Metapopulation structure of the specialized herbivore *Macrosiphoniella tanacetaria* (Homoptera, Aphididae)

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**Abstract**

We investigated population dynamics, genetic diversity and spatial structure in the aphid species *Macrosiphoniella tanacetaria*, a specialist herbivore feeding on tansy, *Tanacetum vulgare*. Tansy plants (genets) consist of many shoots (ramets), and genets are grouped in sites. Thus, aphids feeding on tansy can cluster at the level of ramets, genets and sites. We studied aphid population dynamics in 1997 and 2001 and found that within sites: (i) at any time, aphids used only a fraction of the available ramets and genets; (ii) at the level of ramets, most aphid colonies survived only one week; (iii) at the level of genets, mean survival time was less than 4 weeks; and (iv) colonization and extinction events occurred throughout the season. We sampled aphids in seven sites in the Alsace region, France (4–45 km apart) and two sites in Germany in 1999 to study genetic structure within and between populations. Genetic analyses using nine microsatellite loci showed that: (i) genotypic variability was high, (ii) none of the populations was in Hardy–Weinberg equilibrium, (iii) heterozygote deficits and linkage disequilibria were frequent, and (iv) all populations were genetically differentiated, even at a small geographical scale. Renewed sampling of the Alsace sites in 2001 showed that three populations had become extinct and significant genetic changes had occurred in the remaining four populations. The frequencies of extinction and colonization events at several spatial scales suggest a hierarchical metapopulation structure for *M. tanacetaria*. Frequent population turnover and drift are likely causes for the genetic differentiation of *M. tanacetaria* populations.

**Keywords:** aphid, colonization, extinction, genetic differentiation, metapopulation, microsatellite

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**Introduction**

Most species display population structure at some spatial scale. The effects of spatial subdivision on genetic variation have long been of great interest to evolutionary biologists because of its importance for evolutionary processes like local adaptation and speciation (Wright 1931; Pannell & Charlesworth 2000). Within a metapopulation framework, the resulting genetic structure strongly depends on the details of the processes of local extinction, re-colonization and migration (McCauley 1991; Harrison & Hastings 1996; Pannell & Charlesworth 2000). For example, population turnover can lead to an increase or a decrease in population differentiation depending on whether colonizers are likely to originate from the same or different local populations, and whether their number is high or low compared to the number of migrants into established populations (Wade & McCauley 1988; Whitlock & McCauley 1990; Barton & Whitlock 1997). Population size fluctuations, the magnitude and duration of population bottlenecks and the degree of inbreeding can also qualitatively affect total genetic variation and population differentiation (Harrison & Hastings 1996; Hedrick & Gilpin 1997; Pannell & Charlesworth 2000; Keller et al. 2001). For this reason, it is difficult to infer the causes of a particular population genetic structure solely from measures of genetic differentiation within and among populations. Additional
ecological data are needed to provide insight into the spatial structure of populations.

Many examples of metapopulations come from insects feeding on patchily distributed resources (Harrison & Taylor 1997; Hanski 1999; Roslin 2001). Natural causes of extinction for local populations include demographic stochasticity, local resource depletion, sensitivity to weather conditions, or the action of natural enemies (e.g. McCauley 1989; Whitlock 1992; Thomas 1994; Hanski 1999; Weisser 2000). Most studies on insect metapopulations are, however, ecological rather than genetic in their approach, and studies combining ecological and genetic approaches to understand genetic differentiation in metapopulations are rare (but see McCauley 1989; Whitlock 1992; Eber & Brandl 1994; Lewis et al. 1997; Amos & Harwood 1998; Roslin 2001). In addition, there is a strong bias in the current literature towards studies of butterfly metapopulations, such that empirical information on other insect taxa is still scarce (Roslin 2001).

