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Intra-specific variation and inheritance of BYDV-PAV transmission in the aphid *Sitobion avenae*

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Abstract

Vector efficiency of 44 clonal lines (clones) of *Sitobion avenae* belonging to 31 different genotypes (distinct patterns for five microsatellite loci) originating from Western France was evaluated by transmitting the isolate PAV4 of BYDV-PAV to barley seedlings. Variation in transmission rates from 3.7% to 92.5% was observed, with significant effects of the aphid clone, of the plant species on which clones were collected, and of the reproductive mode of the clones. When genotypes are considered instead of clones, a continuum in transmission rates was observed. A subset of *S. avenae* clones was tested for transmission of one (10 clones) and 13 (4 clones) other BYDV-PAV isolates, and a clear clone effect modulated by an isolate effect was observed. Crosses were made between clones with different vectoring phenotypes and their F1 progeny were tested for PAV4 transmission. The narrow sense heritability of the PAV transmission character was rather high in the F1 families ($h^2 = 0.5$) and the segregation analyses suggested an oligo/polygenic determinism of this character. The possibility of generating new transmission variants by sexual reproduction and its consequences on transmission mechanism studies and on BYD epidemics are discussed.

Introduction

Aphids are important vectors of plant viruses in temperate countries (Matthews, 1991). Unlike other insects, they typically reproduce by cyclical parthenogenesis, a reproductive mode involving alternation between a single sexual generation produced in autumn (decreasing photoperiod and temperature) and several (ca. 20) clonal generations produced during spring and summer conditions. Moreover, in some species (including many agricultural pests), the sexual phase can be totally or partly lost by some genotypes or by the whole species (Simon et al., 2002). Consequently, natural populations of a given aphid species encompass a 'mixture' of different sympatric clonal lines (generally termed 'clones' in the literature), which are most often genetically and phenotypically distinct, and which reproduce by apomictic parthenogenesis for several months (if the sexual phase occurs) up to several years (if not) before disappearing. Thus, aphids are particularly convenient organisms for studying (i) the stability in time of phenotypic characters, since sexual reproduction and consequently annual recombination can be prevented, by rearing aphids under a permanent parthenogenetic reproduction at appropriate light and temperature regime, and (ii) the genetic bases of such characters, by crossing different phenotypes and studying their inheritance in the next generations. In several aphid species, clones were shown to differ for molecular patterns (microsatellite and RAPD markers) (Carvalho et al., 1991; Black et al., 1992; Simon et al., 1996, 1999) and/or

for biological traits such as (i) the ability to produce sexual morphs (Blackman, 1971; Simon et al., 1991; Dedryver et al., 1998) or winged morphs (Dedryver et al., 1990), (ii) host-plant preference (Caillaud et al., 1995; Bournoville et al., 2000) and (iii) transmission of plant viruses (Rochow and Eastop 1966; Bourdin et al., 1998).

Assessing variation for virus transmission by an aphid species is of special interest in epidemiological studies. Thus, it was shown (Fenton et al., 1998; Fuller et al., 1999; Haack et al., 2000) that a limited number of genetically distinct aphid clones (genotypes) are locally or regionally prevalent in populations of different aphid species, and it can be hypothesized that seasonal prevalence of good- or poor virus-transmitting genotypes in a given area affects the course of epidemics. Consequently, future epidemiological scenarios should consider the transmission abilities of prevalent vector genotypes, the probability of emergence of new transmission phenotypes by local sexual reproduction, migration or mutation, and their success in the face of selection. On the other hand, comparing a range of aphid genotypes for efficiency of virus transmission and crossing selected variants could help to identify the genes involved in virus transmission and provide a useful biological system for identifying the molecular sites involved in virus-aphid interactions (Papura et al., 2002).

Inter-clonal variation in virus transmission by aphids has been reported for both stylet-borne (Sohi and Swenson 1964; Thottappilly et al., 1972; Jurik et al., 1980; Singh et al., 1983; Lupoli et al., 1992) and circulative viruses (Björling and Ossiannilsson, 1958; Robert and Maury 1970; Guo et al., 1996, 1997a, b). In most cases, variations in the virus-transmitting abilities of different clones were detected. When many clones were compared, a continuum in virus transmission generally was found from inefficient to very efficient vectors.

