Limited genetic exchanges between populations of an insect pest living on uncultivated and related cultivated host plants

Aude Vialatte^{1,2}, Charles-Antoine Dedryver¹, Jean-Christophe Simon¹,
Marina Galman¹ and Manuel Plantegenest^{1,*}

¹INRA/Agrocampus Rennes, Unité Mixte de Recherche Biologie des Organismes et des Populations appliquées à la Protection des Plantes (UMR BiO3P), Equipe Biologie et Génétique des Populations d'Insectes, Domaine de la Motte, 35653 Le Rheu, France

²Makhteshim Agan France, 12 Bd des Iles, 92441 Issy Les Moulineaux Cedex, France

Habitats in agroecosystems are ephemeral, and are characterized by frequent disturbances forcing pest species to successively colonize various hosts belonging either to the cultivated or to the uncultivated part of the agricultural landscape. The role of wild habitats as reservoirs or refuges for the aphid *Sitobion avenae* that colonize cultivated fields was assessed by investigating the genetic structure of populations collected on both cereal crops (wheat, barley and oat) and uncultivated hosts (Yorkshire fog, cocksfoot, bulbous oatgrass and tall oatgrass) in western France.

Classical genetic analyses and Bayesian clustering algorithms indicate that genetic differentiation is high between populations collected on uncultivated hosts and on crops, revealing a relatively limited gene flow between the uncultivated margins and the cultivated part of the agroecosystem. A closer genetic relatedness was observed between populations living on plants belonging to the same tribe (Triticeae, Poeae and Aveneae tribes) where aphid genotypes appeared not to be specialized on a single host, but rather using a group of related plant species. Causes of this ecological differentiation and its implications for integrated pest management of *S. avenae* as cereals pest are discussed.

Keywords: host-plant specialization; population structure and differentiation; *Sitobion avenae*; pest management; landscape genetics

1. INTRODUCTION

Land cover and spatial heterogeneities at the landscape scale can affect the spatial distribution of organisms, in particular, through their influence on their movement patterns (Crist *et al.* 1992; Johnson *et al.* 1992; Wiens & Milne 1993; Morales & Ellner 2002). Agroecosystems, especially in Europe, are characterized by a high spatial and temporal heterogeneity and can offer a large panel of putative habitats to pest populations. Many agricultural pests and diseases are not restricted to a unique host and can exploit various cultivated or uncultivated plant species. However, studies have traditionally focused on the field scale or, at most, have considered a set of cultivated fields planted with the same crop without taking into account the neighbouring landscape (Pashley 1989; Via 1999; Vives *et al.* 2002; Volkova *et al.* 2002; Chin *et al.* 2003; Lin *et al.* 2003).

Although the role of landscape structure on biodiversity and population structure in cultivated areas has been advocated (Di Giulio *et al.* 2001), only a few studies have considered pest populations in both uncultivated and cultivated areas (but see De Barro *et al.* 1995a; Bourguet *et al.* 2000; Martel *et al.* 2003). Consequently, the exchanges of individuals and genes between populations inhabiting the cultivated system and the uncultivated adjacent environment remain poorly understood.

However, analysing relationships between populations living in cultivated and uncultivated areas is very important for pest management at the landscape scale, both for improving integrated and ecological control of pest populations and for forecasting the sustainability of control methods such as transgenic crops or pesticides (Gould 1998). Uncultivated margins may constitute not only a temporary or a permanent source of pest migrants, but also a source of their natural enemies (Thies *et al.* 2003). In addition, when environmental conditions become unfavourable to pests on cultivated plants (owing to the phenology of the host plant or the agricultural practices such as pesticide sprays or harvesting), uncultivated habitats may act as a refuge zone (Kennedy & Storer 2000). Finally, if gene flow exists between cultivated and uncultivated parts of the agroecosystem, then the uncultivated system may play the role of gene reservoir, while the cultivated system may be responsible for genetic pollution of the adjacent environment (Martel *et al.* 2003).

