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Evidence for gene flow and local clonal selection in field populations of the grain aphid (*Sitobion avenae*) in Britain revealed using microsatellites

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Samples of the grain aphid, *Sitobion avenae* (F.), a major European pest of cereals, were collected in June and July 1997 from fields sown with winter wheat in a rough transect south-west of Rothamsted, UK. These aphids were genotyped at four microsatellite loci known from previous studies to be highly polymorphic. Allelic frequencies were similar between samples collected in the fields and in the 12.2 m high suction trap at Rothamsted, and there were many widespread genotypes (clones), providing evidence that the species is highly migratory. However, field samples were found to display a high level of genotypic heterogeneity (= variable clonal composition), most probably the result of clonal selection. The suction trap genotypes sample were slightly different from the field samples, indicative of the inclusion of genotypes from plant hosts (cereals and grasses, Poaceae) other than winter wheat and/or genotype-biased emigration from the field. The relevance of these data to modelling of aphid outbreaks is briefly discussed.
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Introduction

Winged aphids constitute a significant part of what is colloquially termed the 'aerial plankton' (Drake and Farrow, 1989) and, as such, some species can traverse large vertical distances as well as be displaced by winds above their flight speed in still air (~2 km/h) over large geographic distances (Loxdale *et al*, 1993). At higher altitudes, they may be carried in the jet stream above large continental land masses, for example, North America (Taylor, 1965; Berry and Taylor, 1968). However, the so-called 'plankton' cannot be treated as a single, homogeneous entity of aphids of similar flight behaviour, but rather must be viewed in a case-specific way. That is to say, different aphid species show differing flight behaviours, with differing urges to migrate and abilities to traverse differing geographic areas. Evidence for this has come from both wind tunnel experiments (Hardie, 1993) and population genetic studies using a range of molecular markers (see below). In addition, evidence has been accumulated that, even within a single aphid species, differences occur in flight behaviour (ie there are short- and long-distance migrants; Kidd and Cleaver, 1984, 1986).

There have been many studies over recent years using molecular markers to elucidate the flight behaviour and migratory ambit of a range of aphid species, both pest and non-pest (see Loxdale *et al*, 1993; Hales *et al*, 1997; Loxdale and Lushai, 2001 for reviews). Similar studies have also revealed differences in the genetic structuring of natural aphid populations, relating to lifecycle strategy and clonal selection (eg Simon *et al*, 1999; Guillemaud *et al*, 2003). The genetic structuring reflects flight behaviour (eg low genetic differentiation over a wide area for highly migratory species), although selection for lifecycle types better adapted to the climate in particular regions is also apparent (eg Massonnet *et al*, 2002; Guillemaud *et al*, 2003).

For example, in a previous paper (Llewellyn *et al*, 2003), we showed, using a range of microsatellite markers, that populations of the grain aphid, *Sitobion avenae* (F.), a major European pest of cereals (Vickerman and Wratten, 1979), were strongly influenced by climatic selection acting on lifecycle genotypes in the face of extensive aerial geographic displacements (ie migration). This aphid has a range of lifecycle types, all of which reproduce parthenogenetically for most of the year, but which may or may not have an annual sexual phase (Dixon, 1998). These lifecycles comprise cyclical parthenogens (holocyclic, which are facultative asexuals with an annual sexual phase), obligate parthenogens (anholocyclic, which never recombine) and lineages for which only some of the autumnal offspring contribute to the sexual phase, that is, androcyclic forms that can produce a few males and intermediate forms that produce a few male and female sexuals (see Delmotte *et al*, 2001; Simon *et al*, 2002 for further details). Survival rates of the different types are related to the severity of the winter, since sexuals produce cold-hardy eggs, whereas lifecycle

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types without this facility overwinter as live individuals, which are more susceptible to low temperature mortality. The success of these lifecycle strategies in differential climatic conditions has been modelled by Rispe and coworkers (Rispe and Pierre, 1998; Rispe *et al*, 1998).

Llewellyn *et al* (2003) showed that, on a large geographic scale, *S. avenae* populations have similar allele frequencies, reflecting extensive dispersal by the insect. Contrastingly, genotype structuring was strongly differentiated regionally and appeared to be related to differential climatic selection on the various lifecycle types. In other words, although migration is extensive, annual events are important in structuring the population. Other relevant ecological factors may be more important at smaller spatial scales and, indeed, may be overlooked in studies considering larger-scale movements. Such factors could include the existence of 'generalist' genotypes or those adapted to particular host plants (Haack *et al*, 2000; Lushai *et al*, 2002) as well as microclimate, crop density, natural enemy pressures and potentially, resistance to pesticides (although there is no evidence at present that *S. avenae* is resistant to any of the commonly used insecticides in the UK).

Clearly, clonal selection is a very important consideration in studies of the population structure and dynamics of parthenogenetic organisms. This is because the rapid propagation of asexual offspring (potentially billions *per annum* from a single foundress in the case of aphids theoretically subject to minimal mortality factors, that is, climate/weather, predators, parasites and pathogens; Harrington, 1994) is a very powerful evolutionary force which allows ecological adaptations to occur quickly (Loxdale and Lushai, 2003; Lushai *et al*, 2003). Discovering just how particular clones wax and wane within populations and the consequences for the genetic structure of the population (effectively a 'snap shot' in time for the purposes of most studies bar historical trends in genetic structure) is a crucial task in population ecology. Aphids, because of their short generation times (~10 days in *S. avenae*), including telescoping of generations (Dixon, 1998) and significant aerial displacements, make ideal subjects for such studies.

