ARLEQUIN. The 150 means were then tested for temporal differences using analysis of variance in SPSS, version 16 (SPSS, 2007). Fourth (grouping D), the 610 genotyped parasitoids were grouped according to the 11 sites and time of collection (i.e. early summer, mid-summer or late summer) but only five of the 11 sites where a minimum of 10 or more individuals were sampled for each of the three time points were included in the AMOVA analysis. These were: Jena Nord-1, Burgau-2, Göschwitz-Bhf, Jena West-Bhf, and Jena Nord-2 (Appendix S1). The lowest number of individuals at a given time point was: Jena Nord-1 = 24, Burgau-2 = 23, Göschwitz-Bhf = 12, Jena West-Bhf = 11, and Jena Nord-2 = 45. Random subsampling of the other time points was performed individually for each site using the statistical package R aiming to randomly select a uniform sample number. AMOVA was carried out separately for each of the five sites. Grouping D was performed to establish the spatial (site)-temporal genetic variability patterns. For the fifth grouping (E) and to establish fine-scale spatial genetic differentiation, the 610 parasitoids were grouped into the 11 sites and the 72 genets from which they were sampled, and analyzed for spatial variation, ignoring the temporal component. Lastly (grouping F), to establish fine-scale spatial-temporal genetic variability, the 72 genets were split further into two, three or four groups over time where the number of individuals allowed for this. Twenty-four genets spread over nine sites could be split thus: 17 genets could be split into two, six genets could be split into three, and one genet could be split into four temporal groups of four to ten individuals. Overall, 103 groups were obtained in this way (Appendix S1) and analyzed using AMOVA.

RESULTS

Aphid and parasitoid population dynamics

Tansy plant phenology was not identical within or between sites. Thus, flowering was observed starting late June and, although some plants were wilting and senescing, others were seen to be sprouting (Fig. 1).

At the start of sampling (4–13 June), between 285 (Burgau-1) and 6755 (Jena Nord-2) *M. fuscoviride* aphids were counted. The rates of colony increase and fluctuations varied from one site to another (Table 1). When all the sites were aggregated and analyzed as a function of time, approximately 11 300 aphids were counted at the start of season, dropping to approximately 6900 by mid June and again building up in late June/early July before finally undergoing a continuous decline in early September (Fig. 2). The highest percentage of genet colonization by the aphid

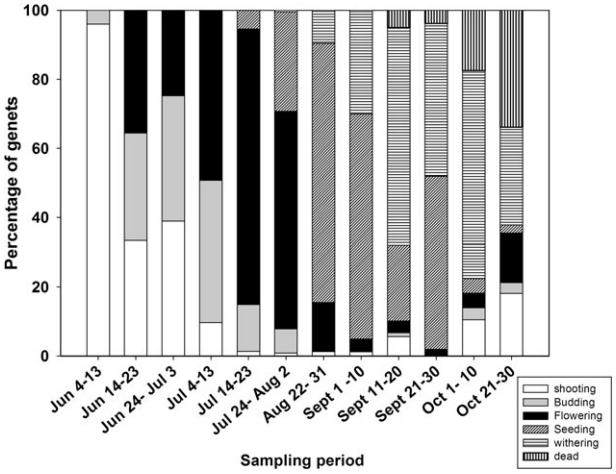

Figure 1. Over all 11 sites sampled over time, tansy (*Tanacetum vulgare*) plants of varying phelonogies were sampled. When some were sprouting, others were senescing and drying.

was observed over the period 24 June to 3 July and new colonization occurred until mid September (Fig. 3). Aphid extinction events on genets were noted from 14–23 June and continued throughout the season, peaking from 11–20 September. A number of genets in which aphid extinction had occurred were again recolonized (Fig. 3). At the level of genets over all sites, mean ± SE aphid colony survival on a genet was 8.9 ± 0.33 weeks (range 3–20 weeks), with a modal survival time of 3 weeks.

From 24 June to 3 July, aphid mummies were recorded at all sites sampled and found to be present until late August when three of the 11 sites had no aphid mummies; thereafter, the sites with no mummified aphids increased until early October (Table 1). The peak of aphid mummification was recorded between 4–13 July and thereafter showed a general declining pattern (Fig. 2). There was no relationship between aphid abundance and parasitoid abundance ($r = 0.069$, $F_{1,257} = 1.223$, $P = 0.270$).

Overall microsatellite diversity

Over all the 610 parasitoids grouped as one population (grouping A), the number of alleles varied from two at locus *Lysi08* and *Lhirt08* to 23 at locus *Lhirt01* (mean ± SD, 8.3 ± 7.07). Overall expected heterozygosity ranged from 0.215 at locus *Lysi08* to 0.828 at *Lhirt04* (Table 2). When parasitoids were analyzed according to site of collection, *Lysi08* and *Lysi6b12* showed significant Hardy–Weinberg deviations ($P < 0.05$) in one site each (Table 2) but, over all sites, did not deviate significantly from expectations (*Lysi08*: $\chi^2 = 20.7$, d.f. = 20, $P = 0.416$; *Lysi6b12*:

Table 1. The total number of aphids/aphid mummies as a result of *Lysiphlebus hirticornis* counted on each of the 11 sites at various time periods