Aphids are particularly prone to host specialization (Dixon 1998) and frequently exhibit host-specialized genotypes often termed 'biotypes' or 'host races', as for example in *Schizaphis graminum* (Puterka & Peters 1988), *Aphis gossypii* (Vanlerberghe-Masutti & Chavigny 1998) and *Acyrtosiphon pisum* (Via 1999; Simon *et al.* 2003). Plants with long vegetative cycles, such as perennial grasses, are especially likely to favour specialization of aphids to their host (Via 1999), while plants with brief cycles, such as annual grasses and cereals, should mainly

* Author for correspondence (manuel.plantegenest@agrocampus-rennes.fr).

host generalist genotypes (Futuyma & Moreno 1988; Haack *et al.* 2000). Many aphid species can develop populations both on crop and on related weeds or non-crop plants (e.g. Robert & Le Gallic 1991; Carré & Bournoville 2003), offering good models for studies on ecological specialization and gene flow in agricultural landscapes. Typically, aphids reproduce by cyclical parthenogenesis, which is the alternation of many parthenogenetic generations in spring and summer, and a single sexual generation in autumn (Dixon 1998). However, aphid species frequently encompass other lineages that are parthenogenetic all year round, or that invest in both sexual and asexual reproduction at the same time of the year (Blackman 1972; Dedryver *et al.* 1998; Simon *et al.* 2002). Parthenogenetic reproduction ensures a rapid succession of many clonal generations that amplify the response to selection, while sexual reproduction allows genetic mixing between aphid lineages. In addition, aphid populations are usually composed of a mixture of wingless forms that ensure rapid development of populations after colonization (De Barro *et al.* 1994) and of winged individuals that can disperse over long distances (greater than 20 km; Loxdale *et al.* 1993; Llewellyn *et al.* 2003), and may be locally very mobile (Simon *et al.* 1999; Loxdale & Lushai 2001).

The grain aphid, *Sitobion avenae*, can colonize a wide range of species belonging to the *Poaceae* family, including cereals and uncultivated or pastured grasses (Hand 1989), but host specialization in this species remains controversial. Performance experiments have shown some degree of specialization in *S. avenae* populations on wheat, oat and cocksfoot, *Dactylis glomerata* (Weber 1985; Caillaud *et al.* 1995; De Barro *et al.* 1995a; Sunnucks *et al.* 1997). However, examining the distribution of *S. avenae* genotypes in the field among different cereal crops revealed the existence of generalist genotypes (Haack *et al.* 2000).

The aim of this study was to examine the genetic relationships between *S. avenae* populations living in cultivated versus uncultivated parts of the agroecosystems, in order to assess the extent of host specialization in this species and the role of uncultivated margins as reservoirs or refuges for aphid populations colonizing cereal crops.

2. MATERIAL AND METHODS

(a) Aphid collection

Aphids were collected in the Rennes Basin (Brittany, France) in spring 2003 at two sites (Le Rheu and Domagné) 28 km apart. At each site, *S. avenae* was sampled in fields of various cultivated *Poaceae* including triticale ($\times$ *Triticosecale*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and oat (*Avena sativa*), and in adjacent margins on cocksfoot (*Dactylis glomerata*), Yorkshire fog (*Holcus lanatus*), bulbous oatgrass (*Arrhenatherum bulbosum*), tall wild oat (*Avena fatua*) and bent (*Agrostis* sp.). All of these uncultivated plants are perennial, apart from tall wild oat, which is annual and dies out at the end of summer. The distance between two sampling locations (fields) in each site never exceeded 1 km. In each sampling location, aphid colonies separated by at least 0.5 m were collected, and only one individual per colony was randomly chosen and used for genetic analysis. To ensure that aphids actually developed on the host plant on which they have been collected, only wingless forms were used. Each aphid was stored in an Eppendorf tube at -20°C prior to genetic

analysis. In the following text, a sample refers to aphids collected on the same plant at the same site while a host population refers to all the samples from the same plant.

(b) DNA extraction and analysis

DNA from individual aphids was extracted by using a phenol-chloroform protocol (Fabre *et al.* 2003). The DNA was then re-suspended in 20 μl of ultra pure (UP) water.

Each individual of *S. avenae* was genotyped at five microsatellite loci. Sm10, Sm17 and S16b were isolated from *Sitobion miscanthi*, while Sa4 Σ and S5.L were developed from *S. avenae* (Wilson *et al.* 2004).