In the present paper, we again use the same suite of microsatellites as Llewellyn *et al* (2003) to further understand the population structure and dynamics of *S. avenae*, but here at the field scale rather than the national, geographic scale as in our previous study. In that study, we were particularly interested in trying to disentangle the effects of movement and clonal selection, something that has been performed in few other studies of aphids to date. Unless the importance of migration and selection is better understood, then, for a given aphid species, its population dynamics is likely to be misinterpreted, giving a false picture of its flight behaviour and population ecology. This, of course, has considerable relevance to programmes modelling pest aphid outbreaks, including genotypes resistant to insecticides, and the spread of plant viruses transmitted by aphids.

Materials and methods

Sampling regime

S. avenae were collected from two wheat fields at each of three sites between 28 June and 15 July 1997, that is, Rothamsted Research Farm, Harpenden and Wood Farm, Hemel Hempstead, both in Hertfordshire, and Manor Farm, Garford in Oxfordshire, UK (Figure 1). Around 25 insects were collected from each field (Table 1), giving a sample of approximately 50 aphids per farm, which is

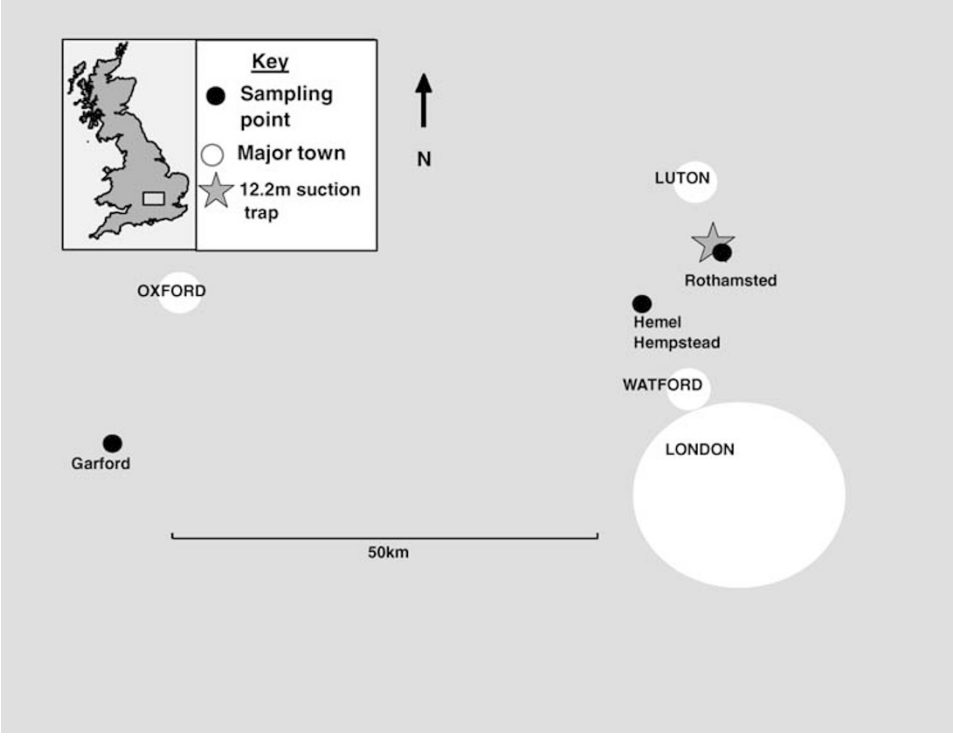

Figure 1 Location of *S. avenae* field collection sites in June and July 1997 and location of the Rothamsted suction trap.

Table 1 Sampling details of S. *avenae* field and suction trap collections

Sample Code	*Farm*	*Field*	*Collection date*	*N*	*Cultivar of winter wheat*	*Growth stage*	*Insecticide applications*	*Location relative to trap*
Field collections								
RA	Rothamsted, Herts	Field A (Delharding)	30 June 1997	25	Genesis	75	Draza 23/10/96	0.3 km NW
RB		Field B (Great Knott II)	1 July 1997	23	Caxton	75	None	1.25 km W
HA	Hemel Hempstead, Herts	Field A (Hill Top)	7 July 1997	24	Consort	69	Hallmark 25/10/96, 16/11/96	9 km WSW
HB		Field B (Water End)	7 July 1997	24	Shangow	73	Hallmark 15/11/96	9 km WSW
GA	Garford, Oxon	Field A	28 June1997 15 July 1997	24	Information not available	85	Autumn spray	65 km WSW
GB		Field B (nr. East Hanney)	15 July 1997	24	Information not available	85	Autumn spray	65 km WSW
Suction trap collections								
R e 97	Rothamsted Tower suction trap		30 June – 10 July 1997	50	N/A	N/A	N/A	N/A
R 97	Rothamsted Tower suction trap		21–26 July 1997	54	N/A	N/A	N/A	N/A

N = sample size. The growth stage of the crop at the time of sampling was recorded (Tottman and Broad, 1987) and information on the cultivar of wheat and the insecticide regime was provided for Rothamsted and Hemel Hempstead, although detailed information was not available for Garford. N/A = Not available.

sufficient to detect alleles present in the populations at a frequency >0.05 (Sjörgren and Wyöni, 1994). Each individual was collected at least 3 m away from other aphids sampled, to minimize the likelihood of sampling direct, clonally produced descendants of individual females. Where possible, winged or wingless adults were collected, but these were scarce in some fields, so nymphs were collected instead. Most collections from a single field were completed within a day, but one field (Garford A) required a second visit to obtain sufficient aphids for analysis (Table 1). Field-collected aphids were stored at -20°C for up to 2 years prior to DNA extraction. Aphids were also collected in the Rothamsted suction trap (Macaulay *et al*, 1988) during two periods in June and July 1997 (Table 1). The earlier collection (R e 97) spanned the same fortnight as the field collections. Suction-trapped insects were kept in 95% ethanol for up to 2 years before DNA extraction. There is ample evidence from previous studies utilizing aphid microsatellites, including the same primers used in the present study for *Sitobion*, that storage by freezing or in 95% ethanol does not lead to alteration of genotypes, for example, genetic assays repeated between Sunnucks *et al* (1996) and Wilson *et al* (1999).

