MOLECULAR ECOLOGY

Molecular Ecology (2012) doi: 10.1111/j.1365-294X.2012.05701.x

Reproduction and dispersal in an ant-associated root aphid community

A. B. F. IVENS,*† D. J. C. KRONAUER,†‡ I. PEN,* F. J. WEISSING* and J. J. BOOMSMA†

*Theoretical Biology, Centre for Ecological and Evolutionary Studies, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands, †Centre for Social Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark, ‡Laboratory of Insect Social Evolution, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

Abstract

Clonal organisms with occasional sex are important for our general understanding of the costs and benefits that maintain sexual reproduction. Cyclically parthenogenetic aphids are highly variable in their frequency of sexual reproduction. However, studies have mostly focused on free-living aphids above ground, whereas dispersal constraints and dependence on ant-tending may differentially affect the costs and benefits of sex in subterranean aphids. Here, we studied reproductive mode and dispersal in a community of root aphids that are obligately associated with the ant *Lasius flavus*. We assessed the genetic population structure of four species (*Geoica utricularia, Tetraneura ulmi, Forda marginata* and *Forda formicaria*) in a Dutch population and found that all species reproduce predominantly if not exclusively asexually, so that populations consist of multiple clonal lineages. We show that population viscosity is high and winged aphids rare, consistent with infrequent horizontal transmission between ant host colonies. The absence of the primary host shrub (*Pistacia*) may explain the absence of sex in three of the studied species, but elm trees (*Ulmus*) that are primary hosts of the fourth species (*T. ulmi*) occurred within a few km of the study population. We discuss the extent to which obligate ant-tending and absence of primary hosts may have affected selection for permanent parthenogenesis, and we highlight the need for further study of these aphids in Southern Europe where primary hosts may occur close to *L. flavus* populations, so that all four root aphid species would have realistic opportunities for completing their sexual life cycle.

Keywords: aphids, clonality, horizontal transmission, mutualism, myrmecophiles, ants

Received 13 December 2011; revision received 30 May 2012; accepted 1 June 2012

Introduction

Asexual reproduction can allow rapid population growth and therefore enable quick colonization of new habitats. Nevertheless, exclusive asexual reproduction is generally considered an evolutionary 'dead end' because prolonged lack of recombination will lead to accumulation of deleterious mutations and slower evolvability in response to parasites and environmental change, ultimately driving asexual species to extinction. Occasional sex in an otherwise asexual species, however, can allow for sufficient recombination to outweigh these costs, without compromising the benefits of asexual reproduction (a.o. Green & Noakes 1995; reviewed in d'Souza & Michiels 2010). Studying species with such mixed reproductive modes can thus enhance our understanding of the evolution and maintenance of sex, one of the major current topics in evolutionary biology.

Aphids are particularly informative in this context, because they are often cyclical parthenogens with a (holocyclic) reproductive cycle consisting of a sequence of asexual generations followed by a single sexual generation (Simon *et al.* 2002). However, some aphids have lost the sexual generation and reproduce exclusively asexually (anholocyclic). These variable reproductive modes, both between species and between populations of the same species, make aphids excellent

Correspondence: Aniek B. F. Ivens, Fax: 0031 50 3633400; E-mail: a.b.f.ivens@rug.nl

model systems for studying the selective forces that affect cyclical parthenogenesis (Simon *et al.* 1996, 1999; Delmotte *et al.* 2002; Gilabert *et al.* 2009; Kanbe & Akimoto 2009; Vantaux *et al.* 2011).

Aphids are also well known for their mutualistic relationships with ants (Way 1963; Stadler & Dixon 2008), which can have profound effects on dispersal and reproduction, and thus on the population structure of mutualistic partners (Herre *et al.* 1999; Leigh 2010). However, most of the previous aphid studies have focused on non-myrmecophiles in above-ground populations, whereas subterranean and obligately ant-associated aphids have been neglected (but see Yao & Akimoto 2009; Yao 2010). Here, we focus on four sympatric species of root aphids (*Geoica utricularia, Tetraneura ulmi, Forda marginata*, *Forda formicaria*) that are known to be obligately associated with ants (Heie 1980; Seifert 2007).

The specific objectives of our study were as follows: (i) to infer the extent of population viscosity of multiple root aphid species across a field transect spanning 7 km (Fig. 1), (ii) to determine the dominant mode of reproduction in these aphids and (iii) to estimate the potential for horizontal dispersal via winged forms.

Materials and methods

Root aphid natural history

Aphids have two alternative and partly overlapping reproductive cycles: (i) Holocyclic reproduction where aphids are propagated asexually during most of the year, but have a single sexual generation in a distinct season often just before overwintering. This form of cyclic asexuality can involve obligate dispersal to a primary host plant, on which mating occurs, and the recurrent colonization of a secondary host plant for asexual reproduction during the rest of the year (Heie 1980; Simon *et al.* 2002). (ii) Anholocyclic reproduction where aphids are propagated by obligate parthenogenesis. These aphids often live on a secondary host plant year-round, because the primary host of their close sexual relative is no longer used. Closely related aphid species may have very different reproductive cycles (reviewed in Simon *et al.* 2002), and even populations of the same species have been found to differ in their mode of reproduction (Heie 1980; Simon *et al.* 1996; Delmotte *et al.* 2002; Gilabert *et al.* 2009; Kanbe & Akimoto 2009).

The sparse available literature suggests that all four focal aphid species of this study (*G. utricularia, T. ulmi, F. marginata* and *F. formicaria*) have been observed to live anholocyclically on the roots of secondary host grasses (*Festuca rubra, Agrostis* spp. and *Elytrigia maritima*) inside ant mounds (Muir 1959; Pontin 1978; Heie 1980; Godske 1991, 1992). The *Forda* species and *G. utricularia* appear to be anholocyclic in Northern and Central Europe (including our study site on the Dutch island of Schiermonnikoog), but holocyclic in Southern Europe, where their primary host *Pistacia* spp. (Anacardiaceae) occurs (Heie 1980; Blackman & Eastop 1994). In contrast to the other three species, *T. ulmi* has been observed to be holocyclic at several sites in Northwestern

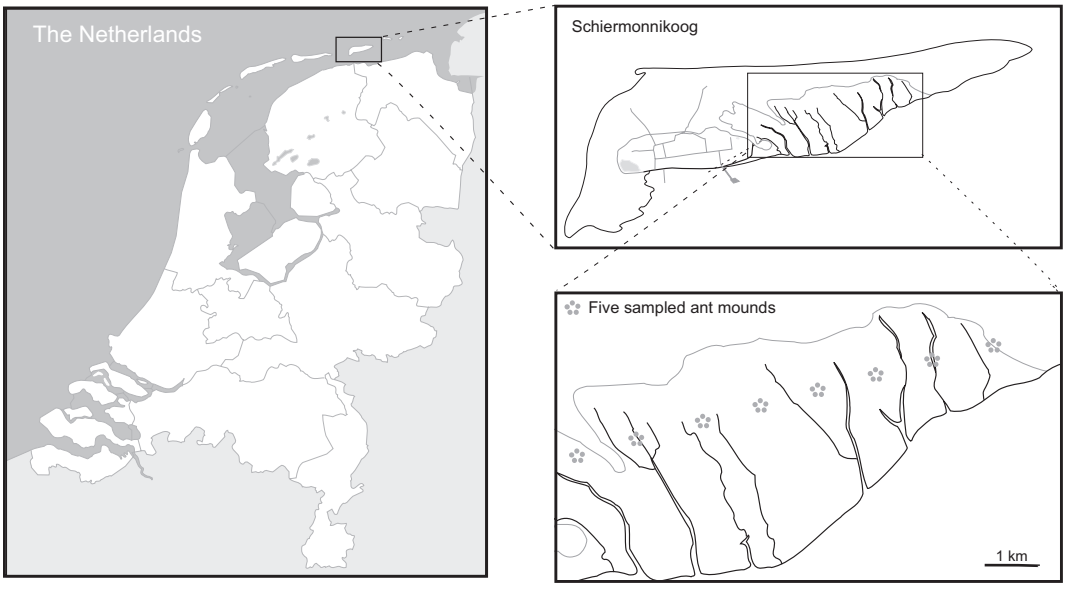

Fig. 1 Sampling site of root aphids associated with *Lasius flavus* mounds. Aphids were collected on the island of Schiermonnikoog (the Netherlands), where ant mounds were sampled in groups of five along a 7 km transect across the salt marsh of the island (maps courtesy of D. Visser).