Sampling period	Burgau-1	Burgau-2	Dornburg-Bhf	Fritz-Winkler-straße	Göschwitz-Bhf	Jena Nord-1	Jena Nord-2	Jena West-Bhf	Porstendorf-Bhf	Saalbahnhof	Zwätzen
4–13 June	285/0	–	–	–	–	–	6 755/15	490/0	–	3400/5	375/0
14–23 June	265/10	755/95	–	–	4850/70	–	–	–	–	–	1025/15
24 June–3 July	–	–	3860/5	1305/110	–	4335/115	6 140/145	585/100	11 255/10	1515/110	–
4–13 July	195/25	272/210	6330/45	750/210	3270/245	4040/450	11 650/290	3490/160	28 710/30	815/110	550/5
14–23 July	80/0	2800/250	7580/50	270/185	2835/175	975/295	6 525/305	820/50	35 020/60	550/65	540/70
24 July–2 August	115/10	4420/235	4050/25	185/50	1345/90	365/95	6 870/255	280/35	41 365/45	865/20	1435/25
22–31 August	370/30	3455/220	2420/100	300/0	710/10	4830/70	11 100/195	270/65	39 655/0	300/0	2010/45
1–10 September	360/60	4025/90	2510/35	30/0	1595/5	4650/70	8 820/160	330/40	19 990/25	300/0	2380/0
11–20 September	30/30	1360/30	–	30/0	965/70	4555/230	7 585/100	215/35	–	70/30	–
21–30 September	–	–	150/35	–	–	–	–	–	6 320/0	–	1200/10
1–10 October	0/0	135/0	5/0	0/0	695/150	–	–	30/5	1 225/0	0/0	35/5
21–30 October	–	–	0/0	–	135/85	–	–	0/0	155/0	0/0	–

–, no data collected.

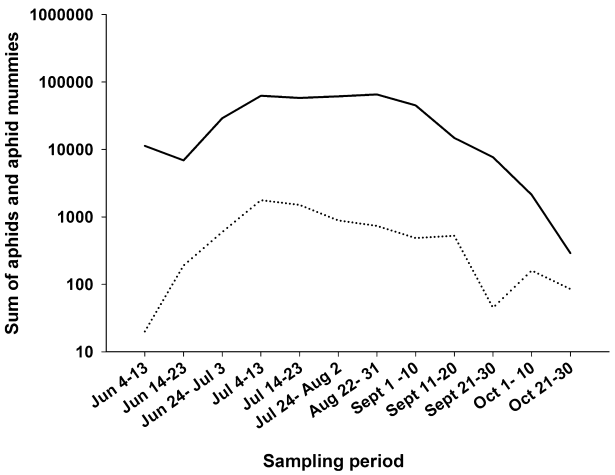

Figure 2. Over all the 11 sites sampled over time, *Lysiphlebus hirticornis* population dynamics as established from mummified aphids (dotted line) was relatively low compared to their aphid host, *Metopeurum fuscoviride* (solid line).

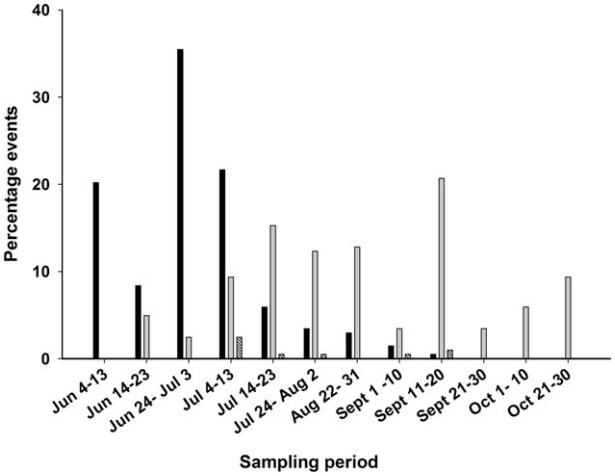

Figure 3. Over all the 11 sites sampled, aphid colonization events (black bars) continued until 11–20 September; aphid extinction events (light grey bars) began on 14–23 June and continued throughout the season, and aphid recolonization following extinction events (patterned bars) were observed starting from 14–23 July until 11–20 September.

$\chi^2 = 20.1$, d.f. = 16, $P = 0.216$). At the other nine loci, between two and 11 sites had samples deviating from Hardy–Weinberg expectations (Table 2), and significantly so over all sites. Between one and 11 sites showed significant heterozygote deficit at the various microsatellite loci tested (Table 2). Exact tests for genotypic linkage disequilibrium using Fisher's method showed 43 significant ($P < 0.05$) values out of the possible 55 pairwise combinations.

Table 2. Diversity indices of the 11 microsatellite loci used in genotyping of *Lysiphlebus hirticornis* obtained from 610 individuals

Locus	Alleles	H_E	H_O	HWE dev	Het. def
Lhirt01	23	0.821	0.369	9***	9
Lhirt02	8	0.395	0.221	7***	7
Lhirt03	10	0.794	0.144	11***	11
Lhirt04	19	0.828	0.482	10***	8
Lhirt06	11	0.615	0.328	8***	7
Lhirt08	2	0.358	0.300	2***	2
Lhirt10	3	0.582	0.382	10***	10
Lhirt15	4	0.513	0.390	6***	6
Lhirt23	6	0.617	0.474	10***	7
Lysi08	2	0.215	0.185	1^{NS}	1
Lysi6b12	3	0.314	0.303	1^{NS}	1

H_E, expected heterozygosity; H_O, observed heterozygosity; HWE dev, number of significant HWE deviations and overall deviation from HWE; Het. def, number of significant heterozygote deficits.
NS, not significant ($P > 0.05$), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Based on the 150 'populations' (grouping C), there were significant differences ($P < 0.05$) between the three time points in number of alleles at seven of the 11 loci. The four loci with no significant differences ($P > 0.05$) were *Lhirt08*, *Lhirt10*, *Lysi08* and *Lysi6b12*. The three time points were significantly different ($P < 0.05$) in terms of H_O and H_E at all 11 loci.