The distances between each of the field sites are given in Table 2. These sites were chosen because they represent the supposed sampling range of a single suction trap of the Rothamsted Insect Survey nationwide network (ie up to $\sim$80 km away; Taylor, 1974) and south-west of the Rothamsted suction trap, that is, roughly upwind of the predominant wind direction (Table 1) and hence potential source populations for aphids caught in the suction trap.

Microsatellite genotyping

DNA from individual aphids was extracted using the salting out protocol described by Sunnucks *et al* (1996), and DNA was resuspended in 25 µl HPLC grade water. Genotypes were examined at four microsatellite loci originally isolated in *S. miscanthi*: Sm10, Sm11, Sm12 and Sm17 (Sunnucks *et al*, 1996, 1997; Wilson *et al*, 1997, 2004; Simon *et al*, 1999). Primers for Sm12 were redesigned and tested to confirm amplification of the same locus (polymerase chain reaction (PCR). Products using the new primers were 21 base pairs (bp) smaller than in the aforementioned studies; see Llewellyn, 2000; Llewellyn *et al*, 2003 for details). PCRs, electrophoresis and silver staining of PCR products were performed as described in Llewellyn *et al* (2003) to determine microsatellite genotypes of each individual for the four loci.

Statistical analysis

To compare the genetic structure of field and suction trap samples, statistical analysis was performed on the data in

Table 2 Distances in km between *S. avenae* field collecting sites

	RA	*RB*	*HA*	*HB*	*GA*
RB	1.3				
HA	8.8	7.5			
HB	9.5	8.3	1.5		
GA	75.0	74.3	66.8	66.3	
GB	76.3	74.8	67.8	67.0	2.8

Field codes as in Table 1.

two ways: (i) multilocus genotype analysis – comparing the diversities and compositions of the samples in terms of four-locus genotypes and (ii) standard population genetic analysis. Standard population genetic analysis was performed using complete data sets, and also, in many specified instances, on data sets reduced to one copy of each genotype, as in Llewellyn *et al* (2003). This approach has become standard procedure in highly resolving population genetic analysis of cyclic parthenogens (see justification in Sunnucks *et al*, 1997). It is necessary because aphids reproduce asexually for at least part of the year, so biased biological or experimental sampling has great potential to skew frequency estimates of alleles and genotypes. Reduction of data sets to 'one-aphid-per-genotype' removes clonal copies and can be thought of as backtracking to the last sexual production of each genotype. Comparing the results from analyses using complete data sets and 'one-aphid-per-genotype' thereby reveals the extent of clonal selection effects.

Multilocus genotype analysis: *S. avenae* reproduce asexually for at least part of the year (Hand, 1989). In this study, individuals with the same multilocus genotype across all four loci were assumed to have been produced asexually from a common ancestor and are henceforth described as being members of the same clone. This relies on aphid asexual reproduction producing offspring that are genotypically identical – this has now been demonstrated by a number of approaches including studies of microsatellites in experimental lineages (Hales *et al*, 2002). The use of only four loci is supported by Wilson *et al* (1999) and Haack *et al* (2000), who showed that addition of seven to 10 further primer sets did not discriminate additional genotypes among individuals previously tested with four to five microsatellites. Note that identical genotypes found in more than 1 year can be assumed to have a lifecycle that does not include an obligate sexual phase, because an intervening sexual phase would break up the genotype by genetic recombination (Sloane *et al*, 2001). As we are accumulating data on *S. avenae* from many years and across Europe and using the same genetic markers, knowledge of individual genotypes continues to increase (Sunnucks *et al*, 1997; Simon *et al*, 1999; Haack *et al*, 2000; Llewellyn *et al*, 2003; present study).

Clonal diversity within populations was calculated as in our earlier study (Llewellyn *et al*, 2003) using the simple measure of clonal (genotypic) diversity: $k = G/N$, where G is the number of different multilocus genotypes present in a sample and N the sample size, and the Shannon–Weaver diversity index, H (Shannon and Weaver, 1949). The clonal diversity of the field samples was compared with that of suction traps and diversity (Shannon–Weaver's H) and was partitioned into various sampling components (total sample, farm and field), following the procedure used by Shufran *et al* (1991).

Distributions of common multilocus genotypes among the fields and two suction trap collections from Rothamsted (R 97 and R e 97) were examined using similarity matrices and principal coordinates analysis. To examine whether the same clones were able to reach particular sites and survive, a similarity matrix based on the presence or absence of clones at particular sites was constructed using Jaccard's similarity coefficient. To assess whether particular clones were equally successful at different sites, another similarity matrix was constructed based on the Euclidean similarity coefficient. For this method, the numbers of aphids with each genotype were standardized by dividing by the sample size. For each similarity matrix, principal coordinates analysis was used to represent the variability in three dimensions (Digby and Kempton, 1987).