In the pioneer work of Björling and Ossiannilsson (1958), more than 100 clones of six different aphid species (mainly *Myzus persicae* sulzer) were compared for transmission of several viruses. Significant differences were found between clones of the same species in transmission of *Potato leaf roll virus* (PLRV, a luteovirus) and 'beet yellowing viruses,' which at that time could be a luteovirus, *Beet mild yellowing virus* (BMYV) or a closterovirus, *Beet yellows virus* (BYV), or a mixture of both. They further attempted to cross *M. persicae* clones differing in transmission abilities and tested the two F1 hybrids obtained. They found that inter-clonal differences for transmission were maintained over several years and that Fl hybrids differed from their parents for transmission abilities, both these results suggest a genetic control of this character.

Barley yellow dwarf (BYD) is one of the most severe cereal diseases in the world (Lister and Ranieri 1995). It is caused by different viruses belonging to the genera *Luteovirus* (BYDV-PAV and BYDV-MAV), *Polerovirus* (CYDV-RPV) or presently unassigned (taxa BYDV-RMV, BYDV-SGV, BYDV-GPV) of the family *Luteoviridae* (D'Arcy and Mayo, 1997). The viruses are all transmitted persistently by different aphid species living on *Poaceae* with a variable degree of vector specificity (Rochow, 1969). Most studies on aphid-BYDV relationships have been focused on variation in species specific transmission among aphid species but inter-clonal variation for BYDV transmission has also been assessed. In the USA, Rochow (1960) described clones of the greenbug *Schizaphis graminum* (Rondani) differing in their ability to transmit a BYDV-SGV isolate, and four biotypes (clones differing by biological characters) of the corn leaf aphid *Rhopalosiphum maidis* (Fitch) were reported to differ in their ability to transmit the AG-1 strain of BYDV (Saksena et al., 1964). Rochow and Eastop (1966) reported variation in the abilities of two morphologically different clones of *Rhopalosiphum padi* (L) to transmit BYDV-RMV, and noticed that differences between clones were less pronounced when experiments were conducted at 30 °C than at lower temperatures. Sadeghi et al. (1997 a, b) showed that two BYDV-MAV isolates were efficiently transmitted by clones of the normally inefficient vector *R. padi*. More recently, similar results have been reported for the transmission of BYDV-RMV isolates by *R. padi* in North America (Lucio-Zavaleta et al., 2001).

In Europe, *Sitobion avenae* (F) is considered one of the most important vectors of BYDV-PAV (Leclercq-Le Quillec et al., 1995), especially in the spring when it spreads the virus from winter hosts (wheat and barley) to spring barley and corn (Haack et al., 2002). Variation in PAV transmission by a limited number of clones of *S. avenae* collected from two French regions has been reported (Guo et a1., 1997a, b).

In the present work, we have assessed the variation in transmission to barley of one PAV isolate by a large sample of locally collected *S. avenae* clones with different phenotypic characteristics and microsatellite patterns. We have also tested the variation in transmission of a number of different PAV isolates by a restricted number of aphid clones. Finally, we have initiated a study of the inheritance of the transmission character by comparing vectoring abilities of some F1 progenies to those of their parents.