In this paper we investigate the metapopulation dynamics and genetic structure of the aphid species Macrosiphoniella tanacetaria Kaltenbach at different spatial scales. This aphid is an oligophagous species feeding mainly on the perennial composite tansy Tanacetum vulgare L., as well as on some tansy relatives, such as Chrysanthemum spp., Matricaria spp. (Heie 1980, 95) which are not common in our study areas. Macrosiphoniella tanacetaria is a cyclical parthenogen (i.e. several parthenogenetic generations followed by a single sexual generation within the annual life cycle) and most individuals are wingless during spring and summer. Winged asexual females occur only sporadically and often colonize new plants (Weisser & Massonnet, unpublished data). Sexual morphs of M. tanacetaria are induced in autumn and mate on tansy. After mating with winged males, sexual wingless females lay diapausing eggs that will hatch the following spring and initiate new asexual lineages. Tansy is native to Europe and Asia (Wagenitz 1987). Originally growing on riverbanks, tansy can now be frequently found along roadsides and in wastelands. Individual plants (= genets) consist of one to many shoots (= ramets). In the field, tansy genets can be easily delimited and a few to several hundred genets can be found at a site. Sites are separated from one another by vegetation unsuitable for the survival of M. tanacetaria. Thus, aphids on tansy cluster at the scale of ramets, at the scale of genets, and at the scale of sites. In this paper, we will refer to all aphids on a single ramet as an aphid colony and to all aphids at a particular site as a single population.

In this study, we studied the population dynamics of aphids on labelled tansy genets in the field, and investigated the population genetic structure of closely located aphid populations (a few kilometres separated the closest populations) using microsatellite loci. We aimed to estimate: (i) the level of aphid colony extinction and colonization rates in the field at the level of ramets and genets, (ii) genetic diversity within aphid populations, (iii) population differentiation at a local spatial scale, and (iv) the stability of the genetic structure of aphid populations sampled in a 2-year interval.

Materials and methods

Aphid metapopulation dynamics

As a study area, two sites along the river Rhine in France, close to Basel, Switzerland (N 47°34'00", E 7°36'00") were chosen (cf. Weisser 2000). In 1997, 107 genets at the two sites were marked and inspected every week for the occurrence of Macrosiphoniella tanacetaria. As the emphasis in this study was not on spatial patterns, results from both sites were grouped together. Spatial variability in M. tanacetaria survival among a large number of sites will be the focus of a separate study. All ramets of all genets were inspected weekly (except for week 19, 11–17 September). The study started from the hatching of aphid eggs in spring (week 1, 8–15 May 1997) and continued for the entire season, until the last aphids died in autumn. Macrosiphoniella tanacetaria starts producing sexual individuals (males and sexual females) in October and these lay eggs on ramets or nearby vegetation (B. Massonnet, unpublished data). In this study, eggs produced by sexual females were not counted. If a ramet was colonized by aphids, it was marked individually. The size of marked colonies was noted each week until the last aphid in the colony had died. In this paper, we report the occupancy of ramets and genets. Data on temporal changes in population size will be published elsewhere. Because our aim was to determine the fate of aphid colonies using a noninvasive approach (Addicott 1978), no samples were taken at the field site. From the data, we calculated the percentages of genets and ramets occupied each week, the survival of aphids at the level of ramets and genets and the weekly colonization and extinction rates.

For comparison, a similar study was conducted in 2001 at six sites in Jena, Germany (N 50°53’00”, E 11°34’00”) where a total of 212 genets were marked and followed from egg hatch at the end of April (week 1, 26 April to 2 May) until the last sexual individuals died at the end of November (week 27, 27 November to 3 December).

Sampling for population genetic analysis

To investigate small-scale differentiation between populations of M. tanacetaria, seven populations were sampled in the Alsace region of France (Fig. 1): Mulhouse (N 47°44’47”, E 7°17’36”), Rosenau (N 47°38’16”, E 7°32’11”), Kembs (N 47°41’43”, E 7°30’27”), Breisac (N 48°02’40”, E 7°34’21”), NeufBris (N 48°01’05”, E 7°31’43”), Ruiss
applied in May 2001, when the seven Alsace populations occupied genets at a site, and stored in 100% ethanol at.

The composition of populations was minimized. Adult females foundresses (i.e. asexual females that start a new clone parthenogenetic generations after the emergence of the year, aphid colonies had gone through one to two generations.

For the genetic analysis, we aimed at genotyping 30 aphids from different ramets were genotyped. In addition, in six sites in Jena (Germany).

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Fig. 1 Location of the aphid populations used for the genetic analyses: Bayreuth and Münster, in Germany; and 1, Breisac; 2, NeufBris; 3, Ruiss; 4, Mulhouse; 5, Edf; 6, Kembs; 7, Rosenau; in the Alsace region, France. Location of the aphid populations used for the field surveys: two sites close to Basel (in the Alsace region) and six sites in Jena (Germany).