Standard population genetic analysis: GENEPOP version 3.1b (Raymond and Rousset, 1995a; see also http://wbiomed.curtin. edu.au/genepop/) was used to perform standard population genetic analysis. Data were analysed by comparing all six field collections with each other and with the suction trap collection made in late June/early July 1997 (R e 97). This method of analysis was used because it was considered interesting to compare the genetic structure of *S. avenae* among fields within a farm, although it involved rather small sample sizes ($\leqslant$25 individuals), which reduces the power of the tests performed. Field samples were also combined and whole farm data sets were compared. Allele frequencies were calculated for all collections. For the analysis, two different data sets were used. Firstly, the complete data set was tested followed by the data set with duplicated copies of genotypes removed (see above for the rationale).

The degree of differentiation between populations was examined by allelic and genotypic (single locus) testing and by calculating F_{ST} values for pair-wise comparisons of samples, along with the assessment of deviation from Hardy–Weinberg equilibrium (HWE) to test for deviations from random mating. The unbiased estimate of the exact probability test (Fisher's exact test) for a contingency table of allelic frequencies was made using a Markov chain method. The null hypothesis is that the allelic distribution is identical across populations (Raymond and Rousset, 1995b). The distribution of single-locus genotypes across fields was tested using an unbiased estimate of the P-value of a log-likelihood-based exact test (G test; Goudet *et al*, 1996). The allelic and single-locus genotypic tests of differentiation were performed for all population pairs. F_{ST} values were estimated according to Weir and Cockerham (1984) for all samples and for pairs of samples. In this analysis, F_{ST} measures are not being used in the context of a particular model of migration, since this may not be meaningful in clonal populations (estimation of migration rate from F_{ST} assumes an island model and drift–migration equilibrium). Nonetheless, F_{ST} estimates are convenient measures of the degree to which allele frequencies differ among the samples, and they are thus used here (see Llewellyn *et al*, 2003 for further details). To reduce the likelihood of type I error where multiple tests were performed, the significance level was adjusted using the Dunn–Šidák method (Sokal and Rohlf, 1995).

Results

Diversity within *S. avenae* populations

A large amount of microsatellite variation was found at the loci tested. Allele frequencies for each field collection and the two suction trap collections are given in Table 3. Four alleles were found at locus Sm11, 15 at Sm12 and six

Table 3 *S. avenae* microsatellite allele frequencies for 1997 field samples and for two suction trap collections

	R e 97	*R 97*	*R A*	*R B*	*H A*	*H B*	*G A*	*G B*
N	50	54	25	23	25	24	24	24
Sm10								
152	0.080	0.065	0.080	0.152	0.200	0.021	0.104	0.083
160	0.040	0.019	—	0.022	0.020	0.021	0.021	—
164	0.490	0.509	0.520	0.391	0.320	0.563	0.521	0.396
166	0.180	0.296	0.240	0.304	0.340	0.229	0.229	0.458
168	0.160	0.056	0.020	0.022	0.040	0.042	0.042	—
185	0.010	—	0.020	0.022	0.020	—	0.042	—
200	0.040	0.056	0.120	0.087	0.060	0.125	0.042	0.042
240	—	—	—	—	—	—	—	0.021
Sm11								
144	0.900	0.769	0.860	0.783	0.780	0.750	0.563	0.583
148	0.010	0.028	—	—	—	0.021	0.083	0.021
149	0.090	0.204	0.140	0.217	0.180	0.208	0.313	0.396
155	—	—	—	—	0.040	0.021	0.042	—
Sm12								
112	—	—	—	—	—	—	0.021	0.021
116	—	—	—	—	—	—	—	0.021
118	0.540	0.213	0.160	0.087	0.100	0.125	0.083	—
130	0.130	0.231	0.220	0.196	0.140	0.271	0.250	0.375
132	0.010	0.046	0.020	0.022	—	0.063	—	0.021
134	0.010	0.019	0.020	0.022	0.040	0.021	—	—
136	0.010	—	—	—	0.020	0.042	—	—
138	0.180	0.194	0.240	0.370	0.440	0.083	0.313	0.167
140	—	—	0.020	0.022	—	0.063	0.063	—
142	—	0.009	—	—	0.020	—	0.021	—
144	0.050	0.167	0.100	0.152	0.120	0.188	0.188	0.375
146	—	0.000	0.020	—	—	—	0.021	—
148	0.010	0.046	0.120	0.065	0.080	0.125	0.042	—
150	0.060	0.065	0.080	0.065	0.040	0.021	—	0.021
152	—	0.009	—	—	—	—	—	—
Sm17								
178	0.510	0.565	0.800	0.739	0.740	0.688	0.688	0.646
179	0.040	0.167	0.120	0.130	0.180	0.271	0.208	0.354
180	0.020	0.019	0.040	0.022	0.020	—	—	—
181	0.010	0.009	0.020	—	0.040	—	—	—
182	—	—	—	0.022	—	—	0.021	—
183	0.420	0.241	0.020	0.087	0.020	0.042	0.083	—

N = sample size.

at Sm17. Eight alleles were assigned at locus Sm10, ranging from 152 to 240 bp (Table 3). Owing to stutter bands in the very largest alleles, which made scoring difficult, some rare large alleles were pooled into groups of similar sizes recorded as 185, 200 or 240 bp. Such grouping was found to have little effect on the statistical analysis (Llewellyn, 2000). None of the alleles recorded here are those that characterise two previously- identified cryptic lineages of Sitobion: '*S. fragariae*-like' and 'Wheat-specific' (Sunnucks *et al*, 1997). Thus we consider these lineages absent from the present samples and do not consider them further.