Europe and Scandinavia (Heie 1980). This species was therefore expected to be holocyclic in our sampling area, with an obligate annual host shift to elm trees (*Ulmus* spp.; Ulmaceae) as primary host for sexual reproduction in autumn (Heie 1980; Paul 1977). However, Pontin (1978), Godske (1991) and Blackman & Eastop (1994) suggested that *T. ulmi* can also abandon its sexual phase and occur anholocyclically on grass roots in Northwestern Europe. Alate (winged) individuals have been described for all four species in at least some populations (Heie 1980). These could be indicators of either sexual reproduction and host shift not having been lost completely, or asexually produced dispersers, although the existence of the latter remains unconfirmed in Pemphigidae (Moran 1992). We therefore started this study with the hypothesis that there might be intra- and interspecific variation in reproductive mode and life cycle among the root aphids in our focal Dutch population.

All four aphid species are found in soil nests of the ant *Lasius flavus*. These nests contain specifically constructed aphid chambers, where the ants actively protect their livestock from parasites and predators (Pontin 1959, 1978), and where they both tend and eat them (Pontin 1958, 1961, 1978; Appendix S1, Supporting information). All four root aphid species appear to be obligate myrmecophiles, having lost predator defense mechanisms common in other aphids (Way 1963; Heie 1980; Hölldobler & Wilson 1990), that is, all have poorly developed cornicles (organs for protective wax production) and lack saltatorial legs to jump or actively drop from branches in response to threats (Way 1963; Heie 1980; Paul 1977). Most importantly, they all have a set of long anal hairs that can hold droplets of honeydew, a 'trophobiotic organ' that is only found in myrmecophilous aphids (Way 1963; Paul 1977; Heie 1980; Hölldobler & Wilson 1990) (Fig. 2). Apart from these distinct traits that reveal obligate dependence on ant care, some of these aphids are also dependent on the ants for survival during overwintering (Way 1963; Heie 1980). However, the sparse literature suggests that there may be considerable variation in this overwintering adaptation, as some aphids are known to have maintained holocyclic sexual reproduction (see above) and would therefore have to leave the ant nests during autumn, whereas others are facultatively or obligately anholocyclic (Pontin 1978; Heie 1980; Godske 1991). How ant colonies acquire their mutualistic aphids remains poorly understood, as virgin queens have not been observed to vector aphids during colony founding (A.B.F. Ivens, personal observation). Most likely, ant mounds that have recently gone extinct are recolonized by founding queens or neighbouring smaller colonies so that local aphid lineages may acquire new 'owners' (Ivens *et al.* 2012). In addition, aphids may disperse independently by wind, walking or floating on tidal water (Foster 1978; Foster & Treherne 1978).

Sampling

All aphid samples were collected in July 2008 from *L. flavus* ant mounds on the island of Schiermonnikoog, the Netherlands (53°28′N, 6°09′E). Sampling followed a 7 km transect across the salt marsh of the island

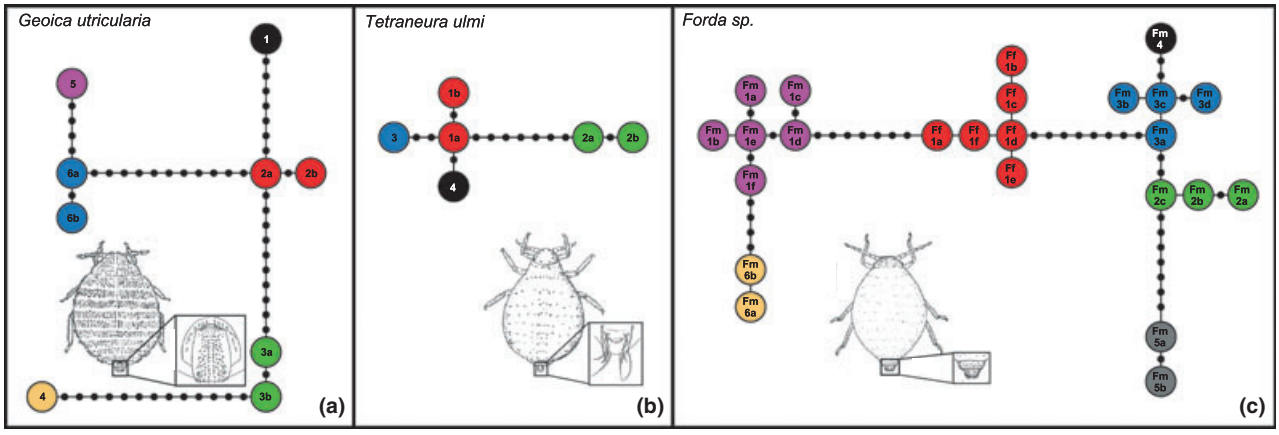

Fig. 2 Typical habitus and minimum spanning MLG trees for the three root aphid genera. Drawings represent apterous viviparous females of *Geoica utricularia* (a), *Tetraneura ulmi* (b) and *Forda marginata* (c). Adaptations to mymecophily, elongated anal hairs to hold honeydew, are highlighted (ventral view in a and b, dorsal view in c). All drawings are reproduced from Heie (1980). Multilocus genotype (MLG) trees were constructed based on shared allele distance (DAS), multiplied by the number of alleles available for comparison, to give the number of unshared alleles. Every circle represents a single MLG and names correspond to those used in Table 2, with colours representing multilocus lineages (MLLs). Connected MLGs differ from each other by a single allele and black dots represent potential unsampled haplotypes that differ from neighbours by a single allele. The *Forda* tree includes both *Forda formicaria* (Ff-MLGs, all belonging to one MLL given in red) and *F. marginata* MLGs.

(Fig. 1), with the westernmost kilometer of the transect located on grazed pasture and the remainder on un-grazed salt marsh. The transect was subdivided into eight locations with 1 km intervals. Nest mound density was high throughout the transect (*c.* 600–3500 mounds per ha). At each location, soil samples of five ant mounds of similar size (Ø *c.* 80 cm) were taken and hand-sorted for the occurrence of wingless and winged (alate) root aphids. The precise location of each ant nest was recorded with a GPS device (eTrexVista™, Garmin, 0.5–5 m precision). A subsample of all collected aphids was used for species identification using a protocol for microscopic preparation modified after Heie (1980). Reference specimens are located at the University of Groningen (access available upon request).

We chose to use small soil cores so that sampling was non-destructive, and we could resample mounds in later years. This sampling scheme was nonetheless sufficient to obtain at least one aphid belonging to each of the focal species from 30 of the 40 mounds, and only one of the 40 mounds yielded no aphids at all in any of our soil core samples. All mounds were confirmed to be inhabited by *L. flavus*, with *c.* 60% of the 21 soil cores taken per mound containing ants.

Molecular analysis

DNA was extracted from entire aphids using 200 μL 20%-Chelex® 100 resin (Fluka) (Walsh *et al.* 1991). We used polymorphic microsatellite markers to genotype specimens of *Forda* spp. (loci Fm1, Fm3, Fm4, Fm6, Gu6, Gu11, Gu13), *G. utricularia* (loci Gu2, Gu3, Gu5, Gu6, Gu8, Gu9, Gu11, Gu13) and *T. ulmi* (loci Tu1, Tu2, Tu3, Tu4, Tu10, Tu11). Marker-specific details and amplification protocols are given in Ivens *et al.* (2011). If a marker failed to amplify in an individual, the amplification process was repeated at least twice. PCR-products were analysed on an ABI-PRISM 3130XL (Applied Biosystems) sequencer and chromatograms were analysed in Genemapper (Applied Biosystems).