(N 47°56’07”, E 7°33’51”) and Edf (N 47°54’36, E 7°32’12”). The mean distance between the Alsace populations was 24.2 km (minimum 4 km from NeufBris to Breisac, maximum 45.5 km from Rosenau to NeufBris). To study genetic diversity at a larger geographical scale, two additional populations were sampled in Germany: Bayreuth (N 50°00’00”, E 11°30’00”) and Münster (N 51°58’00”, E 7°42’00”), at an average of 370 km and 460 km from the Alsace populations.

Sampling took place in May–June 1999. At this time of the year, aphid colonies had gone through one to two parthenogenetic generations after the emergence of foundresses (i.e. asexual females that start a new clone from diapausing eggs). By collecting aphids early in the season the influence of clonal reproduction on the genetic composition of populations was minimized. Adult females of M. tanacetaria were collected from all ramets of all occupied genets at a site, and stored in 100% ethanol at 4 °C until DNA extraction. The same sampling design was applied in May 2001, when the seven Alsace populations were re-visited.

Microsatellite amplification

For the genetic analysis, we aimed at genotyping 30 aphids from 30 different genets per population. We chose aphids from different tansy genets to lower the probability of repeatedly sampling the same aphid genotypes. However, since the number of occupied genets at a site was usually lower than 30, additional aphids from the same genets but from different ramets were genotyped. In addition, in some populations, there were fewer than 30 occupied ramets, thus sample size ranged from 25 to 30 aphids per population.

In total, DNA was extracted from 263 aphids using the salting-out method (Sunnucks et al. 1996). Aphids were genotyped using nine microsatellite loci (Mt1 to Mt9) isolated from M. tanacetaria (Massonnet et al. 2001). Microsatellite amplification was performed on a final volume of 10 µL using 0.2 µM of each primer, 200 µM of each dNTPs, 16 mM (NH4)2SO4, 67 mM Tris–HCl pH 8.8, 0.01% Tween-20, 1.5 mM of MgCl2, 0.5 unit of DNA Taq polymerase (Genecraft, Münster, Germany) and 1 µL of DNA (5 ng). The following amplification programme was used: initial denaturation at 94 °C for 2 min; followed by 30 cycles of denaturation at 94 °C for 20 s, annealing at 60 °C for 20 s and elongation at 72 °C for 30 s; and final extension at 72 °C for 2 min [IR-800]- and [IR-700]labelled fragments (MWG-Biotech, München, Germany) were run on a 6% polyacrylamide gel and detected using a 4200 Licor automated sequencer.

Genetic diversity in aphid populations

Because aphids reproduce by strict apomictic parthenogenesis most of the time, identical multilocus genotypes in two or more individuals within a population may be a result of clonal reproduction. To avoid the bias of identical multilocus genotypes in the analysis, we extracted from the overall data set (i.e. all genotyped aphids of a population) a data set with only one copy of each multilocus genotype per population (Sunnucks et al. 1997). This reduced data set was used for most analyses.

For each aphid population, we calculated the proportion of unique genotypes, $D^*$, as the number of multilocus genotypes divided by the number of aphids analysed (Hunter 1993). To test the power of the microsatellites to distinguish different genotypes, a simulation program was used to calculate the number of genotypes distinguished when only a subset of all microsatellites was used (J. Schumacher, unpublished). For each locus in each population, we used genetix (Belkhir et al. 1996–97) to calculate allele frequencies, observed and expected heterozygosities and the mean number of alleles per locus.

Fisher exact tests were performed to test for deviations from Hardy–Weinberg equilibrium at each locus in each population, using genepop (Raymond & Rousset 1995). When the null hypothesis of Hardy–Weinberg ‘random union of gametes’ was rejected, alternative tests were performed to test whether deviations from Hardy–Weinberg equilibrium were a result of heterozygote deficit or excess, using genepop. We tested for linkage disequilibrium between pairs of loci in each population using genepop. Level of significance of all Hardy–Weinberg and linkage disequilibrium tests were adjusted using sequential Bonferroni corrections (Rice 1989).