Materials and methods

Collection, rearing and crossing of aphids

In February 1990, 100 parthenogenetic females of *S. avenae* were collected in a 50 km^2 area in the Rennes basin (Western France) from winter wheat, winter barley and oat (Dedryver et al., 1998). The progeny of each surviving female (free of parasitoids or entomopathogenic fungi) started a single clonal line which we will refer to as a clone. Sixty clones were obtained and reared in a growth chamber at 20 ± 1 °C; L:16/D:8 to ensure continuous parthenogenetic reproduction. Each clone was reared on a single unvernalized wheat seedling (*Triticum aestivum* 'Arminda') grown on a nutritive medium in a plastic tube (100 × 21 mm) with the leaves enclosed in a Cellophane® bag. The clones were evaluated for different phenotypic traits such as body colour and reproductive mode (Dedryver et al., 2001). Four coloured phenotypes were described: pale green, dark green, brown and orange (Dedryver et al., 1994). The four reproductive modes observed for this sample were (1) holocyclic clones which retained a full commitment to sexual reproduction once a year, with parthenogenetic reproduction followed by the generation of only males and sexual females that lay fertilized diapausing eggs in the autumn; (2) intermediate clones which continue to produce parthenogenetic individuals in autumn but also a full array of sexual forms; (3) androcyclic clones which produce only parthenogenetic forms as well as males in autumn; and (4) anholocyclics which have abandoned sexual reproduction, reproducing all year long by sustained parthenogenesis (Dedryver et al., 1998); Forty four of these clones (22 from wheat, 11 from barley and 11 from oats) were retained for virus transmission experiments (Table 1).

Induction of sexual morphs of some clones (Sa5, Sa1, Sa11, Sa26 and Sa48), crosses and collection of young F1 aphid fundatrices after winter egg diapause were carried out between autumn 1995 and spring 1996 (Dedryver et al., 1998). Initially, we had planned to study the F1 offspring of four crosses including only one highly efficient vector (HEV) (Sa1) and one poorly efficient vector (PEV) (Sa5), i.e. two selfings (Sa5♀ × Sa5♂, Sa1♀ × Sa1♂) and 2 crosses (Sa5♀ × Sa1♂, Sa1♀ × Sa5♂). However, the selfing of Sa1 was sterile and eggs of the cross Sa1 × Sa5 hatched very poorly. Consequently, five F1 offsprings were kept for transmission experiments, from the selfing of the PEV Sa5 (44 clones) and from four crosses : Sa5♀ × Sa1♂ (PEV × HEV: 20 clones), Sa11♀ × Sa48♂ (HEV × HEV: 16 clones), Sa1♀ × Sa26♂ (HEV × PEV: 10 clones), and Sa5♀ × Sa26♂ (PEV × PEV: 5 clones). These clones were reared separately on virus-free wheat plants and allowed to reproduce parthenogenetically at 20 ± 1 °C, L:16/D:8 in a growth chamber until used in experiments.

Genotype characterization of aphid clones

Sitobion avenae clones were characterised at five microsatellite loci, Sm10 (11 alleles), Sm11 (5 alleles), Sm12 (16 alleles), Sm17 (5 alleles) and S4Σ (4 alleles), as described (Simon et al., 1999). Clones presenting the same allelic combinations at these loci were considered as belonging to the same genotype, in so far as they have a very low probability ($P < 0.0001$) to derive from independent recombination events (Papura et al., 2003).

Virus isolates

The isolate PAV4 was used in most experiments. It was collected in le Rheu (France) from barley in 1989 and causes severe symptoms on barley 'Express' (Papura et al., 2002). Thirteen other PAV isolates from the INRA – Le Rheu collection were used in some experiments (Table 2). These isolates were maintained on barley seedlings 'Express' infested with clone Rp1 of *R. padi* in a growth chamber at 20 ± 1 °C; L16/D8 (Sadeghi et al., 1997 a, b).