Data analysis

After omitting 30 samples in which more than half of the markers repeatedly failed to amplify, the total number of individuals included in the data sets was 469: 201 for *G. utricularia*, 92 for *T. ulmi,* 158 for *F. marginata* and 18 for *F. formicaria*.

MLGsim2.0 (http://www.rug.nl/fmns-research/theobio/downloads), an updated version of MLGsim (Stenberg *et al.* 2003), was used to group individuals into diploid multilocus genotypes (MLGs), that is, unique combinations of alleles across all tested marker loci. Where missing data occurred (190 of the 469 individuals had some missing genetic marker data—on average 13% of the alleles was missing), individuals were joined into the MLG of which the genotype for the remaining successfully scored loci was identical. In the two cases where an individual matched two different but very similar MLGs at all successfully amplified loci, it was grouped with the more common MLG of the two. For our further analyses, gaps in an individual's MLG were filled in with the alleles of the MLG to which the individual had been assigned. While this increased sample size for our analyses, it also implied that we may have slightly underestimated overall genetic variability.

Multilocus genotypes were either represented by several to many individuals (*recurrent MLGs*) or (rarely) as a single individual in our samples (*single MLGs*). Asexual reproduction will thus affect population genetic analyses through overrepresentation of clonally produced individuals (Sunnucks *et al.* 1997). To take any possible effects of pseudoreplication into account, all analyses were performed both on the full data set, including all individuals (*ramet data*; i.e., all individuals belonging to a single MLG), and on a subset of the data consisting of only one individual per MLG (*genet data*) (*sensu* Harper 1977). The two analyses yielded comparable qualitative conclusions.

Estimates of genetic variability

Asexual reproduction tends to decrease segregation of alleles within loci and recombination between loci. Over time, this leads to observed heterozygosities (H_O) differing from those expected under sexual outbreeding (H_E), and to deviations from Hardy–Weinberg equilibrium (HWE). Specifically, asexual reproduction can lead to heterozygote excess [i.e. a negative fixation index F_{IS} (Weir & Cockerham 1984; Stoeckel *et al.* 2006; Balloux *et al.* 2003)] through mutation accumulation in clonal lineages, also known as the 'Meselson effect' in ancient asexual lineages (Birky 1996; Welch & Meselson 2000; Halkett *et al.* 2005). Nevertheless, mechanisms such as mitotic recombination or occasional sex can also lead to heterozygote deficiency in clones, particularly when asexual lineages are not very old and stochastic effects determine whether clones happen to be homozygous or heterozygous at neutral markers (Birky 1996). Finally, the lack of recombination under asexual reproduction should lead to significant linkage disequilibrium (LD). GENEPOP 4.0 (Rousset 2008) was used to estimate H_O, H_E, F_{IS}, LD, and deviations from HWE, assuming a panmictic population with random mating.

As a measure for genetic diversity, we used the *G/N* ratio P_d, where *N* is the sample size for a given aphid species and *G* is the number of distinct MLGs in a focal population (Ellstrand & Roose 1987; Dorken & Eckert

2001; Arnaud-Haond *et al.* 2007). We also estimated P_{sex} values for every observed MLG, with P_{sex} being defined as the probability of obtaining at least as many as the observed number of individuals belonging to a given MLG under the null hypothesis of sexual reproduction and population-wide random mating (Tibayrenc *et al.* 1990; Parks & Werth 1993; Young *et al.* 2002; reviewed in Arnaud-Haond *et al.* 2007). The calculation of P_{sex} takes into account the observed frequencies of the alleles constituting the given MLG. A MLG with a low P_{sex} value therefore indicates that the multiple individuals observed for that MLG probably originated from clonal reproduction rather than sexual reproduction. Although P_{sex} calculation was initially developed to confirm strict asexuality, it can also be applied to cyclical parthenogens. This is because P_{sex} estimations are done for each unique MLG separately and as this is based on population-wide allele frequencies, any present genetic variation naturally enters the analysis, regardless its origin. Using MLGsim 2.0, we estimated P_{sex} values and derived P values for these values using Monte Carlo resampling simulations of our study population under HWE with 1000 iterations.

Genetic distance

We calculated genetic distances between MLGs using the shared allele distance DAS (Jin & Chakraborty 1993) in POPULATIONS 1.2.30 (Olivier Langella 1999). Pairs of MLGs with relatively small genetic distances might belong to one multilocus lineage (MLL) (Arnaud-Haond *et al.* 2007), that is, a grouping of MLGs that go back to the same sexual reproduction event and whose genetic differences are because of later mutations, asexual recombination or, possibly, scoring errors. We considered two MLGs as being part of the same MLL when they only differed by one or two alleles over all markers combined. The frequency distribution of the genetic distances between our samples was bimodal, with genetic distances of one or two alleles constituting the first peak of this overall frequency distribution, so that we felt confident that this distinction captured reality rather well. We also used a more formal approach of assessing MLL/MLG distinctions, based on the frequency distribution of genetic distances, following Arnaud-Haond *et al.* (2007). This method was designed for MLL assignments based on at least ten times more MLGs than observed in this study, but the results were very similar to those obtained by our original approach, leading to identical overall conclusions. After multiplying by the number of alleles that were compared for distance to transform proportional distances into absolute allele differences, the distance matrix was used to visualize the relationships between MLGs using minimum spanning MLG trees constructed in HapStar v 0.6 (Teacher & Griffiths 2011). The distance matrix was also used to construct neighbour Joining (NJ) trees (Appendix S1, Supporting information).

Spatial autocorrelation analyses

To assess whether genetically similar individuals tended to occur geographically close to each other, we estimated spatial autocorrelations in all four aphid species. Spatial autocorrelation analysis tests whether the matrix of pairwise genetic distances significantly correlates with the matrix of pairwise geographic distances between individuals (Euclidian distance based on GPS-coordinates). We used a Mantel test of matrix correspondence with 10^4 permutations to test for significant correlation between the matrices (Smouse *et al.* 1986).

The occurrence of spatial autocorrelation can be graphically illustrated using correlograms, in which the estimated matrix correlation coefficient r is plotted against geographical distance, subdivided in classes. We used seven classes (0–1, 1–2, 2–3, 3–4, 4–5, 5–6 and 7–8 km), representing the distances between the eight sampled locations along the transect. Following Smouse & Peakall (1999) and Peakall *et al.* (2003), the estimated r value was plotted in a spatial correlogram with error bars representing the 95% CIs for r determined by bootstrapping with 10^4 replicates. The estimated r values were plotted alongside the 95% CIs of the r values that were expected under the null hypothesis of a random distribution of individuals over locations (10^4 permutations). When an estimated r was larger than 0 and its 95% CI bars fell outside the 95% CIs generated by the null hypothesis, genetic and geographical distance were inferred to be positively correlated, with the first x-axis intercept representing the distance over which significant spatial structure occurred (Smouse & Peakall 1999; Peakall *et al.* 2003). All spatial autocorrelation analyses were performed in GENALEX 6.2 (Peakall & Smouse 2006), and correlograms were drawn using R 2.12.0 and the xyplot() function in the lattice package (Sarkar 2008).