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Population differentiation

Differentiation between populations was based on $F$-statistics (Wright 1951) and estimated according to Weir & Cockerham (1984). Significant deviations from zero of $F$-statistics values were tested using the permutation tests in FSTAT (Goudet 1995). Pairwise $F_{ST}$ values for each population comparison were calculated using GENETIX and their significance was tested using permutation tests.

To test for genotypic differentiation, $G$-tests were applied to the four 1999 and 2001 populations using GENEPOP (Goudet et al. 1996). Mantel tests were performed to test whether there was a correlation between matrices of geographical distances and $F_{ST}/(1 – F_{ST})$ values using GENETIX. Significance of the Pearson correlation coefficient ($r$) was tested using permutation tests using GENETIX.

Statistical analysis

All means are given as means ± standard error. For statistical analysis we used SPSS 10.0.5 (SPSS Inc. 1989–99).

Results

Aphid metapopulation dynamics

In the two Basel sites studied in 1997, the number of genets and ramets colonized by aphids first increased until week 9 (3–9 June) and then declined (Fig. 2a). At the highest level of occupancy, 50% of the genets and 10% of the ramets were colonized by Macrosiphoniella tanacetaria (Fig. 2a). In the Jena sites studied in 2001, occupied genets slightly decreased from week 1 (26 April to 3 May) to week 3 (10–17 May) and then increased from 24% to 68% until week 8 (14–21 June) before it declined (Fig. 2b). At the highest level of occupancy, 68% of the genets and 33% of the ramets were occupied by M. tanacetaria (Fig. 2b). In Basel in 1997, 15.0% of the genets and 75.5% of the ramets were never occupied by the aphid. In Jena in 2001, no genet was found to be continuously occupied throughout the season and 19.8% of the genets were never occupied.

Survival of aphids was low, both at the level of ramets and genets. In Basel in 1997, aphids mostly survived for 1 week only, both at the levels of ramets and genets (Fig. 3). At the level of ramets, mean survival time of aphid colonies was 1.76 ± 0.05 weeks and no aphid colony survived for more than 9 weeks (Fig. 3). At the level of genets, mean survival time was 3.58 ± 0.28 weeks with a maximum survival time of 13 weeks (Fig. 3). In Jena in 2001, mean survival was 3.37 ± 0.11 weeks at the level of genets with a maximum survival time of 12 weeks (Fig. 3).

Colonization and extinction events occurred throughout the season at the levels of ramets and genets (Figs 4a,b and 5a,b). In Basel in 1997, most ramets (76.5%) and genets (73.6%) were colonized only once and 25.5% of ramets and 26.4% of genets were re-colonized after an extinction. At the level of genets, the first extinction event occurred as early as week 5 (24–30 May) and the last colonization event occurred in week 15 (14–20 August), 2 weeks before the last group of aphids on ramets became extinct (Fig. 4a).

Thus, in 1997 in Basel, M. tanacetaria became extinct in the sites before aphids had started to produce sexual forms. In 2001 in Jena, M. tanacetaria became extinct in the sites before aphids had started to produce sexual forms. In
Jena in 2001, 48% of the genets were colonized once, 30% were colonized twice and 22% were colonized more than twice. Sexual females and males were observed in week 23 (18–25 October) on 17 (13.5%) of the labelled genets.

In Alsace in 2001, only four (Ruiss, Mulhouse, Breisac, Rosenau) of the seven tansy sites where aphids were present in 1999 were still inhabited by *M. tanacetaria*. Extinction thus also occurs at the level of sites as well as at the level of ramets and genets.

**Genetic diversity of *M. tanacetaria* populations**

A high allelic diversity was found in the 1999 populations, with four (locus Mt1) to 16 (locus Mt6) alleles per locus. The proportion of unique genotypes (D*) found per population was high, ranging from 0.83 (Bayreuth) to 1.0 (NeufBris) (Table 1). There were no correlations between the number of alleles per population and either the number of genotypes (Pearson correlation, *N* = 9, *r* = −0.498, *P* = 0.17) or the number of aphids collected (*N* = 9, *r* = −0.434, *P* = 0.24). In total, 248 multilocus genotypes were distinguished out of the 263 aphids analysed. Multiple copies of genotypes were only found within sites and most multiple copies of genotypes were found on different, but nearby, genets (10 cases out of 15). The remaining multiple copies of genotypes were found within the same genets but from different ramets (five cases).