Based on grouping D, besides Göschwitz-Bhf in late summer ($N = 12$) and Jena West-Bhf in early summer ($N = 11$) with 9 and 10 polymorphic microsatellite loci (loci with 2 or more alleles), respectively, all other 13 site-time points showed all 11 markers to be polymorphic. There was no relationship between the number of parasitoid samples and number of polymorphic microsatellites ($r = 0.451$, $F_{1,14} = 3.323$, $P = 0.091$).

Alleles and allele frequencies

Based on the 610 individuals sampled over time (grouping B), the number of alleles recorded was 82 in early summer ($N = 179$), 82 in mid-summer ($N = 279$), and 76 in late summer ($N = 152$). Allelic richness based on the minimum sample of 152 diploid individuals (late summer sample) showed a decline over time: 80.6, 77.8, and 76.0 in early, mid and late summer, respectively. New alleles appeared, others were lost, whereas others appeared and disappeared within the single annual season (Appendix S2). Based on the 150 'populations' (grouping C), the mean ± SE number of alleles per locus was 6.93 ± 0.03 in early summer, 6.63 ± 0.03 in mid-summer, and 6.59 ± 0.02 in late summer, and was significantly different between the three time points ($F_{2,147} = 52.34$, $P < 0.001$).

The total number of alleles and allelic richness (R) showed fluctuations over the three time periods for each of the five sites (grouping D) (Table 3). In Jena Nord-1($N = 24$ parasitoids at each time period), the total number of alleles was 47 in early summer, 48 in mid-summer, and 42 in late summer. A similar trend was observed at Burgau-2, Göschwitz-Bhf, and Jena West-Bhf. The total number of alleles at Jena Nord-2 was 57 in early summer, 50 in mid-summer, and 51 in late summer. The total number of alleles was positively correlated with the number of samples analyzed ($r = 0.882$, $F_{1,14} = 45.33$, $P < 0.001$). Allelic richness based on the minimum 11 parasitoid individuals revealed that, in four of the five sites (Jena Nord-1, Burgau-2, Göschwitz-Bhf, and Jena West-Bhf), there was an increase from 'early summer' to 'mid-summer' and thereafter a decrease (Fig. 4). There was a decrease in allelic richness from 'early summer' to 'mid-summer' before an increase in 'late summer' at Jena Nord-2 (Fig. 4). The differences in allelic richness on the five sites over time was not statistically significant ($F_{14,150} = 0.207$, $P = 0.999$).

Gene diversity

On the basis of the 610 individuals sampled over time (grouping B), mean ± SD observed heterozygosity (H_O) were 0.333 ± 0.126 in early summer, 0.328 ± 0.116 in mid-summer, and 0.311 ± 0.111 in late summer. Mean ± SD expected heterozygosity (H_E) was 0.544 ± 0.210 in early summer, 0.542 ± 0.217 in mid-summer, and 0.560 ± 0.207 in late summer. On the basis of the the 150 'populations' (grouping C), mean ± SE H_O declined from 0.332 ± 0.001 in early

Table 3. Descriptive statistics of *Lysiphlebus hirticornis* collected at five sites in Jena analyzed at 11 microsatellite loci

Sites	Jena Nord-1_early	Jena Nord-1_mid	Jena Nord-1_late	Burgau-2_early	Burgau-2_mid	Burgau-2_late	Göschwitz-Bhf_early	Göschwitz-Bhf_mid	Göschwitz-Bhf_late	Jena West-Bhf _early	Jena West-Bhf _mid	Jena West-Bhf _late	Jena Nord-2_early	Jena Nord-2 _mid	Jena Nord-2 _late
N	28	28	28	23	23	23	12	12	12	11	11	11	45	45	45
Total_A	47	48	42	44	47	44	39	43	38	37	40	32	57	50	51
Mean_A	4.27	4.36	3.82	4.00	4.27	4.00	3.55	3.91	3.70	3.89	3.64	2.91	5.18	4.55	4.64
Total_R	38	40	37	39	41	38	39	42	38	37	40	32	43	39	41
Mean_R	3.42	3.65	3.33	3.55	3.73	3.49	3.51	3.82	3.41	3.36	3.64	2.91	3.87	3.51	3.70
H_O	0.315	0.338	0.263	0.300	0.320	0.300	0.273	0.333	0.383	0.273	0.331	0.273	0.408	0.398	0.392
H_E	0.465	0.530	0.516	0.482	0.482	0.486	0.538	0.481	0.569	0.520	0.477	0.462	0.516	0.465	0.547
F_{IS}	0.327	0.367	0.495	0.381	0.341	0.387	0.504	0.317	0.336	0.488	0.317	0.421	0.212	0.146	0.286
Polymorphic loci (*N*)	11	11	11	11	11	11	11	11	10	9	11	11	11	11	11
HW	4	8	8	7	6	5	6	4	4	5	4	5	6	6	8

Number of samples genotyped (*N*), total number of alleles overall loci (Total_A), mean number of alleles over all loci (Mean_A), total allelic richness (Total_R), and mean allelic richness (Mean_R) based on the minimum 11 parasitoid samples. Observed (H_O) and expected (H_E) gene diversity, inbreeding coefficient (F_{IS}), and the number of loci deviating from Hardy–Weinberg equilibrium (HWE).

_early, early summer; _mid, mid-summer; _late, late summer.