Results

Aphid genetic variability and reproduction

All four root aphid species showed significant deviations from HWE both at the *ramet* and the *genet*-level (Table 1). Most of the significant differences from HWE were caused by heterozygote deficiency, but some loci showed heterozygote excess and thus negative F_{IS} values (Table 1). In *G. utricularia*, significant *ramet*-level LD ($P < 0.05$) was present in all 28 pairs of loci, and at the *genet*-level in 17 of the 28 pairs. In *T. ulmi*, *ramet*-level

Table 1 Population genetic statistics for the four species of root aphids

		Ramet-level					Genet-level				
Locus	N_a	N	H_E	H_O	F_{IS}	HWE P value	N	H_E	H_O	F_{IS}	HWE P value
Geoica utricularia											
Gu2	5	201	0.58	0.31	0.465	**<0.001**	9	0.63	0.22	0.680	**0.001**
Gu3	7	201	0.69	1.00	−0.457	**<0.001**	9	0.79	1.00	−0.210	0.096
Gu5	6	201	0.57	0.14	0.746	**<0.001**	9	0.77	0.33	0.607	**0.000**
Gu6	8	201	0.59	0.29	0.513	**<0.001**	9	0.82	0.33	0.631	**<0.001**
Gu8	4	201	0.55	0.06	0.892	**<0.001**	9	0.72	0.22	0.719	**<0.001**
Gu9	8	201	0.78	0.84	−0.068	**<0.001**	9	0.66	0.33	0.539	**0.001**
Gu11	6	201	0.73	0.70	0.043	**<0.001**	9	0.81	0.56	0.365	**<0.001**
Gu13	8	201	0.78	0.90	−0.155	**<0.001**	9	0.81	0.67	0.238	**0.002**
Population*	6.5	201	0.66	0.53	0.196		9	0.75	0.46	0.439	
Tetraneura ulmi											
Tu1	2	92	0.141	0.000	1.000	**<0.001**	6	0.444	0.000	−0.053	0.031
Tu2	2	92	0.141	0.000	1.000	**<0.001**	6	0.444	0.000	0.118	0.031
Tu3	5	92	0.702	0.924	−0.312	**<0.001**	6	0.764	0.667	0.200	0.184
Tu4	5	92	0.621	0.902	−0.449	**<0.001**	6	0.750	0.667	0.216	**0.001**
Tu10	3	92	0.281	0.326	−0.153	0.445	6	0.292	0.333	1.000	1.000
Tu11	4	92	0.384	0.402	−0.043	**0.001**	6	0.514	0.500	1.000	0.532
Population*	3.5	92	0.378	0.426	−0.120		6	0.535	0.361	0.404	
Forda marginata											
Fm1	9	155	0.45	0.25	0.442	**<0.001**	16	0.79	0.56	0.320	**<0.001**
Fm3	6	158	0.46	0.22	0.538	**<0.001**	18	0.79	0.50	0.388	**<0.001**
Fm4	6	158	0.43	0.15	0.665	**<0.001**	18	0.70	0.28	0.622	**<0.001**
Fm6	4	158	0.69	0.94	−0.353	**<0.001**	18	0.73	0.78	−0.030	**<0.001**
Gu6	5	158	0.68	0.71	−0.041	**<0.001**	18	0.58	0.28	0.543	**<0.001**
Gu11	6	158	0.46	0.21	0.548	**<0.001**	18	0.79	0.44	0.460	**<0.001**
Gu13	4	155	0.40	0.00	1.000	**<0.001**	16	0.58	0.00	1.000	**<0.001**
Population*	5.7	157	0.51	0.35	0.311		17	0.71	0.41	0.447	
Forda formicaria											
Fm3†	1	18	0.00	0.00		–	6	0.00	0.00		–
Fm4	3	18	0.50	0.78	−0.551	0.024	6	0.54	0.83	−0.471	0.638
Fm6	2	18	0.50	1.00	−1.000	**<0.001**	6	0.50	1.00	−1.000	0.090
Gu6†	1	18	0.00	0.00		–	6	0.00	0.00		–
Gu11	3	18	0.44	0.28	0.393	0.072	6	0.57	0.33	0.487	0.226
Gu13†	1	18	0.00	0.00		–	6	0.00	0.00		–
Population*	1.8	18	0.24	0.34	−0.409		6	0.27	0.36	−0.262	

HWE, Hardy–Weinberg equilibrium

Data are given both for the *ramet* (including all samples) and the *genet*-level (excluding replicates of the same MLG), and for each tested locus and the population as a whole. N is the number of genotyped aphids, N_a the number of observed alleles, H_E and H_O are expected (under Hardy–Weinberg assumptions) and observed heterozygosities, and F_{IS} is the fixation index. Significant P values for deviations from HWE (at the 0.2% level after Bonferroni correction) are given in bold (see Materials and methods).

*Cumulative population-level tests of deviations from HWE would have been significant in all cases with the possible exception of the *F. formicaria* genets, but have not been performed because significant linkage disequilibrium implies that locus-specific probabilities cannot be considered as independent.

†Loci that are monomorphic in *F. formicaria* and polymorphic in *F. marginata*. The alleles observed for Fm3 and Gu6 were diagnostic for *F. formicaria*.

LD was detected in 11 pairs with only four loci-pairs showing no LD, whereas no significant LD was found at the *genet*-level. *F. marginata* showed *ramet*-level LD in all 23 pairs of loci and *genet*-level LD in 16 of the 23 pairs (Table S1, Supporting information). In *F. formicaria*, only one pair of loci could be tested because of low sample sizes, showing no LD at either the *ramet*- or the *genet*-level. Combined with the consistent deviations from HWE, this predominance of LD is a strong indication for asexuality being the dominant mode of reproduction in three of the four species: *G. utricularia*, *T. ulmi* and *F. marginata*. Despite the low sample size, we infer that this is also likely to be the case for *F. formicaria*, because the biology of the two sibling spe-

cies *F. marginata* and *F. formicaria* appears to be very similar (Heie 1980) and the observed allelic distribution over clonal lineages was similar as well.

In total, we found nine MLGs among the 201 genotyped *G. utricularia* individuals. Three of these were unique '*single*' MLGs, which could all be unambiguously grouped into MLLs with one of the six MLGs occurring in multiple copies (Table 2, Figs 2a and S1a, Supporting information). All MLGs with multiple samples had P_{sex} values significantly below expectation for random mating (Table 2). Genetic diversity P_d for this species was 0.044, with some MLGs being vastly overrepresented among the samples (Table 2). For example, the predominant *G. utricularia* MLG was found 120 times, that is, in 60% of the samples (Table 2). Also these patterns indicate predominant clonal reproduction, and thus that this population of *G. utricularia* is anholocyclic. *T. ulmi* showed a similar pattern, with four MLLs consisting of six MLGs in total, all of which occurred more than once (Table 2, Figs 2b and S1b, Supporting information). The P_{sex} estimates for *T. ulmi* were also significantly reduced when tested against HWE expectation. In this species, the predominant MLG accounted for 52% of the samples and overall genetic diversity P_d was 0.065 (Table 2), indicating that *T. ulmi* is also anholocyclic and clonally propagated in the sampled population.

The same conclusion could be drawn for the two *Forda* species. *F. marginata* samples were more diverse than those of *G. utricularia* and *T. ulmi*, with a total of 18 MLGs, seven of which were encountered only once. All of these *single* MLGs and five of the *recurrent* MLGs could be combined with other MLGs into six MLLs (Table 2, Figs 2c and S1c, Supporting information). All but one of the P_{sex} values were significantly reduced, with the predominant clone accounting for 68% of the samples, and P_d being 0.114 (Table 2). Among the