The simulation program showed that most genotypes could be distinguished with only five randomly selected loci out of the nine used in the study. In addition, the variance in the number of genotypes distinguished declines quickly (Fig. 6). This indicates that genotypic diversity among populations of *M. tanacetaria* was fully resolved with the first five loci. When the full data set was used, between one (NeufBris) and eight (Bayreuth) pairs of loci were in significant linkage disequilibrium (across all populations: 20 pairs of loci in significant linkage disequilibrium). When the reduced data set was used, between one (NeufBris) and four (Bayreuth) pairs of loci were still in significant linkage disequilibrium (across all populations: nine pairs of loci in significant linkage disequilibrium).

Of the 81 Fisher exact tests (nine loci times nine populations) of Hardy–Weinberg equilibrium, 25 (30.8%) showed significant deviations (Table 1). Probability tests across all loci for each population were all significant but these results have to be treated with caution because of linkage disequilibrium between some loci. Significant heterozygote deficit was found in 28 out of 81 tests (34%). Only three
tests showed significant heterozygote excess (Table 1). Thus, deviations from Hardy–Weinberg equilibrium were essentially the result of heterozygote deficit. Accordingly, multilocus $F_{IS}$ values were significantly positive in five out of nine populations (Table 1). Single-locus $F_{IS}$ values were also significantly positive at five loci across all populations and at four loci across the Alsace populations (Table 2). The multilocus $F_{IS}$ estimate was significantly positive in all populations in 1999 (Table 2).

In 2001, allelic diversity within the four remaining populations was again high, ranging from four (locus Mt3) to 13 (locus Mt6) alleles per locus. In total, 89 multilocus genotypes were distinguished among the 120 aphids analysed. All multiple copies of genotypes were found within sites, on different but nearby genets. When the full data set was used, between three (Mulhouse) and 20 (Breisac) pairs of loci were in significant linkage disequilibrium (across all populations: 31 pairs of loci in significant linkage disequilibrium). When the reduced data set was used, between none (Breisac) and five (Ruiss) pairs of loci were still in significant linkage disequilibrium (across all populations: 11 pairs of loci in significant linkage disequilibrium). Thus, in 2001 as in 1999, linkage disequilibria were common in $M. tanacetaria$ populations.

Of the 35 Fisher exact tests (nine loci times four populations, but Mt1 in Mulhouse was monomorphic) of Hardy–Weinberg equilibrium performed on the 2001 data set, seven (20.0%) showed significant deviations (Table 1). Six out of 35 tests (17.1%) for heterozygote deficit were significant. In contrast, no significant heterozygote excess was detected within the four populations (Table 1). The multilocus $F_{IS}$ estimate was significantly positive across the four Alsace populations in 2001 (Table 2).

**Table 1** Genetic diversity in nine populations of *Macrosiphoniella tanacetaria* in 1999 and 2001

<table>
<thead>
<tr>
<th>Populations</th>
<th>$N_{genets}$</th>
<th>$N_{aphids}$</th>
<th>$N_{geno}$</th>
<th>$D^*$</th>
<th>$N_a$ (SE)</th>
<th>$H_E$ (SE)</th>
<th>$H_O$ (SE)</th>
<th>$F_{IS}$</th>
<th>HW</th>
<th>HD</th>
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<td>0.62 (0.04)</td>
<td>0.57 (0.06)</td>
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<td>0.43 (0.07)</td>
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For each population: $N_{genets}$, number of tansy genets occupied by the aphids; $N_{aphids}$, number of aphids analysed; $N_{geno}$, number of genotypes found; $D^*$, proportion of multilocus genotypes found per population; $N_a$ (SE), mean number of alleles per locus (standard error); $H_E$ and $H_O$, expected and observed heterozygosities; $F_{IS}$, estimates of $F_{IS}$ values and level of significance, number of tests (out of nine) indicating significant: (i) deviations from Hardy–Weinberg equilibrium (HW), (ii) heterozygote deficit (HD), and (iii) heterozygote excess (HE). Blanks rows indicate no data due to no sampling (Bayreuth, Münster) or no aphids (Kembs, NeufBrisac, Edf).

NS, $P > 0.05$; *0.05 > $P > 0.01$; **0.01 > $P > 0.001$; ***$P < 0.001$.