Multilocus genotype analysis

A total of 78 multilocus genotypes were found in the sample of 238 individuals tested from field and suction traps. There were 59 aphids with genotypes unique in the sample, while the remaining 179 aphids shared just 19 multilocus genotypes (= clones), some of which were very common and some were rarer (Table 4). Of the 19 multilocus genotypes sampled multiple times here (ie in southern UK in 1997), seven (clones 20, 25, 27, 53, 58, 61 and 71) were also sampled in southern UK in 1994–1995 (Sunnucks *et al*, 1997) and four others (107, 114, 179 and 181) in 1994–1995 in France (Simon *et al*, 1999). Thus, at least 11 of the 19 common clones in the present study do not have an obligate sexual phase in their lifecycle. A number of these clones appear to be quite successful among and/or within years, and are perhaps quite generalist.

Clone 53 was the most common clone in the present sample, being found in all samples, and was one of the two common clones sampled from diverse crops in France in 1997–1998 (Haack *et al*, 2000). However, this clone was sampled only a single time in southern UK in 1994–1995, on cocksfoot grass, *Dactylis glomerata* L. (Sunnucks *et al*, 1997), so may have become common in the UK relatively recently. In the present study, clones 114 and 179 were found in five out of six fields, and clone 107 was found in four out of six fields. All three were recorded from French cereal fields in 1994–1995 (Simon *et al*, 1999), as well as in northern UK suction traps in 1997 (Llewellyn *et al*, 2003). Nine clones in the present study were found in two out of six fields sampled and in all cases, these were fields on different farms. The

proportions of the collections represented by the most common clones varied greatly among fields. Clone 58 was common in suction trap collections, but rare in field collections, while conversely, clone 179 was rare in the suction trap, but somewhat more common in field samples.

Two diversity measures for each population, genotypic diversity, *k*, and the Shannon–Weaver Index, *H*, are given in Table 4. A similar level of diversity was found in suction trap and field collections. The proportion of unique aphid genotypes in each of the samples was between 15 and 35%. The Garford B field was the least diverse, with 24 individuals representing just six genotypes. This is possibly because the collection was made so late in the season (15th July) when any clonal selection would be well advanced (De Barro *et al*, 1995a; Sunnucks *et al*, 1997). However, the collection made from the Garford A field on the same date contained more than twice as many genotypes. Owing to the small size of the samples, diversities between the Garford A collections made on different dates (Table 1) were not compared. The measure of genotypic diversity, *k*, is sample size dependent.

The mean clonal diversity (Shannon–Weaver's, *H*) was partitioned between fields, farms and all field collections (Table 5). Most of the diversity (72.5%) was among aphids in a field, while only 13.5% of diversity was found by sampling different fields in a farm and an additional 14.3% of the total diversity was found by sampling from different farms. This indicates that while there is a high diversity of clonal types overall, there is low differentiation between fields (ie in terms of genotypes being restricted to a particular field). A large proportion of the total number of clones were found within individual fields; hence there is as much variation at this scale as over a much greater area sampled (up to 80 km). This indicates wide distribution of the genotypes sampled. If the suction trap collections are included in the analysis of partitioning of diversity (calculations not shown), twice as much diversity is added by including the two suction trap samples as by sampling an additional farm.

Figure 2 shows the results of principal coordinates analysis examining the distribution of common multilocus genotypes among fields and suction trap collections. Using the Jaccard similarity coefficient, 62.9% of the variability was represented in three dimensions and, with the Euclidean similarity coefficient, 73.9% of the variability was shown. Based on the first two dimensions, the two Hemel Hempstead fields (HA and HB) appeared similar in terms of clonal composition using both similarity coefficients. Such similarities among fields on a farm were not observed in the Rothamsted and Garford samples. In fact, collections from two fields on different farms (Garford A and Rothamsted B) were very similar, suggesting that factors other than distance between fields are important in determining clonal compositions of these aphid populations. The suction trap collections from Rothamsted were no more similar to field collections from Rothamsted than to those from other farms.

Table 4 *S. avenae* multilocus genotypes identified from field and suction trap collections

Genotype	*Suction trap collections*		*Field collections*						*Total*
	R e 97	*R 97*	*R A*	*R B*	*H A*	*H B*	*G A*	*G B*	
Uniques	10	13	8	5	6	8	5	4	59
27	0	1	1	0	0	0	0	0	2
181	1	1	0	0	0	0	0	0	2
U.K. 10	0	0	1	0	1	0	0	0	2
U.K. 12	0	0	1	0	1	0	0	0	2
U.K. 19	0	1	0	0	1	0	0	0	2
U.K. 8	0	0	0	0	0	0	2	0	2
61	1	2	0	0	0	0	0	0	3
U.K. 11	0	0	0	1	0	2	0	0	3
U.K. 7	0	0	0	1	0	0	2	0	3
U.K. 9	0	0	0	0	0	1	2	0	3
25	0	1	2	0	0	1	0	0	4
71	3	1	0	0	0	0	0	0	4
177	0	2	1	0	0	1	0	0	4
20	4	1	0	1	0	0	1	0	7
107	2	1	1	1	1	0	2	0	8
179	0	1	4	1	3	2	1	0	12
58	13	4	0	1	0	0	1	0	19
114	6	6	3	6	7	0	3	3	34
53	4	16	2	5	5	9	5	17	63
N	44	51	24	22	25	24	24	24	238
G	18	26	17	13	13	14	14	6	78
k	0.41	0.51	0.71	0.59	0.52	0.58	0.58	0.25	0.33
H	2.424	2.688	2.694	2.237	2.220	2.239	2.474	1.034	3.224
e^H	11.29	14.70	14.79	9.36	9.21	7.41	11.87	2.81	25.13

Genotypes labelled only with a number are identical to those identified in previous studies. Those labelled 'U.K.' were new to this study. Genotypes with code numbers <103 were found previously in southern U.K. (Sunnucks *et al*, 1997), while those with numbers >102 were first recorded in France (Simon *et al*, 1999). Unique genotypes were unique in the sense that they were found once only in the collections here and in Llewellyn *et al* (2003), although some of them had been found in the previous studies. G = total number of multilocus genotypes (clones) in the sample; N = sample size; k = genotypic diversity (G/N); H = Shannon–Weaver diversity index; e^H = exponential of Shannon–Weaver diversity index.