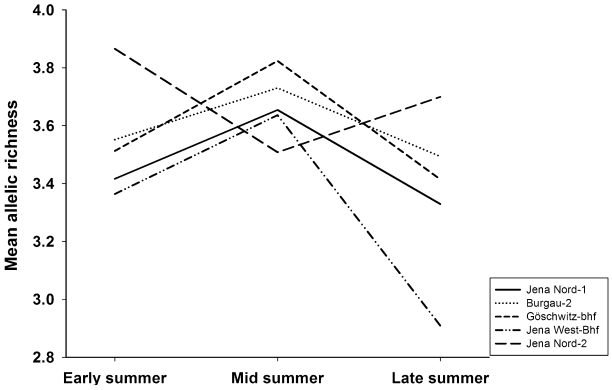

Figure 4. Mean allelic richness based on 11 parasitoid samples for five sites at three time points. At four sites, allelic richness increased in mid-summer in tandem with the high colonization events and declined in late summer when higher extinction events and reduced parasitoid population densities were recorded. The differences in allelic richness within and among the five sites over time were not statistically significant ($F_{14,150} = 0.207$, $P = 0.999$).

summer to 0.327 ± 0.002 in mid-summer, and to 0.313 ± 0.002 in late summer. The differences between the three time points in H_O were significantly different ($F_{2,147} = 32.40$, $P < 0.001$). Mean ± SE H_E decreased from 0.545 ± 0.001 in early summer to 0.540 ± 0.001 in mid-summer, and then increased to 0.562 ± 0.001 in late summer. The differences between the three time points in H_E were also significantly different ($F_{2,147} = 102.18$, $P < 0.001$).

At the five sites (grouping D), H_E ranged from 0.462 at Jena West-Bhf in late summer to 0.569 at Göschwitz-Bhf in late summer (Table 3). Mean H_O increased from early summer to mid-summer and decreased by late summer at Jena Nord-1, Burgau-2, and Jena West-Bhf. At Göschwitz-Bhf, mean H_O increased throughout the season, whereas, at Jena Nord-2, it decreased from 0.408 to 0.392 (Table 3). Neither the differences in H_E ($F_{14,150} = 0.278$, $P = 0.995$) and H_O ($F_{14,147} = 0.833$, $P = 0.633$) at the five sites over time, nor their relationship to the number of samples analyzed (H_E: $r = 0.017$, $F_{1,14} = 0.004$, $P = 0.952$; H_O: $r = 0.471$, $F_{1,14} = 3.701$, $P = 0.079$) was significant.

INBREEDING COEFFICIENTS (F_{IS})

Average F_{IS} increased from 0.390 in early summer to 0.394 in mid-summer and to 0.446 in late summer based on the 610 parasitoids sampled over time (grouping B). On the basis of the 150 'populations' (grouping C), mean ± SE F_{IS} increased from 0.392 ± 0.002 in early summer to 0.396 ± 0.004 in mid-summer, and to 0.444 ± 0.002 in late summer, and the

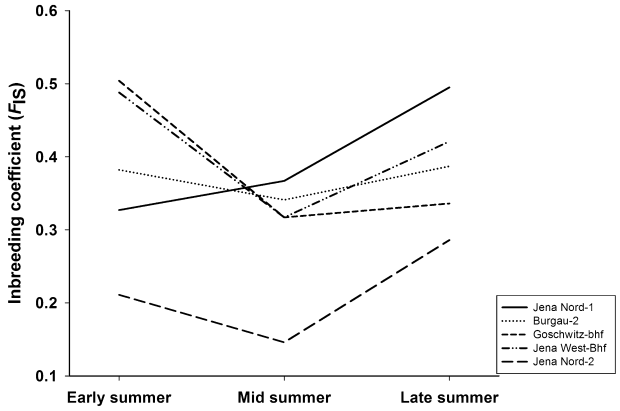

Figure 5. At four of the five sites, the inbreeding coefficient (F_{IS}) declined in mid-summer, consistent with colonization events, and increased in late summer, an indication of reduced population densities consistent with extinction events. Differences in F_{IS} within and among sites at the five sites over time were not significant ($F_{14,147} = 1.121$, $P = 0.345$).

differences between the three time points were highly significant ($F_{2,147} = 114.43$, $P < 0.001$). At the five sites over time (grouping D), average F_{IS} values ranged from 0.218 at Jena Nord-2 to 0.40 at Jena West-Bhf (Table 3). The general trend was an increase in mean F_{IS} at Jena Nord-1 throughout the growing season, whereas the other four sites showed an initial decrease in mid-summer followed by an increase in late summer (Fig. 5). The differences in F_{IS} on the five sites over time were not significant ($F_{14,147} = 1.121$, $P = 0.345$). F_{IS} was not dependent on number of samples analyzed ($r = 0.467$, $F_{1,14} = 3.632$, $P = 0.079$).

AMOVA

Analysis of molecular variance for the 610 parasitoids over time (grouping B), namely early summer ($N = 179$), mid-summer ($N = 279$), and late summer ($N = 152$), with 10 000 permutations revealed that percent variance 'among the time periods' was 0.77% (d.f. = 2, $P < 0.001$), 'among individuals within time periods' was 40.28% (d.f. = 607, $P < 0.001$), and 'within individuals' was 58.95% (d.f. = 610, $P < 0.001$).