Microsatellite amplifications were carried out in 15 μl reaction mixtures consisting of 0.2 U of *Taq* polymerase (Promega), 1 $\times$ MgCl₂-free reaction buffer, 2 mM MgCl₂, 200 μM dNTP, 0.2 mM of each primer (unlabelled and IRD) and 1 μl of the dilute aphid DNA. Amplifications were made in a PTC-100 programmable thermal controller (MJ Research, Inc.) using a regime of initial denaturation at 94 $^{\circ}\text{C}$ for 3 min, followed by 40 cycles of denaturation at 94 $^{\circ}\text{C}$ for 1 min, annealing for 1 min with temperature depending on locus and elongation at 72 $^{\circ}\text{C}$ for 45 s, with a final extension at 72 $^{\circ}\text{C}$ for 4 min. For loci Sm10 (IRD 700) and S16b (IRD 700), annealing took place at 56 $^{\circ}\text{C}$ and for Sm17 (IRD 800), S5.L (IRD 800) and Sa4 Σ (IRD 800), annealing took place at 60 $^{\circ}\text{C}$. The Sm10 polymerase chain reaction (PCR) products were diluted by half with ultra pure water. Then, PCR products Sm10 (0.3 μl) and Sm17 (4 μl) were pooled together in 10 μl of denaturing buffer as S16b (1 μl) and Sa4 Σ (2 μl). 2.2 μl of S5.L PCR products were used in 10 μl of denaturing buffer. All these mixes were completed by a volume of UP water up to 20 μl .

For each sample, about 1 μl of pooled PCR products was resolved by denaturing 7.5% polyacrylamide urea gelling electrophoresis (19 : 1 acrylamide : bisacrylamide), run for 3 h at 1000 V and 75 W in 1 $\times$ TBE buffer, according to the LI-COR (global IR2 system double laser sequencing) protocol. The size of the alleles of each locus was estimated by SAGA software using a sequencing size ladder made in our laboratory.

(c) Analysis of genetic data

Observed heterozygosities, unbiased estimates of expected heterozygosities under Hardy-Weinberg assumptions (Levene 1949), *F*-statistics (Weir & Cockerham 1984) and linkage disequilibrium were calculated for each sample and statistics host population using GENEPOP (Raymond & Rousset 1995). Fisher exact tests were performed to test for deviations from Hardy-Weinberg equilibrium. Significance of *F*-statistics was assessed by a permutation test using ARLEQUIN v. 2 (Schneider *et al.* 2000). Following Sunnucks *et al.* (1997), genetic analyses were carried out both using only one copy per genotype and with all individuals to account for the clonal reproduction of aphids. To quantify genotypic diversity, the ratio of the number of multilocus genotypes over the total number of individuals (*G/n*) was calculated for each host population.

Population structure was inferred using a Bayesian model clustering algorithm implemented in the computer programme STRUCTURE (v. 2; Pritchard & Wen 2000). To run the programme, a number *K* of genetic clusters characterized by the matrices of allele frequencies at each locus is first assumed. Then, for each individual, the proportion of its genome derived from each genetic cluster (proportion of ancestry) is estimated. Five independent runs for

Table 1. Genetic diversity in nine host populations of *S. avenae* with one copy per genotype.

(For each host population: n , number of individuals; G , number of genotypes; G/n , genotypic diversity index; n all, mean number of alleles per locus; F_{IS} , estimates of F_{IS} values and level of significance; H_E and H_O , expected and observed heterozygosities; s.e., standard error; *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$; n.s. $p > 0.05$.)