Table 2 Multilocus genotypes (MLG) of the four root aphid species

MLG	*n*	P_{sex}
Geoica utricularia		
Gu-MLL1	17	0.000***
Gu-MLL2a	1	–
Gu-MLL2b	2	0.000***
Gu-MLL3a	1	–
Gu-MLL3b	11	0.000***
Gu-MLL4	46	0.000***
Gu-MLL5	2	0.000***
Gu-MLL6a	1	–
Gu-MLL6b	120	0.000***
N	201	
G	9	
P_d	0.044	
Tetraneura ulmi		
Tu-MLL1a	48	0.000***
Tu-MLL1b	7	0.000***
Tu-MLL2a	3	0.000***
Tu-MLL2b	4	0.000***
Tu-MLL3	24	0.000***
Tu-MLL4	6	0.000***
N	92	
G	6	
P_d	0.065	
Forda marginata		
Fm-MLL1a	1	–
Fm-MLL1b	1	–
Fm-MLL1c	1	–
Fm-MLL1d	2	0.009
Fm-MLL1e	108	0.000***
Fm-MLL1f	1	–
Fm-MLL2a	4	0.000***
Fm-MLL2b	5	0.000***
Fm-MLL2c	6	0.000***
Fm-MLL3a	5	0.000***
Fm-MLL3b	7	0.000***
Fm-MLL3c	3	0.000***
Fm-MLL3d	1	–
Fm-MLL4	2	0.000***
Fm-MLL5a	7	0.000***
Fm-MLL5b	1	–
Fm-MLL6a	1	–
Fm-MLL6b	2	0.000***
N	158	
G	18	
P_d	0.114	
Forda formicaria		
Ff-MLL1a	1	–
Ff-MLL1b	4	0.072
Ff-MLL1c	6	0.009*
Ff-MLL1d	4	0.061
Ff-MLL1e	1	–
Ff-MLL1f	2	0.037
N	18	
G	6	
P_d	0.333	

Name (clone acronym) and sample size (*n*) for each MLG; P_{sex}, the probability that the MLG occurred at least *n* times under the assumption of random mating is given for each *recurrent* MLG. All significant P_{sex} values remained significant after Bonferroni correction with the exception of *Forda formicaria* MLL1c. Total sample size (*N*), total number of MLGs (*G*) and genetic diversity P_d (*G/N*) are given for each species. Clone acronyms of MLGs consist of the species name abbreviation (Gu, Tu, Fm, Ff), the MLL-number (1–6) and a letter (a–f) that is unique for the MLG within its MLL.
***P value of $P_{sex} < 0.001$.
**P value of $P_{sex} < 0.01$.
*P value of $P_{sex} < 0.05$.

F. formicaria samples, we found six MLGs, all belonging to a single MLL. In this species, P_{sex} values were also generally low, although mostly not significant, which appears to be due to low sample size. Most MLGs in this species were also recurrent, with 33% of the individuals belonging to the predominant clone, and P_d being 0.333 (Table 2). Interestingly, the *F. formicaria* MLGs formed a single clade within the *F. marginata* tree, confirming the close relationship between the two species and suggesting that further work is needed to unambiguously establish species identities in this group (Figs 2c and S1c, Supporting information).

Aphid dispersal

Figure 3 shows the relative frequencies of all detected MLGs and MLLs over the 7 km transect for *G. utricularia*, *F. marginata* and *T. ulmi*. Although all species had at least one MLG that was relatively abundant and distributed over more than two locations on the island (except for *F. marginata*), most MLGs only occurred at one or two sampled locations. Furthermore, the MLGs belonging to the same MLL tended to occur in a clustered manner (Fig. 3), suggesting that dispersal levels are generally low. The Mantel test showed a significant correlation between genetic and geographic distances in *G. utricularia* and *F. marginata* (Fig. 4a,c). The correlograms were consistent with the outcome of the Mantel test, with significantly positive coefficients only being found for short (<3 km) distances in *G. utricularia* and *F. marginata*, with 95% confidence bars well above expectation based on a random distribution of genotypes over the island (Fig. 4). No significant autocorrelation was found in the populations of the other species, *T. ulmi*. The low sample size for *F. formicaria* (n = 18) did not allow for conclusive estimation of dispersal in this species. For completeness, the results of this analysis are given in the Appendix S1 (Supporting information) (Figs S2 and S3, Supporting information).

We found alate aphids for all species except *F. formicaria*, but numbers were always very small (five for *G. utricularia*, one for *T. ulmi*, and four for *F. marginata* out of a total of 505 aphids sampled in our entire study). Four of these alates were genotyped, which showed that three of them belonged to the same MLG as the other aphids sampled from the same ant nest, whereas the fourth alate had a different genotype (but one known from another colony nearby). On the basics of the observed alate frequencies, we estimated that these alate phenotypes had upper 95% CLs of 6% at most in the three species for which we had sample sizes to arrive at reasonable estimates (Table 3).

Discussion

Our population genetic estimates showed a strong signature of clonal reproduction for all four root aphid species. We therefore infer that *G. utricularia*, *T. ulmi*, *F. marginata* and *F. formicaria* have predominantly if not exclusively asexual reproduction in the sampled Schiermonnikoog population, which largely confirms earlier findings and records (Muir 1959; Pontin 1978; Heie 1980; Godske 1991; Blackman & Eastop 1994). A single clone accounted for the majority of individuals in all species. In *G. utricularia* and *F. marginata*, genetic differentiation and geographical distance correlated significantly, further corroborating that the clonal aphid populations are viscous.

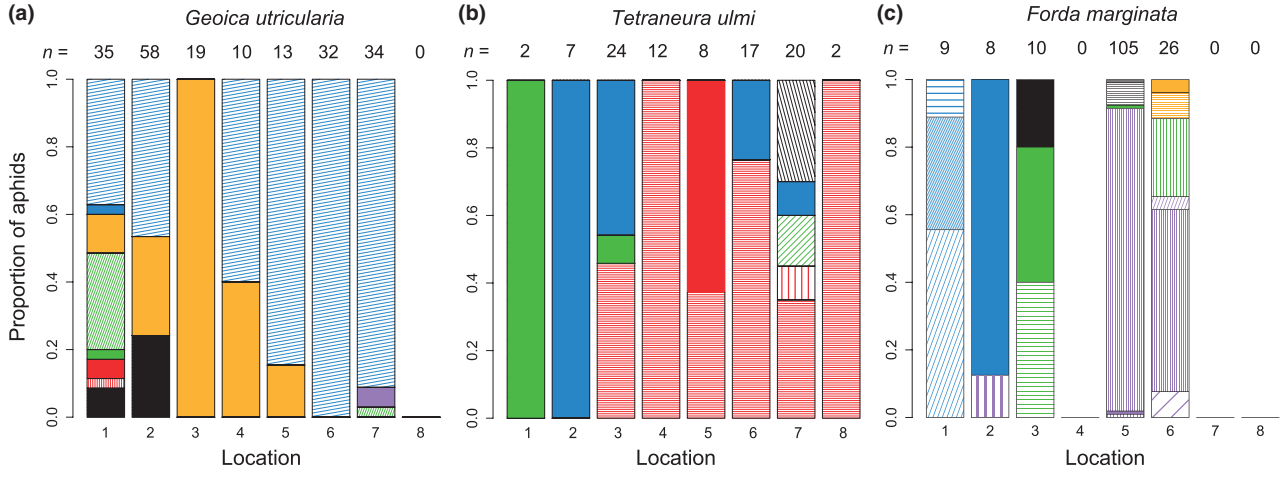

Fig. 3 Spatial distribution of cumulative frequencies of multilocus lineages (MLLs) and multilocus genotypes (MLGs) for *Geoica utricularia* (a), *Tetraneura ulmi* (b) and *Forda marginata* (c). Hatching patterns within each column represent the MLGs, whereas colours represent the MLLs and correspond to the colours used in Fig. 2. n is the sample size per aphid species for each transect location (location 1–8, with adjacent sample sites being 1 km apart—see Fig. 1).