When the AMOVA was carried out for five sites only (grouping D) at the three time periods, between 1.1% (Jena Nord-1) and 6.8% (Burgau-2) (mean ± SE, 3.29 ± 0.965%) was accounted for by 'among time periods', between 21.3% (Jena Nord-2) and 39.4% (Jena Nord-1) (mean ± SE, 34.45 ± 3.406%) by 'among individuals within time period', and between 57.4% (Jena West-Bhf) and 76.4% (Jena Nord-2) (mean ± SE, 62.28 ± 3.538%) of the variance was attributed to variation within individuals (Table 4).

AMOVA of the 11 sites split into 72 genets (grouping E) revealed that 9% (d.f. = 10, $P < 0.001$) of the variance was accounted for by 'among sites' variation and 11.8% (d.f. = 61, $P < 0.001$) 'among genets within sites', 20.9% (d.f. = 538, $P < 0.001$), 'among individuals within genets' and 58.3% (d.f. = 610, $P < 0.001$) 'within individuals'.

At the level of genets at all sites over time (grouping F), 13.3% (d.f. = 71, $P < 0.001$) of the variance was accounted for by 'among genets', 10.1% (d.f. = 31, $P < 0.001$) 'among time periods within genets', 17.6% (d.f. = 507, $P < 0.001$) 'among individuals within genets', and 58.9% (d.f. = 610, $P < 0.001$) 'within individuals'.

Genetic differentiation among and within populations

Pairwise F_{ST} of 'grouping B' (Table 5) revealed that there was significant genetic differentiation between the three time periods ($P < 0.05$).

Pairwise F_{ST} values for the five sites over the three time points (Grouping D), analyzed as 15 subgroupings, showed that among site-time comparisons, significant genetic differentiation was detected in all cases, except between Göschwitz-Bhf-early summer and Jena West in late summer. Only at Jena Nord-2 was there significant genetic differentiation among the three time points (Table 6). Mean F_{ST} for the five sites was not correlated with the number of samples analyzed ($r = 0.061$, $F_{1,4} = 0.011$, $P = 0.922$).

DISCUSSION

The present study aimed to establish the temporal genetic dynamics of the primary wasp parasitoid, *L. hirticornis*, in its natural environment. The approach chosen was to monitor the population dynamics of the parasitoid, and to sparingly collect parasitoid samples for DNA molecular analysis with minimal disturbance to the aphid–parasitoid colonies, and hence population structuring.

Both the specialist tansy aphid, *M. fuscoviride* and, consequently, its specialist parasitoid, *L. hirticornis*, show local extinction and recolonization phases on tansy (Weisser, 2000; Weisser & Härri, 2005; Zheng *et al.*, 2009). Over a single annual season, parasitoid genetic structuring and a decline in genetic diversity was recorded. There were differences in the levels of genetic diversity over time within parasitoid metapopulations, and genetic divergence among such populations (i.e. there was both a statistically significant genetic structural change as well as nonsignificant differences among samples taken from the same locality at different sampling times). These results reveal that regional genetic structure in *L. hirticornis* can be

Table 4. Analysis of molecular variance table for five sites, each analyzed as three temporal subpopulations (early summer, mid-summer, and late summer)

Site	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Jena Nord-1	Among time points	2	11.38	0.032	1.13
	Among individuals within time points	81	316.05	1.112	39.39
	Within individuals	84	141.00	1.679	59.48
	Total	167	468.43	2.822	
Burgau-2	Among time points	2	25.44	0.197	6.84
	Among individuals within time points	66	242.17	0.990	34.44
	Within individuals	69	116.50	1.688	58.72
	Total	137	384.11		
Göschwitz-Bhf	Among time points	2	11.63	0.076	2.60
	Among individuals within time points	33	131.33	1.115	37.91
	Within individuals	36	63.00	1.750	59.50
	Total	71	205.96	2.941	
Jena West-Bhf	Among time points	2	11.14	0.090	3.41
	Among individuals within time points	30	107.59	1.036	39.22
	Within individuals	33	50.00	1.515	57.37
	Total	65	168.73	2.641	
Jena Nord-2	Among time points	2	19.05	0.068	2.36
	Among individuals within time points	132	451.66	0.613	21.30
	Within individuals	135	296.50	2.196	76.35
	Total	269	767.20	2.877	

Table 5. Pairwise F_{ST} test for the 610 parasitoid samples over the three time points

Time point	Early summer	Mid-summer	Late summer
Early summer		0.00089	0.00000
Mid-summer	0.00519		0.00000
Late summer	0.01237	0.01057	

F_{ST} values are below the diagonal, whereas *P*-values are above the diagonal.

highly pronounced and very unstable. Examination of genetic differentiation results further revealed that not only were some sites isolated temporally, but also spatially, and that the events unfolding at each of the sites (resource patch) were largely independent of each other.

At three of the five sites analyzed as a function of time, there was a lack of genetic differentiation between early summer and mid-summer subpopulations, indicative of temporary stability. By way of contrast, on all five sites, significant genetic differentiation was found either between early summer and late summer, or between mid-summer and late summer, an indication of temporal instability. The genetic homogenization of sub-populations at some sites did not result in a general loss of genetic diversity among populations comprising the metapopulation, which as demonstrated in the present study, appears stable at some sites, whereas it is increasing or decreasing in others. Such differences are consistent with the effects of different micro-evolutionary processes affecting both effectively neutral as well as adaptive genetic variation in natural populations.