host populations	n	G	G/n	F_{IS}	H_O (s.e.)	H_E (s.e.)	n all (s.e.)
Yorkshire fog	44	30	0.68	-0.044 n.s.	0.62 (0.13)	0.58 (0.07)	4.8 (1.6)
cocksfoot	17	15	0.88	0.198**	0.47 (0.08)	0.56 (0.14)	4 (1.7)
bent	22	12	0.54	0.071 n.s.	0.47 (0.19)	0.47 (0.12)	3.6 (1.2)
tall wild oat	47	40	0.85	0.103***	0.61 (0.23)	0.67 (0.12)	6.4 (2.5)
Bulbous oatgrass	26	21	0.81	0.085 n.s.	0.57 (0.33)	0.61 (0.11)	4.6 (1.5)
Oat	80	61	0.76	0.0001 n.s.	0.62 (0.12)	0.62 (0.07)	5.8 (2)
barley	28	26	0.93	-0.014 n.s.	0.78 (0.21)	0.75 (0.03)	7 (1.4)
wheat	44	38	0.86	-0.116***	0.73 (0.23)	0.64 (0.04)	5.8 (0.7)
triticale	23	20	0.87	0.037 n.s.	0.67 (0.26)	0.66 (0.04)	5.2 (1.5)

Table 2. Matrix of pair-wise F_{ST} measured between the nine hosts populations (n.s., non significant at 5%).

F_{ST}	Yorkshire fog	cocksfoot	bent	tall wild oat	bulbous oatgrass	oat	barley	triticale
cocksfoot	0.05 n.s.							
bent	0.16	0.11						
tall wild oat	0.1	0.17	0.22					
bulbous oatgrass	0.23	0.28	0.31	0.06				
oat	0.2	0.24	0.27	0.12	0.08 n.s.			
barley	0.14	0.16	0.24	0.1	0.11	0.15		
triticale	0.17	0.21	0.28	0.1	0.1	0.1	0.05	
wheat	0.2	0.25	0.32	0.12	0.11	0.15	0.06	0 n.s.

each value of K ranging from 2–20 were performed at 30 000 Markov Chain Monte Carlo (MCMC) repetitions and 300 000 burn-in period. In order to calculate a global rate of assignation, individuals were arbitrarily deemed as assigned to a single genetic cluster when the proportion of ancestry in that cluster was greater than 0.8.

A hierarchical analysis of molecular variance (AMOVA; [Escoffier et al. 1992](#)) including: (i) site within plant and (ii) plant, was performed using ARLEQUIN v. 2 ([Schneider et al. 2000](#)) in order to evaluate the genetic variance attributable to plant and site differentiation.

3. RESULTS

(a) Genetic and genotypic diversity of *S. avenae* populations

Globally, a high allelic diversity was found among the populations of *S. avenae*, with eight alleles at locus Sm17 and S5.L, 11 at S4Σ, 14 at Sm10 and 27 at S16b. A total of 263 different multilocus genotypes were identified from the 331 aphids analysed. Results of AMOVA revealed a highly significant host plant effect (21.3% of variance, $p < 0.01$), and a slight but significant site effect for uncultivated *Poaceae* and wheat (4.1% of variance, $p < 0.05$).

The genotypic diversity (G/n) was high in all populations, ranging from 54% (bent) to 93% (barley) (table 1). When a single copy of each multilocus genotype was considered, deviation from Hardy-Weinberg proportions was significant only for populations from cocksfoot and tall wild oat, which showed significant heterozygote deficit with a significant positive F_{IS} , and for populations from wheat,

which showed significant heterozygote excess with a significant negative F_{IS} (table 1). Results were very similar when all aphids were used in the analyses, because of the low number of copies of the same genotypes.

(b) Genetic differentiation of *S. avenae* according to host species

Genetic differentiation among host populations was substantial within uncultivated *Poaceae*, while populations from cereals were genetically closer. F_{ST} values indicated a high genetic differentiation between all pairs of host populations originating from uncultivated *Poaceae*, except for the pairs wild oat/ bulbous oatgrass and Yorkshire fog/cocksfoot, for which genetic differentiation was moderate (table 2). Conversely, all host populations from crops showed a low genetic differentiation except that from oat, which differed moderately from the other three. All the populations from uncultivated *Poaceae* were strongly differentiated from any populations from crops, except in the case of oats where populations from cultivated and uncultivated species were moderately differentiated. Population from bent was the most genetically different to populations from cereals. Individuals sharing the same genotype were generally found on the same host plant species. Only five genotypes were found both on crops and on uncultivated *Poaceae*, and all were found on wild oats (table 3). Among the 15 genotypes found on more than one plant species, 13 were found on wild or cultivated oat. Striking allele frequency differences were found at some loci between either populations from different plant species or