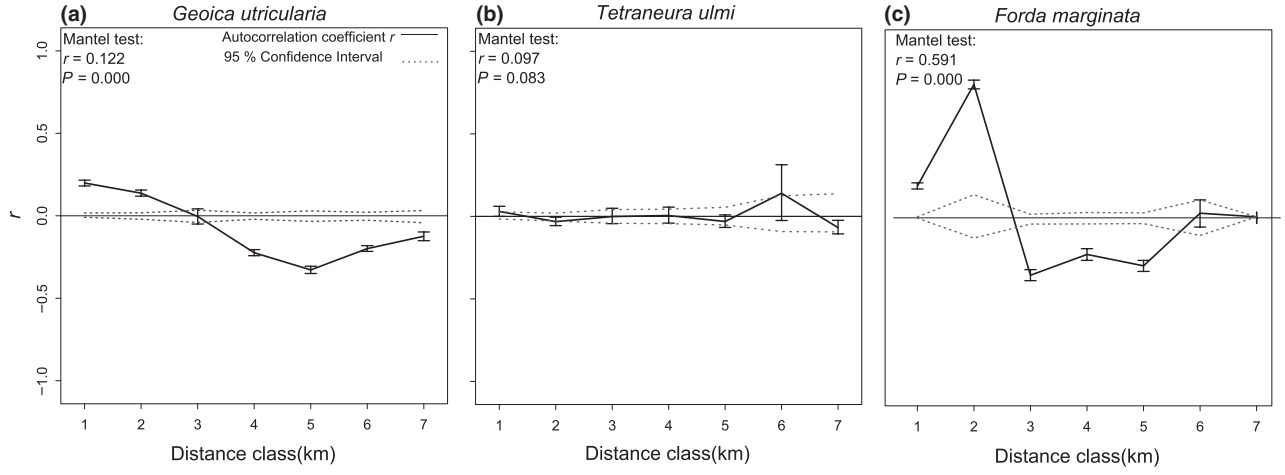

Fig. 4 Spatial autocorrelation patterns for *Geoica utricularia* (a), *Tetraneura ulmi* (b) and *Forda marginata* (c). Plots show genetic correlation coefficients *r* (solid line), their 95% confidence bars as determined by bootstrapping, and the 95% confidence intervals around zero as expected from random distributions of genotypes (dashed lines), plotted across the seven distance classes (1–7 km). Results for the Mantel tests for each correlation are provided in each panel.

Table 3 Percentages of alates for the four root aphid species

Species	*n*	Observed alates (%)	Confidence intervals (%)
Geoica utricularia	225	2.2	0.6–4.8
Tetraneura ulmi	93	1.1	0.0–5.7
Forda marginata	169	2.4	0.5–5.4
Forda formicaria	18	0.00	0.0–15.1

Total number of collected aphids (*n*), the percentage of alates and the 95% confidence intervals around them (Rohlf & Sokal 1981).

The reproductive modes of ant-tended root aphids

Anholocyclic reproduction and overwintering as asexual females (instead of sexually produced eggs) in ant nests in Northwest Europe has previously been inferred for *G. utricularia* and both *Forda* species (Muir 1959; Pontin 1978; Heie 1980; Godske 1991; Blackman & Eastop 1994), and our molecular data confirm this. However, finding the same extent of asexual reproduction in *T. ulmi* was interesting, as this result matched observations by Muir (1959), Pontin (1978) and Godske (1991), but contrasted with Heie's (1980) statement that *T. ulmi* reproduces sexually on elm trees in NW-Europe. Moderately mature (*c.* 30 years old) elm trees do occur in the single village on the island of Schiermonnikoog, but leaf-galls of *T. ulmi* were not found (A.B.F. Ivens & M. Schrama, unpublished data). The recurrence of identical *T. ulmi* clones in the same nest-mounds over consecutive years (Ivens *et al.* 2012) further corroborates that *T. ulmi* does not reproduce sexually on the island at any appreciable frequency. Until the 1980s there were much older elm trees in the village, which died from Dutch elm disease. These might have provided better opportunities for sexual reproduction in *T. ulmi.*

It is important to note that currently available statistical tests for inferring modes of reproduction from microsatellite data are designed to test the null hypothesis of 100% sexual reproduction and random mating, so that rejecting this null hypothesis does not imply that sexual reproduction is completely absent. Low frequencies of sex (<5%) could also account for the high variance in F_{IS} values in combination with the strong LD that we found in most of the loci-pairs (De Meeus & Balloux 2004). Statistically, we cannot rule out, therefore, that a small proportion of reproduction, particularly in *T. ulmi*, was in fact sexual (Balloux *et al.* 2003; A.B.F. Ivens, J.J. Boomsma, F.J. Weissing and I. Pen, in preparation), consistent with the primary host plant for this species being available and spatial autocorrelation being low, in contrast to *G. utricularia and F. marginata* where the host shrub for sexual reproduction is lacking (Heie 1980; Blackman & Eastop 1994) and autocorrelation patterns are distinct (Fig. 4a, c).

Population viscosity and dispersal

The aphid populations under study were viscous with low frequencies of alates and significant autocorrelation in two species, *G. utricularia* and *F. marginata.* These autocorrelations were 10-fold stronger that the largely non-significant, previously observed autocorrelations in two species of holocyclic aphids (Abbot & Chhatre 2007; Michel *et al.* 2009). Despite this strong spatial structuring, some clonal lineages had managed to spread along the studied transect (Fig. 3), most likely by wind-dispersal of winged individuals (alates). As

our genetic data indicate that there is negligible recruitment from sexual reproduction on Schiermonnikoog, this must imply that the few alates that we genotyped were either sexual migrants destined to fail (i.e. to never result in spring offspring recolonizing ant mounds) or unusual asexual dispersers headed for other ant mounds rather than for primary host trees. Both scenarios would be consistent with the alate MLGs being identical with those of wingless aphids in the same or a neighbouring mound, so that our data do not allow us to discriminate between these possibilities.

Morphological analysis of the embryos borne by the alates could have allowed us to resolve whether alates were sexual or not, because embryos of sexuparae are arostrate, whereas embryos of asexual dispersers are rostrate (Blackman & Eastop 1994). Unfortunately, by the time we realized this, we had used the alates for DNA extraction and microscopic preparation. However, the timing of their appearance (mid-summer) suggests that we were dealing with unusual asexual dispersers, as sexupara are expected to occur only towards the autumn (Heie 1980). Moreover, as the primary host of three species (the Mediterranean shrub *Pistacia*) has not occurred even close to the Island of Schiermonnikoog since the last glaciation, it seems hard to imagine how the island could have ever been colonized if alates of these species could only migrate to and from *Pistacia* shrubs.

What we can infer is that clonal aphid gene flow between ant mounds would indeed be very low if dispersal remains restricted to wingless aphids walking on the soil surface (even when helped somewhat by wind), which has been observed for both *Forda* and *Tetraneura* (Muir 1959) or passive floating during tidal inundations, which has been observed for the salt marsh root aphid *Pemphigus trehernei*, a non-ant-attended relative of *Forda* and *Geoica* (Foster 1978; Foster & Treherne 1978). *Pemphigus trehernei* also reproduces predominantly clonally and produces alates at very low frequencies (Foster 1975), but nevertheless colonizes new host plants very effectively (Foster & Treherne 1978). Although dispersal by tidal floating is poorly understood, it is conceivable that the aphid symbionts of *L. flavus* employ the same mechanism during occasional summer inundations, to colonize existing ant mounds where they might then establish a new MLG that can be vertically propagated after adoption by the ants (Ivens *et al.* 2012).

Why aphids may lose sex altogether and does ant-tending matter?

Although it has long been known that aphids either have holocyclic (with sex) or anholocyclic (completely parthenogenetic) life cycles, we lack an overall understanding of the selection forces that make aphids lose sex and whether such development is always irreversible. This is because populations of only a few species have been studied in sufficient genetic detail and few of these studies have targeted metapopulations consisting of holocyclic and anholocyclic patches. Polymorphisms of this kind are known to occur (Simon *et al.* 2002) and genetic studies of such populations would thus be highly informative. The strong LD, significant deviations from HWE and the observed heterozygote excess match the genetic population structures of other anholocyclic aphids (Simon *et al.* 1996, 1999; Delmotte *et al.* 2002; Gilabert *et al.* 2009; Kanbe & Akimoto 2009). However, all of these studies concern free-living aphids, while an association with ants is likely to affect the genetic population structure as well. Yao (2010) for example showed that F_{IS} values for ant-tended *Tuberculatus* aphids were higher than those for unattended aphids. However, Vantaux *et al.* (2011) did not find any effect of ant attendance on the genetic population structure of facultatively ant-tended aphids above ground. The root aphids studied here may have such metapopulations consisting of sexual and asexual patches in Southern Europe, but their genetic analysis would only reveal whether close proximity of primary host plants increases the frequency of sex, and not whether ant-tending matters, because there are no free-living underground populations of these aphids (see Fig. 2 for illustrations of their adaptations to myrmecophily).