Fig. 6 Number of different multilocus aphid genotypes found using different numbers of microsatellite loci.
Pairwise $F_{ST}$ values showed that all aphid populations were significantly differentiated from each other (Table 3). There was no correlation between pairwise $F_{ST}/(1 - F_{ST})$ values and geographical distances, both when all populations were analysed ($r = -0.26$, $P > 0.5$) and when the Alsace populations only were analysed ($r = -0.09$, $P > 0.5$), indicating no isolation by distance. For the 2001 data set, single-locus and multilocus $F_{ST}$ values were also significantly positive for the Alsace populations (Table 2). All aphid populations collected in 2001 were significantly differentiated from each other (Table 4). No Mantel test was performed because of the low number of aphid populations.

### Discussion

Metapopulation dynamics are often suspected in insects because of habitat discontinuities and small sizes of local populations. However, case studies have revealed that extinctions of local insect populations are often rare (Harrison & Taylor 1997; Roslin 2001) and/or that migration rates are relatively high (e.g. Roslin 2001) such that there is often little differentiation between local populations (e.g. De Barro et al. 1995; Johannesen & Loeschcke 1999; Simon et al. 1999; Roslin 2001). Theory predicts that spatial separation leads to genetic differentiation between local populations which will, under
most circumstances, be increased if extinction and colon-
ization events are common (Harrison & Hastings 1996;
Barton & Whitlock 1997; Pannell & Charlesworth 2000).
The most striking results of our study on
Macrosiphoniella
tanacetaria
are the high frequency of extinctions at several
spatial scales and the strong degree of genetic differenti-
ation between local populations.

Hanski (1999) presented four necessary conditions for
metapopulation persistence: (i) local habitats support local
breeding populations, (ii) no single population is large
enough to avoid extinction, (iii) local populations are not
too isolated so that colonization can occur, and (iv) local
dynamics are sufficiently asynchronous to make simult-
aneous extinction of all local populations unlikely. We will
discuss the results of our field study in the light of these
conditions.

**Condition 1 — discrete breeding populations**

Sites with tansy plants are separated by a matrix of
unsuitable habitat for M. tanacetaria. Wingless individuals,
which make up the vast majority of a population, cannot
migrate to other sites and most of them probably spend
all of their life in the same genet. Thus, it can be safely
concluded that sites support local breeding populations.

**Condition 2 — extinction risk of populations**

Within sites, the high extinction rate and the low
occupancy of ramets and genets by M. tanacetaria were
particularly striking. Both in 1997 and 2001 there was an ‘expansion’ phase from spring to summer, when
colonizations outnumbered extinctions, leading to an
increase in occupancy of ramets and genets, and a later
‘retraction’ phase, when occupancy dropped to low levels in
autumn. Extinctions, however, occurred throughout the
season and no genet or ramet was occupied from the
emergence of foundresses to the autumn. Because of
this high turnover within sites, there are no genets where
aphids survive indefinitely and consequently, it is likely
that in some years ramet and genet colonization rates
within a site will not compensate for the extinction rates, as
happened in the field study in 1997. Further evidence for
extinction the level of sites comes from the sampling of
populations for the genetic study when four out of seven
populations sampled in 1999 were extinct in 2001. Thus, it
is fair to conclude that each population has a high risk of
extinction.

Interestingly, habitat deterioration is unlikely to be an
important cause of extinction in M. tanacetaria. Tansy
ramets do not dry up until the end of September so most of
the extinctions of aphids were not caused by tansy death.
A more likely cause for many aphid extinctions, in par-
ticular when aphids live in small colonies, is the action of
natural enemies. Even though it is difficult to attribute a
particular extinction to the action of natural enemies
(Weisser, unpublished results), ladybirds, mirid bugs,
parasitoids and syrphid flies were frequently observed in
tansy genets and are known to have a major impact on
aphid colonies (e.g. Dixon 1998; Haack et al. 2000; Minoretti
& Weisser 2000).