Table 3. Host populations of *S. avenae* sharing common genotypes.

genotype	host plants on which genotype was found	comments
1	Yorkshire fog and tall wild oat	genotypes found on wild hosts only
2	Yorkshire fog, cocksfoot and tall wild oat	
3	Yorkshire fog and tall wild oat	
4, 5, 6, 7	tall wild oat and bulbous oatgrass	genotypes found on both wild and cultivated hosts
8	bulbous oatgrass and oat	
9	bulbous oatgrass and barley	
10, 11	bulbous oatgrass and wheat	genotypes found on cultivated hosts only
12	tall wild oat, bulbous oatgrass and wheat	
13	oat, triticale and wheat	
14, 15	triticale and wheat	

populations from uncultivated versus cultivated *Poaceae* (see Electronic Appendix). For example, S16b₁₇₅ was at very high frequency in populations from bent (67% of individuals shared this allele), while Sm10₁₆₆ was especially frequent in populations from bent (82% of individuals) and cocksfoot (70% of individuals). Similarly, alleles S5.L₂₂₇ and S4Σ₁₅₉ were in high frequency in populations from uncultivated hosts (except wild oats), while allele S4Σ₁₅₇ appears characteristic of populations from cereals and wild oats. Moreover, alleles restricted to some host populations were found in the sample, for example, Sm17₁₇₇ was restricted to populations from cereals, while Sm10₁₇₀ was only found in populations from barley.

(c) Bayesian clustering

The highest assignment rates were obtained for $K=2$ (92%) and $K=3$ (82%; figure 1). For $K=2$, the clustering analysis tended to discriminate populations on crops from those on uncultivated plants. For $K=3$, the grouping was dependent on the plant tribe (*Poaceae*, *Aveneae* or *Triticeae*) from which aphids were collected. Within the tribes, the subgroups were not characteristic of the host plant and the rate of assignment of individuals to a subgroup was relatively low. Although the likelihood was maximized for $K=9$, the assignment rate dropped to 49%. For $K=9$, structure proposed splitting the three groups correlated to host plant tribes into subgroups. Clusters 1–3 in $K=9$ contributed mainly to the populations found on *Agrostis* sp., *D. glomerata* and *H. lanatus* and secondarily to the population found on *A. fatua* and barley. Clusters 4–7 contributed to the populations found on *A. fatua*, *A. sativa* and *A. bulbosum* and secondarily to the populations found on wheat, barley and triticale. Finally, clusters 8 and 9 contributed mainly to populations found on wheat, barley and triticale. The results obtained with $K=3$ and $K=9$ were then very consistent (figure 2).

4. DISCUSSION

(a) A high genic and genotypic diversity in *S. avenae* populations

In the present study, substantial allelic variation was detected in all populations of *S. avenae* at five microsatellite loci, with a mean number of alleles/locus between 4 and 7, which is consistent with prior results on this aphid species with a similar number of microsatellites (Sunnucks *et al.* 1997; Simon *et al.* 1999; Haack *et al.* 2000). In our sample, we did not find Sm17 and Sm10 alleles restricted to the *Sitobion fragariae* and to the wheat-specific *Sitobion* reported by Sunnucks *et al.* (1997) in the UK. Hence, we considered all our aphids as 'typical' *S. avenae* in the sense that these authors used the term.