Table 1. S. avenae clones used in PAV transmission to barley experiments

Clone	Date	Plant[a]	Life-cycle[b]	Color[C]	Genotype[d]	% Transmission (S.E.)	
						%PAV4	%PAV13
Sa1	16/02/90	W	I	Pg	1	56.67 (17.4)	80 (8.6)
Sa2	16/02/90	W	A	O	2	31.67 (10.9)	
Sa3	16/02/90	W	I	O	3	6.67 (4.4)	66.67 (10.9)
Sa4	16/02/90	W	H	Dg	4	58.33 (17.6)	
Sa5	16/02/90	W	H	Dg	5	3.67 (3.6)	66.67 (2.9)
Sa6	16/02/90	W	I	O	6	48.33 (13)	
Sa7	16/02/90	W	I	Pg	7	77.50 (2)	
Sa8	16/02/90	W	I	Pg	1	61.67 (7.2)	
Sa11	16/02/90	W	I	O	6	51.67 (7.2)	
Sa12	16/02/90	W	H	B	12	33.33 (11.6)	
Sa13	16/02/90	W	I	Pg	13	53.33 (14.2)	
Sa15	16/02/90	W	H	B	15	45.0 (24.6)	
Sa16	16/02/90	W	H	B	16	55 (25.5)	
Sa17	16/02/90	W	H	B	15	30 (7.6)	
Sa18	16/02/90	W	I	B	13	36 (10.2)	
Sa20	16/02/90	W	I	Pg	20	23.33 (18)	88.3 (7.3)
Sa22	16/02/90	W	An	O	22	76.67 (14.5)	95 (5)
Sa23	16/02/90	Oa	H	B	23	18.33 (15.9)	
Sa24	16/02/90	Oa	I	Pg	24	55 (20.2)	
Sa25	16/02/90	Oa	An	O	25	40 (11.5)	
Sa26	16/02/90	Oa	An	B	26	15 (15)	
Sa28	23/02/90	Oa	H	Dg	28	8.33 (8.3)	73.3 (9.3)
Sa29	23/02/90	Oa	A	Pg	29	75 (13.2)	
Sa30	23/02/90	Oa	An	Pg	30	11.67 (9.3)	60 (12.6)
Sa31	23/02/90	Oa	H	B	16	55 (18.9)	
Sa32	23/02/90	Oa	H	Pg	16	58.33 (15.2)	
Sa35	23/02/90	Oa	An	O	25	55 (15.2)	
Sa36	23/02/90	Oa	An	O	36	38.33 (7.2)	
Sa39	23/02/90	W	I	O	6	56.67 (1.7)	
Sa40	23/02/90	W	I	O	40	60 (18.9)	
Sa41	23/02/90	W	I	Pg	1	70 (12.5)	
Sa42	23/02/90	W	H	Pg	1	55 (17.5)	
Sa43	23/02/90	B	I	O	43	43.33 (4.4)	
Sa44	23/02/90	B	H	Pg	44	62 (11.7)	
Sa45	23/02/90	B	A	Pg	1	79.67 (6.7)	
Sa46	23/02/90	B	H	O	46	53.33 (11.6)	
Sa47	23/02/90	B	A	B	1	65 (5.7)	
Sa48	23/02/90	B	H	B	48	68.33 (12)	
Sa49	23/02/90	B	H	Pg	49	48.33 (14.2)	
Sa50	23/02/90	B	I	O	50	66.67 (8.8)	91.67 (1.7)
Sa51	28/02/90	B	A	B	51	92.67 (6.1)	81.67 (15.9)
Sa52	28/02/90	B	An	Pg	52	77.33 (9)	85.67 (12.9)
Sa53	28/02/90	W	I	Pg	44	50.33 (2.6)	
Sa54	28/02/90	Oa	H	B	54	52.67 (18.3)	

[a]B: barley; Oa: oat; W: wheat.
[b]A: anholocyclic; An: androcyclic; I: intermediate; 4: holocyclic.
[c]B: brown; Dg: dark green; O: orange; Pg: pale green.
[d]According to 5 microsatellite loci pattern.

Viral inoculum

Barley seedlings 'Express' at the 2 leaf-stage, grown in plastic pots were each inoculated by three fourth instar larvae of *R. padi* collected on the source plants. Each seedling was caged with a cellophane bag for a 5 day inoculation access period (IAP) and then sprayed with an insecticide