**Condition 3 — recolonization**

The migration rate between aphid colonies on ramets
within a genet is likely to be high. Wingless individuals
can frequently be observed walking from one ramet to
another and this is also indicated by the high number of
recolonization events of ramets within genets. In contrast,
walking from one genet to another is difficult for aphids,
as there are risks of starvation and especially predation
(e.g. Losey & Denno 1998). Thus, colonization of genets by
wingless individuals will occur only very rarely. Most
genets will be colonized by winged morphs. In the 1997
field study, all adults were winged in 44% of the founding
colonies that initiated genet occupancy, suggesting that
these genets were colonized by winged morphs. This is a
conservative estimate as winged individuals founding a
colony may also have died before the next census date. In
M. tanacetaria colonies in 1997, winged morphs occurred in
43.2% of all colonies until the middle of July but only in 3.7% thereafter, so dispersal by winged asexual females is largely restricted to the first half of the season. Some of these winged females may have come from other sites. Colonization of empty sites was not a focus of the study, but winged individuals can fly large distances and colonization of empty sites can frequently be observed (S. Haerri, unpublished data). Thus, the third condition is also fulfilled in the system.

**Condition 4 — asynchrony in local dynamics**

A full analysis of asynchrony of local dynamics would require the monitoring of a number of different sites for several years (cf. Hanski 1999), which was beyond the scope of the present study. Within sites, asynchrony in dynamics is clearly seen as the duration of occupancy on the levels of both ramet and genet was very low, despite the persistence of aphids in a site. The within-site patterns and the observation that only some of the populations sampled in 1997 were extinct in 1999 suggest that there is some degree of asynchrony also on the level of sites. Taken together therefore the analysis of conditions 1–4 of Hanski (1999) suggests that *M. tanacetaria* persists as a metapopulation.

Because of the high turnover within sites, it is tempting to speculate about a substructuring of the aphid populations within a site. At the level of a ramet, an aphid colony can grow up to 15 generations until autumn (Massonnet & Weisser, unpublished data). Sexual females feeding on a particular genet lay their eggs somewhere on or close by the genet on which they feed. The next spring, foundresses start feeding on one of the new ramets of the same genet. At the level of genets therefore mixing of lineages takes place at least once a year. Aphids occupying a particular genet therefore seem to conform to the model of a patchy population, in the sense of Harrison & Taylor (1997).

Within a site, mixing between different genets only occurs when winged individuals leave their colony and settle on another genet of the same site. The percentage of aphids in a genet that are winged is very small (on average 8% in 2001) and most of the reproduction occurs in the wingless individuals. This prevents mixing of individuals to a degree that would synchronize the population dynamics of different genets. Thus, at each site, the genets seem to form a network with semi-independent dynamics of *M. tanacetaria*. Studying aphid populations on the level of genets in a number of adjacent sites would be required to determine the degree of synchrony in dynamics at various spatial scales.

At the level of sites, there was strong genetic differentiation between *M. tanacetaria* populations, suggesting that migration between sites is limited. This high level of population differentiation contrasts with results from polyphagous aphids. For example, populations of *M. tanacetaria* were four times more differentiated at a regional scale than populations of *Sitobion avenae* F. or *Rhopalosiphum padi* L. at the scale of a country (Simon et al. 1999; Delmotte et al. 2002). Our results emphasize that restricted gene flow can also be found in aphids, particularly in monophagous species or species that show host-specialized populations (i.e. host races) (Loxidale & Brooks 1990; Loxidale 1990; Via 1999; Via et al. 2000). Genetic differentiation of *M. tanacetaria* populations was not correlated with geographical distance. However, our sampling was not designed to test specifically for isolation by distance in this specialized phytophagous insect (cf. Peterson & Denno 1998) and sampling of more populations over a large distance is necessary to rule out isolation by distance for this species.

The temporal variability observed in the four Alsace populations sampled in 1999 and 2001 is likely to be a result of a combination of genetic drift through bottlenecks during the season and re-colonization of the sites after extinction. The fact that at present we are unable to distinguish between these two possibilities emphasizes again the necessity to combine genetic with ecological studies.