Table 5 Partitioning total diversity (Shannon–Weaver index, *H*) for field-collected *S. avenae* populations during 1997 (Shufran and Wilde, 1994)

Shannon-Weaver Index

Population	*Field*	*Farm*	*Total*
Rothamsted A	2.694		
Rothamsted B	2.237	2.867	
Hemel A	2.220		
Hemel B	2.239	2.667	
Garford A	2.474		
Garford B	1.034	2.115	2.965
Mean diversity	$H_{field} = 2.150$	$H_{farm} = 2.550$	$H_{all} = 2.965$

Partitioning of total diversity

Among aphids in a field	H_{field}/H_{all}	72.5%
Among fields in a farm	$(H_{farm}–H_{field})/H_{all}$	13.5%
Among farms	$(H_{all}–H_{farm})/H_{all}$	14.0%
Total		100.0%

Standard population genetic analysis

Most field and suction trap data deviated from H–W equilibrium (mainly heterozygote deficits) at Sm12 when all-aphid data sets were tested (data not shown; see Llewellyn, 2000 for details). Heterozygote excesses were found at Sm10 and Sm11, and the Garford population was the furthest from H–W equilibrium. However, using one-aphid-per-genotype data sets, no heterozygote excesses were found; indeed, Rothamsted and Hemel Hempstead samples showed heterozygote deficits at Sm11 and Sm12. The deviations from expectations using the complete data sets appear to be largely due to the duplications of a number of clones in the samples examined, although the lack of significance in some of the tests using one-aphid-per-genotype may be an artefact of sample size. The heterozygote deficiencies that remain after removal of duplicated genotypes may be due to the presence of null alleles (alleles that do not amplify due to mutations in the primer binding sites; Goldstein and Schlötterer, 1999).

Almost all pairs of loci were in genotypic (linkage) disequilibrium in all populations using the complete data sets ($P < 0.001$ for most loci). This is due to the presence of clonal copies in the samples. When using one-aphid-per-genotype data, most loci are in genotypic equilibrium, although there is some reduction in the power of this test due to the sample sizes (Table 6).

Exact tests for allelic and genotypic differentiation among samples gave similar results to each other (data not shown; see Llewellyn, 2000 for details). When all-aphid data were examined, pairwise comparisons revealed greater allelic and genotypic differentiation between suction trap and field samples than among field samples. The samples from Garford, which is furthest from the Rothamsted suction trap, were most differentiated from the suction trap collection. The Garford B field was also slightly differentiated from some other field samples. Using one-aphid-per-genotype, no significant allelic or genotypic differentiation was found using pairwise comparisons: in other words, there was more differentiation in relative clone frequencies than in genetic characteristics of clones present. Again, nonsignificant test results with the one-copy-per-genotype samples may be due partially to small sample size.

Using all-aphid data, there were a large number of high pairwise F_{ST} values (38/84 single locus values >0.05) (Table 7). Using one-aphid-per-genotype, values were generally low or negative, indicating low levels of differentiation (8/84 single locus values >0.05). These differences, when replicated clones are removed from the analysis, provide strong evidence that clonal selection is the differentiating force among the field populations. The largest F_{ST} values were found in comparisons of the suction trap collection from Rothamsted (R e 97) with the field collections. The collection from the Garford B site was also well differentiated from other samples. Sm11 and Sm17 were the most differentiated loci.

When field data were combined by farm for this analysis, some large pairwise F_{ST} values were found using all-aphid data sets. For Sm12 and Sm17, these were between the Rothamsted suction trap collection and the

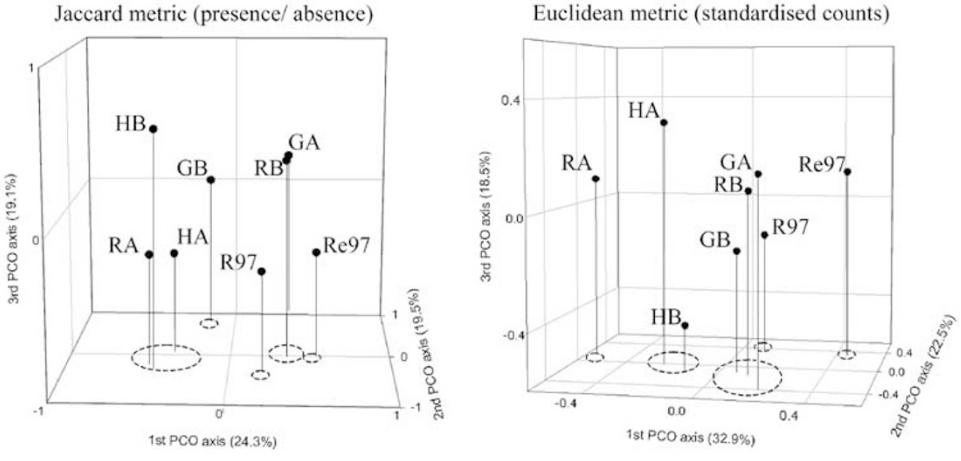

Figure 2 Results of principal coordinates analysis of similarity matrices examining the distributions of common multilocus genotypes among samples. Circles are drawn for clarity to indicate grouping of samples that are most similar according to the first and second axes. They are not part of the formal statistical analysis. Sample codes as in Table 1.