The strong patterns of genetic differentiation at the site level, and temporally within a site, suggest a small population size as shown in the present study through low number of mummified aphids, and reduced contemporary dispersal (Nyabuga *et al.*, 2010). At Jena Nord-2, there was a significant genetic differentiation at all three time points, such that there was either a lack of inter-colony gene flow with time or a revolution in terms of gene/genotype frequencies, or both. Significant population structure and genetic differences detected at the time points at a local level are likely the result of aphid population density fluctuations at the local spatial scales tested. The persistence of differentiation among populations over all three time points in the season shows that the spatial genetic structure may not be directly connected to seasonal or density dependent factors. Indeed, the results obtained in the present study revealed that parasitoid abundance was not correlated with aphid abundance. Variance effective population size is affected by the sex ratio in the breeding population, inter-individual variation in offspring

Table 6. Pairwise F_{ST} test for each of the five sites, each at three time points analyzed as 15 populations

Site and time point	Jena Nord-1_early	Jena Nord-1_mid	Jena Nord-1_Late	Jena Burgau-2_early	Jena Burgau-2_mid	Jena Burgau-2_late	Göschwitz-Bhf _early	Göschwitz-Bhf _mid	Göschwitz-Bhf _late	Jena West-Bhf _early	Jena West-Bhf _mid	Jena West-Bhf _late	Jena Nord-2 _early	Jena Nord-2 _mid	Jena Nord-2 _late
Jena Nord-1_early		–	+	+	+	+	+	+	+	+	+	+	+	+	+
Jena Nord-1_mid	0.006		–	+	+	+	+	+	+	+	+	+	+	+	+
Jena Nord-1_Late	0.037	0.013		+	+	+	+	+	+	+	+	+	+	+	+
Burgau-2_early	0.116	0.120	0.139		–	+	+	+	+	+	+	+	+	+	+
Burgau-2_mid	0.119	0.122	0.139	–0.003		+	+	+	+	+	+	+	+	+	+
Burgau-2_late	0.159	0.158	0.169	0.111	0.110		+	+	+	+	+	+	+	+	+
Göschwitz-Bhf _early	0.119	0.118	0.138	0.066	0.065	0.082		–	–	+	+	–	+	+	+
Göschwitz-Bhf _mid	0.148	0.155	0.182	0.161	0.155	0.135	0.027		+	+	+	+	+	+	+
Göschwitz-Bhf _late	0.136	0.128	0.147	0.123	0.127	0.136	0.024	0.075		+	+	+	+	+	+
Jena West-Bhf _early	0.105	0.106	0.125	0.098	0.093	0.169	0.127	0.174	0.137		–	+	+	+	+
Jena West-Bhf _mid	0.105	0.103	0.144	0.107	0.128	0.172	0.124	0.162	0.125	0.042		–	+	+	+
Jena West-Bhf _late	0.117	0.122	0.149	0.053	0.062	0.134	0.053	0.142	0.105	0.072	0.045		+	+	+
Jena Nord-2_early	0.098	0.094	0.110	0.088	0.086	0.183	0.054	0.123	0.084	0.109	0.096	0.054		+	+
Jena Nord-2_mid	0.107	0.108	0.126	0.124	0.127	0.193	0.067	0.141	0.083	0.128	0.121	0.071	0.014		+
Jena Nord-2_late	0.147	0.126	0.123	0.124	0.126	0.183	0.070	0.139	0.095	0.136	0.123	0.074	0.019	0.044	

Lower diagonal shows F_{ST} values. Upper diagonal with + indicates significantly different populations ($P < 0.05$) and – indicates non-significant populations (i.e. $P > 0.05$). _early, early summer; _mid, mid-summer; _late, late summer.

number, generation time, and the mating system (Amos & Harwood, 1998). *Lysiphlebus hirticornis* is characterized by female biased sex ratios in the field, short and overlapping lifecycles and is considered to inbreed on the natal patches (Mackauer & Völkl, 2002; Nyabuga *et al*., 2010).

The results of the field study showed that the population dynamics of the aphid, *M. fuscoviride*, were subject to large fluctuations at the site/deme level over time. Aphid colony survival at the genet level was highly variable, with a mean of approximately 9 weeks, although high numbers of extinctions were observed within approximately 3 weeks. The causes of aphid extinction were not only a result of hymenopterous parasitoids, as shown by low rates of mummified aphids, but also predators, including hoverfly larvae (Diptera: Syrphidae), ladybird larvae and adults (Coleoptera: Coccinelidae), and lacewing larvae (Neuroptera: Chrysopidae) (Weisser & Härri, 2005; Loxdale, Nyabuga & Weisser, 2008). Obviously, aphid host extinction affects the parasitoid population. The tansy plant itself could also be a likely cause of aphid extinction: plants at different growth stages were observed within a site and, even on a single genet, ramets at different stages of growth were common (i.e. when some genets and ramets were wilting and dying, others were sprouting or flowering) (Fig. 1). Extinctions followed by recolonization events, as observed in the present study, may explain the loss of alleles and appearance of new ones over time (Appendix S2). Parasitoid allelic richness (Fig. 4) and genetic diversity trends (Table 3) showed similar patterns of fluctuations to population dynamic trends (i.e. reduced genetic diversity with increase in extinction events). Loss of allelic diversity is also an indicator of historical bottlenecks (Leberg, 1992; Brookes *et al*., 1997) and, in *L. hirticornis* within a single season, extinctions of aphid colonies also accounts for such losses. Hitherto, microgeographic genetic differences had only been reported in a number of studies of small winged insects such as aphids (De Barro *et al*., 1995; Sunnucks *et al*., 1997; Haack *et al*., 2000; Vorburger, 2006), where it was suggested that stochastic events (i.e. genetic drift or founder events) were mainly responsible for the observed changes in population gene/genotype frequencies.