Table 2. BYDV-PAV isolates used in transmission

Isolate name	Origin			Reference
	Plant	Location[a]	Year[b]	
RG	Rye-grass	Yvelines	1988	Guo et al. (1997)
21	Maize	Drôme	1997	Fabre et al. (2003)
4	Barley	Ille et Vilaine	1989	Papura et al. (2002)
13	Barley	Loire atlantique	1985	Papura et al. (2002)
B	Barley	Yonne	1996	Papura et al. (2002)
L14	Maize	Landes	1990	Beuve et al. (1999)
2T	Barley	Yvelines	1991	Chaloub et al. (1994)
Clout	NR	Canada	1978	Rochow and Carmichael (1979)
C9	Maize	Haut-Rhin	1997	Fabre et al. (2003)
Vd34	Maize	Vendée	1997	This report
Fr	NR	Ille et Vilaine	NR	This report
B 12	Maize	Haute-Garonne	1997	Fabre et al. (2003)
Rp	NR	Ille et Vilaine	NR	This report
Vd29	Maize	Vendée	1997	Fabre et al. (2003)

NR: Not Recorded.
[a] French department except for BYDV-PAV Clout, which was collected in Canada.
[b] After being collected in the indicated year from original hosts, the isolate was maintained in a growth chamber using the barley susceptible cv. Express as host.

solution (Décis EC, Bayer Cropscience, deltamethrin 1 $ml.l^{-1}$) After a 15 day period allowing virus multiplication in the plants, each plant was tested for virus content with a triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA) Plants with similar optical densities (OD) were used as virus inoculum for transmission experiments.

Virus acquisition and transmission to test plants

Experiments were done in a growth chamber at 20 ± 1 °C, L:16/D:8. For each *S. avenae* clone, groups of 100 virus-free third (apterous) instar larvae were removed from the colony and placed on the virus inoculum plants for a two-day acquisition access period (AAP). Aphids were then removed from the virus inoculum plants and three aphids were transferred onto each test plant. By this time, most of them had moulted into adults. After a 5 day infection access period (IAP), aphids were killed with an insecticide as described above. In a first experiment, 44 clones were tested in five different trials during 6 months with PAV4 only and barley 'Express' as test plants: each trial included nine different clones and the clone Sa1 as control, with 20 test plants per clone; each trial was repeated at three different periods. Ten genotypically different aphid clones (genotypes) were selected from the results of experiment 1 and tested for PAV4 and PAV13 transmission to barley 'Express'. Each treatment was repeated three times over a 4 month period, under the same conditions as described above. Four selected clones were tested in the same conditions for transmission of 14 isolates to barley. The F1 clones were tested for PAV4 transmission to barley (three replicates) in the same conditions as for the first experiment.

Virus detection in test plants

Detection of virus antigen in leaves of infected plants was by TAS-ELISA (Torrance et al., 1986; Leclercq-Le Quillec et al., 1995). Anti PAV-like purified IgG were obtained from H. Lapierre: (INRA, Laboratoire de Virologie, Versailles, France) and anti-PAV monoclonal antibody Mac91 was purchased from Adgen diagnostic systems (Ayr, Scotland, UK). The optical density (OD) was measured at 405 nm with a Dynatech MR5000 spectrophotometer after 1–2 h incubation at room temperature. Samples were considered positive when OD values were greater than three times the mean of the results from uninfected control leaves. Virus transmission efficiency was

calculated for each repetition as the percentage of infected plants among the 20 plants infested with viruliferous aphids.

Data analyses

Analyses of variance (ANOVAs) were performed using the GLM procedure in the SAS system (SAS Institute Inc. 1999). Better stabilization of variances was obtained for data transformation by Arcsin$\sqrt{x}$. Normality of the data was checked (W of Shapiro-Wilk). Homogeneity of variances was assessed by Levene's test and significant effects were followed by comparison of means using Duncan's-tests. Groups were compared using contrasts.