Living in obligate mutualistic symbiosis has been argued to promote the irreversible loss of sexual reproduction when the symbiotic environment becomes highly predictable and uniform (Law & Lewis 1983; Wulff 1985). Although originally developed for endosymbionts, this hypothesis might also apply to ectosymbionts such as the aphids of our present study, because they are surrounded by a highly protective host colony (i.e. they are endosymbionts at the colony-level). The ants thus provide a protective underground environment in which potentially (i) sexually produced frost-resistant eggs are no longer necessary and (ii) well-known sex-inducing environmental cues such as lower temperature and shorter daylight are less likely to be effective as cues (Rispe *et al.* 1998; reviewed in Moran 1992; Hales *et al.* 1997; Simon *et al.* 2002). In addition, ants actively keep aphid densities low by culling (Ivens *et al.* 2012), decreasing the potential for crowding, a factor also known to induce dispersal and sexual reproduction in aphids (reviewed in Hales *et al.* 1997). Dispersal constraints due to lack of primary host plants nearby and year-round underground nursing may thus have tipped the balance towards permanent parthenogenesis, relative to aphids that are facultatively ant-tended on food plants above ground where holocyclic life cycles are more often maintained. We would

thus be surprised if underground ant-tending would ever be compatible with substantial aphid sexuality, even when primary host plants grow nearby. This logic seems to match the sparsely available comparative data as many above-ground aphids such as *Aphis fabae* combine a holocyclic life cycle with facultative ant-association (Heie 1986), whereas some other obligately ant-tended aphids and coccids have become anholocyclic and even use the dispersing virgin queens of the host ants for transmission (Hölldobler & Wilson 1990).

It is interesting to note that some studies have found that 'host-ant management' can delay aphid dispersal and reduce alate production (Way 1963; Kindlmann *et al.* 2007; Oliver *et al.* 2007; Yao 2010; Tegelaar *et al.* 2011). In addition to the culling mentioned earlier, underlying mechanisms for such practices as described for *Lasius* ants vary from inhibition through semiochemicals that slow aphids down (Oliver *et al.* 2007) to ants actively cutting the wings of their 'dairy farm' dispersal morphs (Way 1963; Hölldobler & Wilson 1990). We note, however, that our study population does not allow a convincing test of the possible role of 'ant management' in the restriction of root aphid sex, because the primary host plant was absent for three species and quite possibly unsuitable for the fourth species. Such a study would be possible in the southern European range of *Pistacia*, at sites where these bushes and mature elm trees co-occur with *L. flavus* and their root aphids. The microsatellite markers developed for the present study would provide efficient tools to address these questions.

Acknowledgements

We thank Sylvia Mathiasen for help in the laboratory, Thijs Janzen for field and laboratory assistance, Ole E. Heie and Maurice Jansen for help with aphid taxonomy, David Nash for constructing the minimal spanning trees of Fig. 2 and Subject Editor Jacob Russell and three anonymous reviewers for their constructive comments on the manuscript. This study was supported by a PhD-Grant of the Centre for Ecological and Evolutionary Studies, University of Groningen and grants by the Langerhuizen Fund and Nicolaas Mulerius Fund, awarded to A.B.F.I, as well as by a grant from the Danish National Research Foundation to J.J.B that funded D.J.C.K's contributions before he obtained support from the Harvard Society of Fellows. We are grateful to the Vereniging Natuurmonumenten for granting us permission to conduct field work in the National Nature Reserve Schiermonnikoog and to the publishers Apollo Books, Stenstrup, Denmark and Brill, Leiden, the Netherlands for granting permission to reproduce figures from Heie (1980) in Fig. 2.

References

Abbot P, Chhatre V (2007) Kin structure provides no explanation for intruders in social aphids. *Molecular Ecology*, **16**, 3659–3670.

Arnaud-Haond S, Duarte CM, Alberto F, Serrao EA (2007) Standardizing methods to address clonality in population studies. *Molecular Ecology*, **16**, 5115–5139.

Balloux F, Lehmann L, de Meeus T (2003) The population genetics of clonal and partially clonal diploids. *Genetics*, **164**, 1635–1644.

Birky CW (1996) Heterozygosity, heteromorphy, and phylogenetic trees in asexual eukaryotes. *Genetics*, **144**, 427–437.

Blackman RL, Eastop VF (1994) *Aphids on The World's Trees – An Identification and Information Guide*. CAB International, Oxon, UK.

De Meeus T, Balloux F (2004) Clonal reproduction and linkage disequilibrium in diploids: a simulation study. *Infection, Genetics and Evolution*, **4**, 345–351.

Delmotte F, Leterme N, Gauthier JP, Rispe C, Simon JC (2002) Genetic architecture of sexual and asexual populations of the aphid *Rhopalosiphum padi* based on allozyme and microsatellite markers. *Molecular Ecology*, **11**, 711–723.

Dorken ME, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *Journal of Ecology*, **89**, 339–350.

Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant-species. *American Journal of Botany*, **74**, 123–131.

Foster WA (1975) Life-history and population biology of an intertidal aphid, *Pemphigus trehernei* Foster. *Transactions of the Royal Entomological Society of London*, **127**, 193–207.

Foster WA (1978) Dispersal behavior of an intertidal aphid. *Journal of Animal Ecology*, **47**, 653–659.

Foster WA, Treherne JE (1978) Dispersal mechanisms in an intertidal aphid. *Journal of Animal Ecology*, **47**, 205–217.

Gilabert A, Simon JC, Mieuzet L *et al.* (2009) Climate and agricultural context shape reproductive mode variation in an aphid crop pest. *Molecular Ecology*, **18**, 3050–3061.

Godske L (1991) Aphids in nests of *Lasius flavus* F. in Denmark I: faunistic description. *Entomologiske Meddelelser*, **59**, 85–89.

Godske L (1992) Aphids in nests of *Lasius flavus* F. in Denmark II: population dynamics. *Entomologiske Meddelelser*, **60**, 21–26.

Green RF, Noakes DLG (1995) Is a little bit of sex as good as a lot? *Journal of Theoretical Biology*, **174**, 87–96.

Hales DF, Tomiuk J, Wohrmann K, Sunnucks P (1997) Evolutionary and genetic aspects of aphid biology: a review. *European Journal of Entomology*, **94**, 1–55.

Halkett F, Simon J, Balloux F (2005) Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology & Evolution*, **20**, 194–201.

Harper J (1977) *Population Biology of Plants*. Academic Press, London.

Heie OE (1980) The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. I. *Fauna Entomologica Scandinavica*, **9**, 1–236.

Heie OE (1986) The Aphidoidea (Hemiptera) of Fennoscandia and Denmark. III. *Fauna Entomologica Scandinavica*, **17**, 1–314.

Herre EA, Knowlton N, Mueller UG, Rehner SA (1999) The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology & Evolution*, **14**, 49–53.

Hölldobler B, Wilson EO (1990) *The Ants*. Harvard University Press, Cambridge, Massachusetts.

Ivens ABF, Kronauer DJC, Boomsma JJ (2011) Characterisation and cross-amplification of polymorphic microsatellite loci in

ant-associated root-aphids. *Conservation Genetics Resources*, **3**, 73–77.