The tansy plant provides a resource with a patchy distribution at various spatial scales: (1) aphid colonies form on single tansy ramets; (2) as a result of asexual plant growth, tansy genets provide the next spatial level in the system; and (3) individual genets in field sites (Weisser, 2000). A single *L. hirticornis* female lays some 40–70 eggs on a single aphid colony on a ramet (Mackauer & Völkl, 2002). Large numbers of *L. hirticornis* mummies were found on a single ramet and even on several ramets on a single genet.

Such a system undoubtedly creates a good breeding ground involving sib-mating for eclosing adult parasitoids, which in turn results in local inbreeding. Inbreeding may create fine-scale genetic structuring (Chesser, 1991b) and, indeed, the results of an AMOVA in the present study showed that the genet rather than the site represents the level of genetic sub-structuring in *L. hirticornis*.

Positive inbreeding coefficients (F_{IS}) and deficiency of heterozygotes were also observed at particular sites and temporally within some sites. The deficiency of heterozygotes appears to be biologically founded, rather than a result of purely stochastic influences (i.e. drift). *L. hirticornis* has an arrhenotokous mode of reproduction (parthenogenetic production of haploid males from unfertilized eggs) and, as already mentioned, females have been reported to lay many eggs on the aggregated aphid colonies (Mackauer & Völkl, 2002). In cases where the parasitoids emerge and no suitable aphid hosts are found, either as a result of the unavailability of aphid hosts (extinctions) or because those available have already been parasitized, the adult wasps have no choice but to disperse in search of new resources. Such dispersal might create 'temporal Wahlund effects' (Van Rossum & Triest, 2006) and thus positive F_{IS} values as a result of homozygous excess or insignificant pairwise population differentiation, as found in some sites over time in the present study.

Departures from HWE always involved heterozygote deficiency, which could have been as a result of the presence of null alleles, Wahlund effects, and/or inbreeding as discussed above. Null alleles occur when a mutation prevents annealing of one of the oligonucleotide primers at a given locus and hence prevents amplification of the products, resulting in an underestimation of the number of heterozygotes (Pemberton *et al*., 1995). Earlier evidence showed that such null alleles were rare at the loci screened in *L. hirticornis* (Nyabuga *et al*., 2009).

The intensity of sampling is known to influence genetic parameters. As observed by Leberg (2002), there was a nonlinear relationship between total number of alleles and number of samples. Small sample sizes may under estimate genetic diversity and overestimate population subdivision (Sinclair & Hobbs, 2009). At Göschwitz-Bhf ($N = 12$) and Jena West-Bhf ($N = 11$), the number of polymorphic loci was reduced, which probably led to an underestimation of genetic diversity. Both significant and nonsignificant within-site differentiation results were obtained, although there was no relationship between sample size and mean F_{ST}. Furthermore, there was no relationship between sample size and other genetic parameters such as H_O, H_E, and F_{IS}. Because the broad aim of the present study was to show genetic patterns over time (in which case, we standardized sample sizes within site), and not to compare sites, the variability in sample size among sites is not a serious problem.

In conclusion, the present study provides a unique demonstration of genetic variation and population sub-structuring in a specialist primary parasitoid attacking a specialist aphid in a metapopulation system over a single growing season. This metapopulation is characterized by extinction and recolonization, and parasitoid inbreeding at the genet level, which explains the disappearance of existing alleles/genotypes, as well as the appearance of new alleles, and enhances parasitoid population differentiation, whilst reducing genetic diversity over the season. In *L. hirticornis*, a high molecular variance at the genet level compared with the site level strongly implies that population differentiation occurs at the fine-scale level of the genet.

ACKNOWLEDGEMENTS

We thank Professor John Allen and the two anonymous reviewers for their helpful comments on the manuscript. F.N.N. thanks the Deutscher Akademischer Austauschdienst (DAAD) for financial support.

REFERENCES

Abbott JK, Bensch S, Gosden TP, Svensson EI. 2008. Patterns of differentiation in a colour polymorphism and in neutral markers reveal rapid genetic changes in natural damselfly populations. *Molecular Ecology* **17:** 1597–1604.

Amos W, Harwood J. 1998. Factors affecting levels of genetic diversity in natural populations. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **353:** 177–186.

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.

Brookes MI, Graneau YA, King P, Rose OC, Thomas CD, Mallet JLB. 1997. Genetic analysis of founder bottlenecks in the rare British butterfly *Plebejus argus*. *Conservation Biology* **11:** 648–661.

Chesser RK. 1991a. Gene diversity and female philopatry. *Genetics* **127:** 437–447.

Chesser RK. 1991b. Influence of gene flow and breeding tactics on gene diversity within populations. *Genetics* **129:** 573–583.

De Barro PJ, Sherratt TN, Brookes CP, David O, Maclean N. 1995. Spatial and temporal genetic variation in British field populations of the grain aphid *Sitobion avenae* (F) (Hemiptera: Aphididae) studied using RAPD-PCR. *Proceedings of the Royal Society of London Series B, Biological Sciences* **262:** 321–327.

Dempster JP, Atkinson DA, Cheesman OD. 1995. The spatial population dynamics of insects exploiting a patchy food resource. 1. Population extinctions and regulation. *Oecologia* **104:** 340–353.

Eber S, Brandl R. 1994. Ecological and genetic spatial patterns of *Urophora cardui* (Diptera: Tephritidae) as evidence for population structure and biogeographical processes. *Journal of Animal Ecology* **63:** 187–199.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1:** 47–50.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes – application to human mitochondrial-DNA restriction data. *Genetics* **131:** 479–491.