Table 6 Results of pairwise exact tests for allelic and genotypic differentiation using complete data (above diagonal) and one copy per genotype (below diagonal)

(a) Allelic differentiation

	R e 97	*R A*	*R B*	*H A*	*H B*	*G A*	*G B*
R e 97	x	12, 17	12, 17	12, 17	12, 17	11, 12, 17	10, 11, 12, 17
R A	—	x	—	—	—	—	12
R B	—	—	x	—	—	—	—
H A	—	—	—	x	—	—	12
H B	—	—	—	—	x	—	12
G A	—	—	—	—	—	x	—
G B	—	—	—	—	—	—	x

	R e 97	*Rothamsted*	*Hemel*	*Garford*
R e 97	x	12, 17	12, 17	11, 12, 17
Rothamsted	—	x	—	—
Hemel	—	—	X	—
Garford	—	—	—	x

(b) Genotypic differentiation

	R e 97	*R A*	*R B*	*H A*	*H B*	*G A*	*G B*
R e 97	x	10, 12	12, 17	12, 17	12, 17	11, 12, 17	11, 12, 17
R A	—	x	—	—	—	—	—
R B	—	—	x	—	—	—	—
H A	—	—	—	x	—	—	12
H B	—	—	—	—	x	—	—
G A	—	—	—	—	—	x	—
G B	—	—	—	—	—	—	x

	R e 97	*Rothamsted*	*Hemel*	*Garford*
R e 97	x	12, 17	12, 17	11, 12, 17
Rothamsted	—	x	—	11
Hemel	—	—	x	—
Garford	—	—	—	x

Comparisons for each locus significant at $P<0.05$ (following Dunn-Šidák correction for 108 tests) are represented by the number of the locus (10 = Sm10, 11 = Sm11 etc.). — = no significant differences found.

field samples, although for Sm11, the greatest differences were found between the Garford farm and the other collections (data not shown; see Llewellyn, 2000 for details). Smaller F_{ST} values were found when one-aphid-per-genotype was used. F_{ST} values >0.05 were found in comparisons of the Rothamsted suction trap with the Garford farm collection at Sm11 and Sm12, and with the Hemel Hempstead farm collection at Sm17.

Discussion

In this paper, we describe the genetic analysis of *S. avenae* samples collected from the field in Britain at small spatial scales ($\leqslant$80 km) relative to those geographic scales studied in Llewellyn *et al* (2003) and compare the data obtained with samples trapped in the 12.2 m high suction trap at Rothamsted. We provide evidence for the disproportionate proliferation of a number of successful aphid clones, the extensive dispersal of this species over the study area and factors other than migration (eg clonal selection, see below) as being responsible for the clonal composition observed.

The differences in deviation from H–W expectations and linkage disequilibrium between the complete data set and the set with duplicated genotypes removed indicate a disproportionate proliferation of a number of successful clones. Evidence for migration is as follows. Levels of differentiation (including allelic, genotypic and F_{ST} measures) using one-aphid-per-genotype were low over distances of $<$80 km and identical clones were present in different fields, farms and the suction trap. This indicates that migration over distances equivalent to those between the sample sites is common, supporting the view that *S. avenae* is capable of long-distance aerial movements (Llewellyn *et al*, 2003). Partitioning of the genetic diversity sampled also provides evidence for a wide distribution of genotypes, and therefore extensive movement (although the time scale of these movements is not known, as many of the clones sampled appear to have overwintered for a number of years).

Even so, other evidence clearly points towards the persistence of certain genotypes at different sites, indicative of clonal selection. The evidence for such selection causing population differentiation over and above any restriction of migration (and hence gene flow)

Table 7 Single-locus pairwise F_{ST} values for suction-trapped and field-collected *S. avenae*

Sm10	*R e 97*	*R A*	*R B*	*H A*	*H B*	*G A*	*GB*
R e 97		−0.0112	−0.0021	0.0322	−0.0277	−0.0253	**0.0935**
R A	0.0085		−0.0183	0.0004	−0.0088	−0.0288	0.0356
R B	0.0220	0.0010		−0.0122	−0.0041	−0.0248	0.0065
H A	0.0437	0.0305	−0.0088		0.0403	−0.0078	−0.0135
H B	0.0104	−0.0131	0.0239	**0.0601**		−0.0230	**0.0934**
G A	−0.0009	−0.0143	0.0015	0.0265	−0.0042		0.0358
G B	**0.0670**	0.0379	0.0110	0.0145	**0.0593**	0.0400	
Sm11							
R e 97		−0.0165	−0.0149	0.0190	−0.0306	**0.0839**	0.0389
R A	−0.0051		−0.0346	−0.0050	−0.0095	0.0436	0.0030
R B	0.0431	−0.0023		−0.0322	−0.0131	0.0037	−0.0574
H A	0.0333	−0.0019	−0.0184		0.0088	−0.0171	−0.0479
H B	**0.0546**	0.0099	−0.0198	−0.0149		**0.0615**	0.0258
G A	**0.2015**	**0.1152**	0.0417	**0.0503**	0.0275		−0.0911
G B	**0.2445**	**0.1507**	**0.0621**	**0.0795**	**0.0528**	−0.007	
Sm12							
R e 97		0.0275	0.0438	0.0227	0.0197	0.0489	**0.0946**
R A	**0.0896**		−0.0132	−0.0054	−0.0140	−0.0115	−0.0045
R B	**0.1400**	−0.0082		−0.0381	−0.0019	−0.0273	−0.0369
H A	**0.1488**	0.0085	−0.0189		−0.0051	−0.0264	−0.0314
H B	**0.1290**	0.0035	0.0362	**0.0699**		0.0010	0.0243
G A	**0.1457**	−0.0019	−0.0136	0.0033	0.0203		−0.0278
G B	**0.2439**	**0.0701**	**0.0666**	**0.1107**	0.0396	0.0375	
Sm17							
R e 97		**0.0606**	−0.0002	0.0477	0.0479	0.0010	**0.0995**
R A	**0.1875**		−0.0145	−0.0172	−0.0274	−0.0108	−0.0119
R B	**0.1230**	−0.0110		0.0000	−0.0208	−0.0329	−0.0015
H A	**0.1689**	−0.0091	−0.0102		−0.0185	−0.0170	0.0478
H B	**0.1617**	0.0269	0.0077	−0.0020		−0.0287	−0.0068
G A	**0.1222**	0.0113	−0.0122	−0.0079	−0.0125		0.0278
G B	**0.2033**	**0.0782**	**0.0550**	0.0323	−0.0021	0.0166	
All loci							
R e 97		0.0195	0.0137	0.0309	0.0079	0.0248	**0.0882**
R A	**0.0829**		−0.0178	−0.0060	−0.0145	−0.0060	0.0079
R B	**0.0900**	−0.0050		−0.0216	−0.0076	−0.0219	−0.0204
H A	**0.1100**	0.0112	−0.0140		0.0077	−0.0175	−0.0147
H B	**0.0960**	0.0040	0.0185	0.0407		0.0005	0.0417
G A	**0.1087**	0.0200	0.0021	0.0176	0.0092		−0.0128
G B	**0.1858**	**0.0774**	0.0477	**0.0627**	0.0397	0.0244	