Ivens ABF, Kronauer DJC, Pen I, Weissing FJ, Boomsma JJ (2012) Ants farm subterranean aphids mostly in single clone groups–an example of prudent husbandry for carbohydrates and proteins? *BMC Evolutionary Biology*. In press.

Jin L, Chakraborty R (1993) A bias-corrected estimate of heterozygosity for single-probe multilocus DNA fingerprints. *Molecular Biology and Evolution*, **10**, 1112–1114.

Kanbe T, Akimoto SI (2009) Allelic and genotypic diversity in long-term asexual populations of the pea aphid, *Acyrthosiphon pisum* in comparison with sexual populations. *Molecular Ecology*, **18**, 801–816.

Kindlmann P, Hulle M, Stadler B (2007) Timing of dispersal: effect of ants on aphids. *Oecologia*, **152**, 625–631.

Law R, Lewis DH (1983) Biotic environments and the maintenance of sex – some evidence from mutualistic symbioses. *Biological Journal of the Linnean Society*, **20**, 249–276.

Leigh EG (2010) The evolution of mutualism. *Journal of Evolutionary Biology*, **23**, 2507–2528.

Michel AP, Zhang W, Jung JK, Kang S, Mian MAR (2009) Population genetic structure of *Aphis glycines*. *Environmental Entomology*, **38**, 1301–1311.

Moran NA (1992) The evolution of aphid life-cycles. *Annual Review of Entomology*, **37**, 321–348.

Muir DA (1959) The ant-aphid-plant relationship in West Dunbartonshire. *Journal of Animal Ecology*, **28**, 133–140.

Olivier L (1999) Populations, 1.2.30 , CNRS UPR9034. Available from http://bioinformatics.org/tryphon/populations/#ancre_bibliographie.

Oliver TH, Mashanova A, Leather SR, Cook JM, Jansen VAA (2007) Ant semiochemicals limit apterous aphid dispersal. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 3127–3131.

Parks JC, Werth CR (1993) A study of spatial features of clones in a population of Bracken Fern, *Pteridium aquilinum* (Dennstaedtiaceae). *American Journal of Botany*, **80**, 537–544.

Paul RG. (1977) Aspects of the biology and taxonomy of Britsh myrmecophilous root aphids, PhD Thesis. Department of Entomology, British Museum, London.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution*, **57**, 1182–1195.

Pontin AJ (1958) A preliminary note on the eating of aphids by ants of the genus *Lasius* (Hym., Formicidae). *The Entomologist's Monthly Magazine*, **94**, 9–11.

Pontin AJ (1959) Some records of predators and parasites adapted to attack aphids attended by ants. *The Entomologist's Monthly Magazine*, **95**, 154–155.

Pontin AJ (1961) The prey of *Lasius niger* (L.) and *L. flavus* (F.) (Hym., Formicidae). *The Entomologist's Monthly Magazine*, **97**, 135–137.

Pontin AJ (1978) Numbers and distribution of subterranean aphids and their exploitation by the ant *Lasius flavus* (Fabr.). *Ecological Entomology*, **3**, 203–207.

Rispe C, Pierre JS, Simon JC, Gouyon PH (1998) Models of sexual and asexual coexistence in aphids based on constraints. *Journal of Evolutionary Biology*, **11**, 685–701.

Rohlf FJ, Sokal RR (1981) *Statistical Tables*, 2nd edn. W.H. Freeman and Company, San Francisco, California.

Rousset F (2008) GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Sarkar D (2008) *Lattice: Multivariate Data Visualization With R*. Springer, New York.

Seifert B (2007) *Die Ameisen Mittel- und Nordeuropas*. Lutra, Görlitz.

Simon JC, Carrel E, Hebert PDN, Dedryver CA, Bonhomme J, LeGallic JF (1996) Genetic diversity and mode of reproduction in French populations of the aphid *Rhopalosiphum padi* L. *Heredity*, **76**, 305–313.

Simon JC, Baumann S, Sunnucks P *et al.* (1999) Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. *Molecular Ecology*, **8**, 531–545.

Simon JC, Rispe C, Sunnucks P (2002) Ecology and evolution of sex in aphids. *Trends in Ecology & Evolution*, **17**, 34–39.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity*, **82**, 561–573.

Smouse PE, Long JC, Sokal RR (1986) Multiple-regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, **35**, 627–632.

d'Souza TG, Michiels NK (2010) The costs and benefits of occasional sex: theoretical predictions and a case study. *Journal of Heredity*, **101**, S34–S41.

Stadler B, Dixon T (2008) *Mutualism: Ants and Their Insect Partners*. Cambridge University Press, Cambridge.

Stenberg P, Lundmark M, Saura A (2003) MLGsim: a program for detecting clones using a simulation approach. *Molecular Ecology Notes*, **3**, 329–331.

Stoeckel S, Grange J, Fernandez-Manjarres JF, Bilger I, Frascaria-Lacoste N, Mariette S (2006) Heterozygote excess in a self-incompatible and partially clonal forest tree species – *Prunus avium* L. *Molecular Ecology*, **15**, 2109–2118.

Sunnucks P, DeBarro 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.

Teacher AGF, Griffiths DJ (2011) HapStar: automated haplotype network layout and visualization. *Molecular Ecology Resources*, **11**, 151–153.

Tegelaar K, Hagman M, Glinwood R, Pettersson J, Leimar O (2011) Ant-aphid mutualism: the influence of ants on the aphid summer cycle. *Oikos*, **121**, 61–66.

Tibayrenc M, Kjellberg F, Ayala FJ (1990) A clonal theory of parasitic protozoa: the population structures of *Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium*, *Trichomonas*, and *Trypanosoma* and their medical and taxonomical consequences. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 2414–2418.

Vantaux A, Billen J, Wenseleers T (2011) Levels of clonal mixing in the black bean aphid *Aphis fabae*, a facultative ant mutualist. *Molecular Ecology*, **20**, 4772–4785.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, **10**, 506–513.

Way MJ (1963) Mutualism between ants and honeydew-producing Homoptera. *Annual Review of Entomology*, **8**, 307–344.

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

Welch D, Meselson M (2000) Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. *Science*, **288**, 1211–1215.

Wulff JL (1985) Clonal organisms and the evolution of mutualism. In: *Population Biology and Evolution of Clonal Organisms* (eds Jackson JBC, Buss LW and Cook RE), pp. 437–666. Yale University Press, New Haven, Connecticut.

Yao I (2010) Contrasting patterns of genetic structure and dispersal ability in ant-attended and non-attended *Tuberculatus* aphids. *Biology Letters*, **6**, 282–286.

Yao I, Akimoto S (2009) Seasonal changes in the genetic structure of an aphid-ant mutualism as revealed using microsatellite analysis of the aphid *Tuberculatus quercicola* and the ant *Formica yessensis*. *Journal of Insect Science*, **9**, 1–18.

Young AG, Hill JH, Murray BG, Peakall R (2002) Breeding system, genetic diversity and clonal structure in the sub-alpine forb *Rutidosis leiolepis* F. Muell. (Asteraceae). *Biological Conservation*, **106**, 71–78.

This work is part of the PhD thesis of A.B.F.I. All authors have long standing interest in the evolutionary biology of cooperation and conflict within and between species in all its facets, including reproductive mode and genetic diversity. F.J.W. and I.P. are theoretical biologists using a modeling approach to study social evolution and the causes and consequences of sexual reproduction.

Data accessibility

Sampling locations, sampling details, and microsatellite data are available from DRYAD (entry doi: 10.5061/dryad.d0t63).

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Materials and methods.

Fig. S1. Neighbour-Joining trees for the three root aphid genera.

Fig. S2 Spatial distribution of cumulative frequencies of multilocus genotypes (MLGs) of *Forda formicaria*.

Fig. S3 Spatial autocorrelation pattern for *Forda formicaria*.

Table S1 Results of tests for the presence of linkage disequilibrium for *ramet* and *genet* datasets of *Geoica utricularia*, *Tetraneura ulmi* and *Forda marginata*.

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