Godfray HCJ. 1994. *Parasitoids: behavioural and evolutionary ecology*. Princeton, NJ: Princeton University Press.

Goodisman MAD, Sankovich KA, Kovacs JL. 2007. Genetic and morphological variation over space and time in the invasive fire ant *Solenopsis invicta*. *Biological Invasions* **9:** 571–584.

Goudet J. 2002. FSTAT, A program to estimate and test gene diversities and fixation indices. 2.9.3.2 ed.

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.

Hanski I. 1999. *Metapopulation ecology*. Oxford: Oxford University Press.

Husband BC, Barrett SCH. 1996. A metapopulation perspective in plant population biology. *Journal of Ecology* **84:** 461–469.

Leberg PL. 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution* **46:** 477–494.

Leberg PL. 2002. Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology* **11:** 2445–2449.

Loxdale HD, Brookes CP. 1990. Temporal genetic stability within and restricted migration (gene flow) between local-populations of the blackberry-grain aphid *Sitobion fragariae* in south-east England. *Journal of Animal Ecology* **59:** 497–514.

Loxdale HD, Nyabuga FN, Weisser WW. 2008. The 'cake on the plate' syndrome . . . and how specialist tansy aphids avoid extinction by hungry predators and parasitoids: insights from the use of polymorphic microsatellite markers. In: Rossing W, ed. *Landscape management for functional biodiversity*. Bordeaux: France IOBC/wprs Bulletin, 61–64.

Lynch M, Conery J, Burger R. 1995. Mutation accumulation and the extinction of small populations. *American Naturalist* **146:** 489–518.

Mackauer M, Völkl W. 2002. Brood-size and sex-ratio variation in field populations of three species of solitary aphid parasitoids (Hymenoptera: Braconidae, Aphidiinae). *Oecologia* **131:** 296–305.

Nyabuga FN, Loxdale HD, Heckel DG, Weisser WW. 2010. Spatial population dynamics of a specialist aphid parasitoid, *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae: Aphidiinae): evidence for philopatry and restricted dispersal. *Heredity* **105:** 433–442.

Nyabuga FN, Loxdale HD, Sharbel TF, Todd M, Weisser WW. 2009. Microsatellites from *Lysiphlebus hirticornis* Mackauer (Hymenoptera: Braconidae), a specialist primary parasitoid attacking the specialist tansy aphid, *Metopeurum fuscoviride* Stroyan (Hemiptera: Aphididae). *Molecular Ecology Resources* **9:** 931–934.

Pemberton JM, Slate J, Bancroft DR, Barrett JA. 1995. Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology* **4:** 249–252.

R Development Core Team. 2008. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Rauch G, Weisser WW. 2007. Local and spatial dynamics of a host-parasitoid system in a field experiment. *Basic and Applied Ecology* **8:** 89–95.

Raymond M, Rousset F. 1995. Genepop (Version 1.2) – population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86:** 248–249.

Rhomberg LR, Joseph S, Singh RS. 1985. Seasonal-variation and clonal selection in cyclically parthenogenetic rose aphids (*Macrosiphum rosae*). *Canadian Journal of Genetics and Cytology* **27:** 224–232.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* **392:** 491–494.

Sinclair EA, Hobbs RJ. 2009. Sample size effects on estimates of population genetic structure: implications for ecological restoration. *Restoration Ecology* **17:** 837–844.

Slatkin M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* **16:** 393–430.

SPSS. 2007. *SPSS base 16.0 for Windows user's guide*. Chicago, IL: SPSS Inc.

Sugg DW, Chesser RK, Dobson FS, Hoogland JL. 1996. Population genetics meets behavioral ecology. *Trends in Ecology & Evolution* **11:** 338–342.

Sunnucks P, De Barro PJ, Lushai G, Maclean N, Hales D. 1997. Genetic structure of an aphid studied using microsatellites: cyclic parthenogenesis, differentiated lineages and host specialization. *Molecular Ecology* **6:** 1059–1073.

Sunnucks P, Hales DF. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution* **13:** 510–524.

Van Rossum F, Triest L. 2006. Fine-scale genetic structure of the common *Primula elatior* (Primulaceae) at an early stage of population fragmentation. *American Journal of Botany* **93:** 1281–1288.

Vorburger C. 2006. Temporal dynamics of genotypic diversity reveal strong clonal selection in the aphid *Myzus persicae*. *Journal of Evolutionary Biology* **19:** 97–107.

Wade MJ, McCauley DE. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution* **42:** 995–1005.

Weir BS, Cockerham CC. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38:** 1358–1370.

Weisser WW. 2000. Metapopulation dynamics in an aphid-parasitoid system. *Entomologia Experimentalis et Applicata* **97:** 83–92.

Weisser WW, Härri SA. 2005. Colonisations and extinctions at multiple spatial scales: a case study in monophagous herbivores. *Annales Zoologici Fennici* **42:** 363–377.

Whitlock MC, McCauley DE. 1990. Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. *Evolution* **44:** 1717–1724.

Wright S. 1940. Breeding structure of populations in relation to speciation. *American Naturalist* **74:** 232–248.

Zheng CZ, Weisser WW, Härri SA, Ovaskainen O. 2009. Hierarchical metapopulation dynamics of two aphid species on a shared host plant. *American Naturalist* **174:** 331–341.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Sample sizes and their distribution within the sites on genets, and on genets over time.
Appendix S2. Allele frequencies of the 610 genotyped parasitoids grouped into three time points at each of the 11 microsatellite loci.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.