Earlier works have generally found moderate levels of genotypic variation at microsatellites in populations of *S. avenae*, mainly because of clonal amplification of a few genotypes. In the present study, the genotypic diversity ($G/n=0.79$) was higher than those found in previous studies on populations of *S. avenae* from Western Europe where G/n ranged from 0.27 to 0.5 (Sunnucks *et al.* 1997; Simon *et al.* 1999; Haack *et al.* 2000; Llewellyn *et al.* 2003). The only case where *S. avenae* exhibited higher genotypic diversity ($G/n=0.97$) was in Romanian populations with predominantly sexual reproduction (Papura *et al.* 2003). Therefore, it could be that the genetic diversity observed here resulted from recent sexual reproduction events. Supporting this view, most populations of *S. avenae* showed no departure from HW equilibrium or linkage disequilibrium. Populations from cocksfoot and wild oat were, however, in heterozygote deficit, but this is probably owing to population subdivision (see below). Populations from wheat were the only ones with strong heterozygote excess: interestingly, asexual populations of aphids are often characterized by higher heterozygosity than their sexual counterparts (Simon *et al.* 1999; Delmotte *et al.* 2002; Vorburger *et al.* 2003). Thus, asexual lineages of *S. avenae* could be more frequent in wheat than in other cultivated or uncultivated plants. Testing this hypothesis requires the characterization of reproductive modes, through appropriate biological experiments, of *S. avenae* genotypes from different uncultivated and cultivated host plants. If sexual reproduction is the main source of genetic diversity, then the difference between present and earlier studies could be explained by either between-year variation in the relative proportion of sexual and asexual lineages in the populations (Dedryver *et al.* 2001), or by higher proportions of sexual lineages on uncultivated host plants.

(b) A two-level host-based differentiation of *S. avenae* populations

F_{ST} pairwise comparisons and the clustering analysis indicated two levels of genetic differentiation between populations of *S. avenae* collected on various *Poaceae*. The first level essentially separated populations from cultivated and uncultivated *Poaceae*, with the notable exception of aphids on wild oats, which clustered with genotypes from crops. It is worth noting that Lushai *et al.* (2002) obtained a similar pattern among British populations of *S. avenae* on crops (wheat and barley) and uncultivated *Poaceae* (cocksfoot and Yorkshire fog).

Figure 1. Estimated *S. avenae* population structure. Each aphid is represented by a thin vertical line, which is partitioned into K segments, representing the individual's estimated membership fractions in K clusters. Black lines separate individuals of different populations labelled with the host plant. 1, Yorkshire fog; 2, cocksfoot; 3, bent; 4, tall wild oat; 5, bulbous oatgrass; 6, oat; 7, barley; 8, triticale; 9, wheat. Tribe of host plants (Poeae, Aveneae, Triticeae) is specified under the figure.

Figure 2. Mean proportion of ancestry calculated for each host population, from results obtained by clustering analysis on individuals for $K=9$ clusters (named C1–C9).

The present study extends this finding by considering: (i) a broader host spectrum, (ii) wingless aphids that are necessarily born on the plant on which they were collected instead of winged aphids, which could be transient and (iii) a greater number of well-discriminated genotypes on both cultivated and uncultivated plants. The second level differentiated three groups according to the plant tribe on which aphids have been collected:

Triticeae (barley, wheat and triticale), Aveneae (cultivated and wild oats) and Poeae (bent, cocksfoot and Yorkshire fog) tribes. It is noteworthy that the extent of genetic differentiation between populations from Triticeae and from Poeae ($0.2 > F_{ST} > 0.3$) falls within the range usually encountered for divergence between host races in phytophagous insects (Dres & Mallet 2002). Populations collected on Poeae appeared the more

homogeneous, with the specific genetic pool contributing to more than 90% of the genotypes' ancestry. However, some genotypes typical of the Poeae cluster were also found on Aveneae, and to a lesser extent on Triticeae (barley), suggesting a preferential one-way gene flow. In contrast, exchanges between Aveneae and Triticeae tribes were more frequent with the genetic pool specific of Aveneae contributing substantially to populations found on Triticeae and *vice versa*. The Aveneae tribe then seemed to occupy an intermediate position. This was confirmed by the fact that all the multicopy genotypes shared by two different tribes were found on a plant belonging to the Aveneae tribe.

The intermediate position of Aveneae tribe could result from the fact that it was the only tribe in our study with both a coexisting cultivated and uncultivated close relative (*A. sativa* and *A. fatua*), and an annual and a perennial uncultivated member (*A. fatua* and *A. bulbosum*). This would confer to the Aveneae tribe the properties of both the cultivated and of uncultivated plants.

Oats could be a favourable host for *S. avenae* genotypes from either cultivated or uncultivated *Poaceae*, in the same way that host races of the pea aphid *A. pisum* can all develop on the broad bean *Faba vulgaris* (Carré & Bournoville 2003).