A high rate of extinction will eventually lead to a loss of genetic diversity (McCauley 1991; Barton & Whitlock 1997; Amos & Harwood 1998). In our study, however, both allelic and genotypic diversities were high. Similar results were found in a study of metapopulation genetic structure of facultative sexual freshwater invertebrates (Freeland et al. 2000). The high level of genetic diversity observed in *M. tanacetaria* populations is consistent with the occurrence of a regular sexual phase (cf. Delmotte et al. 2002). In our study, the level of observed heterozygosity ranged from 0.41 to 0.63 (mean = 0.52), which was in the range of other sexual aphid populations studied with microsatellites (e.g. *H. curvula* in *Rhopalosiphum padi*, Delmotte et al. 2002; *H. rufipes* = 0.41 in *Sitobion avenae*, Simon et al. 1999). Significant deviations from Hardy–Weinberg equilibrium were common, most often associated with a significant deficit in homozygotes. Linkage disequilibria between pairs of loci were often observed, even when multiple copies of same genotypes were removed.

Could this heterozygote deficit result from the presence of null alleles, i.e. the failure to amplify one of the alleles in an individual? Up to three amplification attempts were carried out for a particular locus in an individual. Following this procedure, only 10 amplifications were unsuccessful (0.4% of all 2367 amplifications, less than two per locus). While we cannot rule out the possibility that null alleles were present, they are unlikely to be a cause for the observed low heterozygosity. Sampling clonal organisms some time after sexual reproduction can also lead to a heterozygote deficit when clonal diversity decreases rapidly because of drift or competition between different clones (Gomez & Carvalho 2000; Delmotte et al. 2002). We sampled aphids early in the season, one to two generations later.
after sexual reproduction, to minimize the effect of clonal reproduction on our data. There was also no relationship between sampling date and the degree of heterozygote deficit (Pearson correlation, $N = 9, r = 0.35, P > 0.05$). Thus, both null alleles and clonal reproduction are an unlikely explanation for the observed heterozygote deficits.

Heterozygote deficit at the level of a population can also arise through the Wahlund effect (Hedrick 1999). Although winged males may move to other genets, most mating in \textit{M. tanacetaria} probably occurred between individuals from the same genet, suggesting that the unit of random mating is within a genet or a group of closely located genets, rather than at the level of the whole site. In this study, the number of aphid colonies found per genet was too low to investigate the occurrence of a finer genetic structure within a site but this will be the focus of further investigations.

In our study, we found linkage disequilibria in all aphid populations, between different pairs of loci. Such disequilibria can be caused by different factors, including physical linkage between loci, inbreeding, genetic drift, or migration events (Hedrick 1999). One microsatellite locus is X-linked (Mt6; B. Massonnet, unpublished data) but the precise locations of the eight remaining microsatellites on the autosomal chromosomes are unknown. As such, we cannot reject the possibility of physical linkage between loci. However, it seems unlikely that all microsatellites are located on the same chromosomes, since they have been isolated randomly from the \textit{M. tanacetaria} genome. In contrast, inbreeding certainly contributed to heterozygote deficit and linkage disequilibrium (cf. Viard et al. 1997).

Most important for the genetic structure of \textit{M. tanacetaria} populations is the combination of genetic drift and bottlenecks which can in itself lead to linkage disequilibrium (Hedrick 1999). It is likely that genetic drift acting on small population sizes will lead to a reduction of the initial genotypic diversity present in aphid populations in spring and the linkage disequilibrium in \textit{M. tanacetaria} may be reinforced by mating between closely related individuals from the same genet. In addition, after extinction of a local population, a site will be colonized by an unknown number of individuals from other sites, and, over the time-course of the season, there may be additional immigrants. These founding effects in association with clonal reproduction probably account for much of the observed linkage disequilibrium and heterozygote deficit seen in \textit{M. tanacetaria} populations. Further studies should be designed to differentiate between these different causes of the occurrence of deviations from Hardy–Weinberg equilibrium and of linkage disequilibrium.

To summarize, \textit{M. tanacetaria} has a metapopulation structure with local populations occurring in sites where the number of tansy genets is often much lower than 100 genets. Colonization and extinction events are frequent, both at the level of ramets and genets, suggesting the existence of metapopulation-like dynamics also within a site. The high rates of turnover at the levels of ramets, genets and sites are reflected in high levels of linkage disequilibrium. Inbreeding is common and is a likely cause of the observed deviations from Hardy–Weinberg equilibrium. Despite population turnover, populations are differentiation at a small spatial scale. While population turnover, genetic drift, inbreeding and migration all influence the observed patterns, further studies are needed to determine the relative importance of these processes for the population structure of \textit{M. tanacetaria}.

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References


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