F_{ST} values for all data below the diagonal, and for one copy per genotype above the diagonal. F_{ST} values >0.05 are highlighted in bold.

is as follows. High levels of differentiation (using allelic, genotypic and F_{ST} measures) with complete data sets, not seen when replicated copies of clones are removed, show that while migration homogenizes allelic diversity, factors other than movement (eg clonal selection) are more important in determining genetic structure at the field scale. Additional evidence is provided by principal coordinates analysis of similarity matrices examining the distribution of common clones (Figure 2), which reveals that the most similar fields are not necessarily those closest geographically.

The variation in clonal composition could be due to stochastic and/or more likely, deterministic differences in clones arriving (ie host preferences by winged foundresses; Lushai *et al*, 2002), surviving and thriving within fields (ie clonal selection and competition). Clonal selection in the microhabitat of a particular field, or interclonal competition of specialist and generalist genotypes, is undoubtedly an important factor in determining observed clonal frequencies. From the present results, the causes of the differences in clonal composition – in effect, a waxing and waning of particular genotypes in time and space (Blackman, 1981) – cannot be unequivocally decided upon, although we can examine the strength of the arguments for various possibilities, including stochastic effects, competition and selection.

In terms of stochastic effects, we consider these as minimal compared with the known host preferences of the aphid (Haack *et al*, 2000; Lushai *et al*, 2002) and the rapid results of asexual propagation with resultant selection for particular genotypes. As for competition, the population density of *S. avenae* in wheat crops during the sampling season was low; hence, direct clonal competition for feeding sites was unlikely, although evidence for such competition has been found in other studies of this aphid (eg De Barro *et al*, 1994).

In terms of selection, proliferation of specific clones, spatially and temporally, may well be dictated by differences in local microclimate (eg temperature, humidity), wheat cultivar, crop density and possibly insecticide regime (Carter *et al*, 1980; Lowe, 1980, 1981;

Honek, 1987; see also De Barro *et al*, 1994, 1995a, b; Fenton *et al*, 2003). Although insecticide resistance has not, to date, been reported in *S. avenae*, it has evolved in several other aphid species, including most recently in the greenbug, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) (Ono *et al*, 1999).

Our last point of consideration concerns the validity of using suction traps to obtain genetically representative samples of aphids over large areas of their preferred host (ie wheat). *S. avenae* suction trap and field collections showed broad similarities in genetic structure (these results). While all collections showed similar diversity, and most common clones were shared between field and trap collections, the trap-field differentiation was greater than that among field collections only. In addition, one clone common in the suction trap (clone 58) was rare in the field and may have a preference for a host other than winter wheat. This clone was found on winter barley (*Hordeum vulgare L.*) and *D. glomerata* in other studies (Sunnucks *et al*, 1997; Llewellyn, unpublished data). Alternatively, this genotype may undertake a relatively early emigration from the wheat crop, and so was common in aerial samples in early July 1997, but had already left wheat fields by the time of field collection. Conversely, clone 53, which showed the opposite pattern, may be a late migrant.

In conclusion, the present evidence provides strong support for migration and, more importantly, clonal selection at the field scale. Clearly, the populations examined do not represent one homogeneous genetic entity but, rather, comprise numerous clones whose frequency, determined in this 'snap-shot' of time, is governed by selective forces. This is not to say that these effects may not be masked to some extent by the compensatory effects of migration and drift. While the data confirm the long-range migratory tendencies of *S. avenae* (ie homogenization of allelic structure), at the small spatial scales sampled, populations are different genetically (ie genotypically) when compared with one another, to the extent that field populations on the same farm or in close geographic proximity (eg Rothamsted and Hemel Hempstead, approximately nine km apart) vary within the same year at the same collection date. The very local differentiating forces must surely include some kind of clonal selection.

This study further emphasizes the importance of using molecular markers in elucidating the population structure of these small and very mobile herbivorous insects and holds promise for future studies, both of a fundamental and applied nature. The economic importance of these major plant pests and virus vectors spans horticulture, agriculture and forestry (eg see Tatchell, 1989).

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