(c) Role of host plants and agricultural landscape on population structure of *S. avenae*

The cultivated system exists in a succession of crops in time and space that mature and become unsuitable for aphids usually after only a few months. This habitat instability leads to frequent and obligate dispersal events of phytophagous insects that may result in a large mixing of individuals, but also in strong population bottlenecks (Cobb & Whitham 1998). Cereal aphids have to alternate between different crops such as wheat or barley (from November to June) and maize (from June to October). Such conditions may favour genotypes that are able to develop and multiply successfully on different cereals as confirmed by Haack *et al.* (2000), who revealed the presence of common genotypes of *S. avenae* in high proportions on these crops.

Conversely, the 'uncultivated' system is probably more stable where uncultivated *Poaceae* have a longer and less synchronized vegetative annual life cycle than cereals and many of them are perennial. In these conditions, obligate host shifts within the uncultivated system could be less frequent than in the cultivated system, reducing genetic mixing and favouring host specialization among aphid populations. This is what we observed in the present study, where contrary to populations found on cereals, which were genetically homogenous, populations found on uncultivated *Poaceae* were highly differentiated.

Host-based population differentiation could result from better performances/preferences of *S. avenae* genotypes on/for one or a few host plants leading to host specialization (e.g., De Barro *et al.* 1995b; Vanlerberghe-Masutti & Chavigny 1998; Via 1999). Plant phenology, surface compounds and sap composition could account for the selection of host-specialized genotypes (Scriber 2002). These biological characteristics could be different enough between *Poaceae* tribes to promote host adaptation in

S. avenae populations. Other factors may also play a role, directly or indirectly in host specialization, such as primary and facultative symbionts (Adams & Douglas 1997; Simon *et al.* 2003; Tsuchida *et al.* 2004) and the reproductive mode of aphid populations (Blackman 1990). Finally, the possibility that the observed population differentiation among local populations of *S. avenae* results from stochastic events (e.g. founder effects, genetic drift) cannot be discarded. However, comparisons of between site population differentiation for the same plant with between plant differentiation at the same site (wild oat, cocksfoot and wheat), showed that host-based population divergence was always much higher than site effect, reinforcing the host specialization hypothesis.

As stated above, plant phenology is likely to influence aphid–host interactions across the seasons. Similarly, aphid population dynamics are very much affected by seasonal changes, in particular the production of winged forms, which is driven by density dependence, temperature and host quality (Robert 1987). This could subsequently modify the genetic stratification of aphid populations according to their hosts along the seasons, as evidenced by De Barro *et al.* 1995a and Sunnucks *et al.* (1997), who showed that the genetic differentiation between populations of *S. avenae* on wheat and cocksfoot dissipated as the growing season progressed.

(d) Implications for population functioning and pest management strategies

Sitobion avenae is one of the most damaging pests on cereals in Europe; directly by feeding on plants and indirectly by transmitting viruses (Rochow 1969). Its population functioning at both the field scale (Dedryver 1978; Carter *et al.* 1982; Entwistle & Dixon 1986; De Barro *et al.* 1994; Plantegenest *et al.* 1996) and regional scale (Harrington *et al.* 2004) has been intensively studied leading to forecasting tools (Pierre & Dedryver 1984; Plantegenest *et al.* 2001). However, there remains a gap in knowledge at the intermediate landscape scale. This is an important scale as field colonization by aphids is partly due to winged individuals originating from local reservoirs. Our data indicated limited exchanges of individuals between cultivated and uncultivated parts of the agricultural system, which thus constitute two largely independent groups of aphids. *Poaceae* of the uncultivated margins seemed to play little role as reservoirs for *S. avenae* populations colonizing cereal crops, and can be ignored when designing integrated pest management strategies against this pest. Such a dichotomy in the agroecosystem has also been found for the European corn borer, in which populations from maize are genetically divergent from those on sagebrush that was previously thought to be a refuge for this moth (Martel *et al.* 2003; Thomas *et al.* 2003).

However, exchanges of individuals, or genes if populations are not yet reproductively isolated, are not scarce events. Populations of *S. avenae* on uncultivated *Poaceae* could then provide a potential reservoir of genetic diversity that could enhance the adaptive potential of this species, particularly in response to the modification of agricultural practices.

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