Results

Overall variation in PAV4 transmission to barley among 44 S. avenae *clones*

For all 44 *S. avenae* clones, the mean transmission percentage of PAV4 was 49%. The 44 clones displayed considerable variation in their ability to transmit PAV4 to barley (Table 1), ranging from 3.7% to 92.5%. Differences between clones are significant ($F = 5.64$; $P < 0.0001$) but the mean's comparison's test (Duncan's multiple range test) showed largely overlapping statistic groups. The mean transmission percentage was 62% for clones from barley, 48.5% for clones from wheat and 39% for clones from oat, and the effect of the plant on which the clones were collected is significant ($F = 6.88$; $P = 0.0016$). The mean transmission percentage was 74.5% for anholocyclic clones, 51% for intermediates, 40% for androcyclics, and 44% for holocyclics. The effect of the reproductive mode is significant ($F = 4.44$, $P = 0.0057$) and there was a weak interaction between 'plant' and 'reproductive mode' factors ($F = 2.2$ $P = 0.0605$). The effect of body colour (pale green, orange, dark green, brown) on PAV4 transmission was not significant ($F = 1.28$; $P = 0.2865$). The clone effect, nested in each of the three above factors, remained significant, but is weaker than the effects of plant and reproductive mode. Contrasts were analysed on the effects of the different modalities of each factor. For the plant effect, differences between barley and oat were highly significant ($F = 12.32$, $P < 0.0001$), differences between wheat and barley were slightly significant ($F = 10.27$, $P = 0.002$), as were differences between wheat and oat ($F = 8.32$, $P = 0.0052$). For the reproductive mode effect, differences between anholocyclics, and the three other categories were highly significant ($P < 0.0001$).

Among the 44 clones, 31 different patterns were characterised at the five microsatellite loci (Tables 1 and 3) and were considered as belonging to different genotypes. There were no significant differences in PAV4 transmission to barley between clones belonging to the same genotype and the clone effect nested in genotype was not significant ($F = 0.57$; $P = 0.8597$). Consequently, the transmission percentages of each genotype in multicopies was obtained as the mean of transmission percentages of the clones belonging to the same

Table 3. Genotypes at five microsatellites loci found more than once in the sample of *S. avenae* tested for PAV4 transmission

	Genotype					N_{90}	$N_{93–97}$
	Sm10	Sm11	Sm12	Sm17	S4Σ		
15	164164	144144	139153	178178	168168	2	3
16	164168	144144	139171	180183	168168	3	1
6	164185	144144	139159	178178	172176	3	0
13	164206	144144	159169	178178	162162	2	0
1	152164	144149	151159	178178	162162	6	14
25	164197	149149	139173	178179	168172	2	0
44	164168	144144	139139	178178	168176	2	0

N_{90} represents the number of copies of each genotype sampled in 1990. $N_{90–97}$ refers to the total number of copies of the corresponding genotype found in a temporal survey (1993–1997) of *S. avenae* populations (Simon et al., l999; Haack et al., 2000).

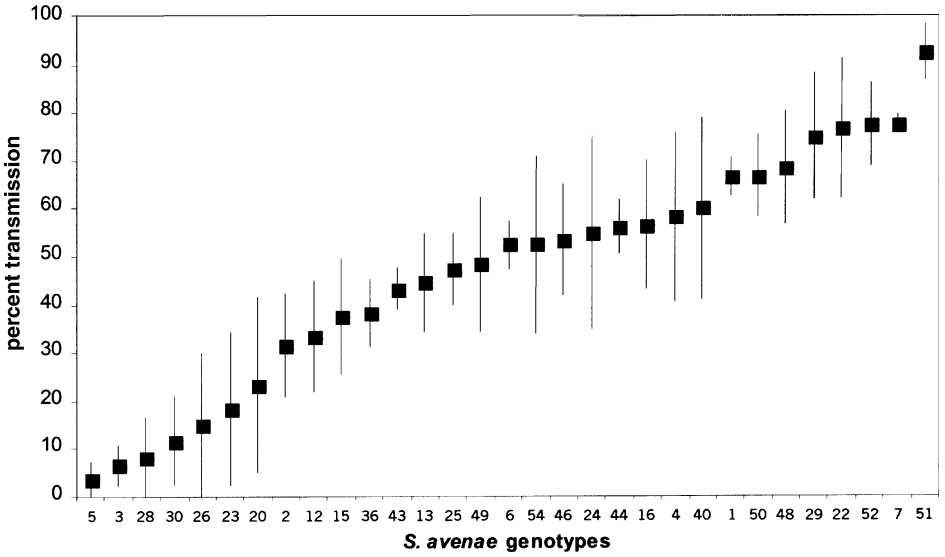

Figure 1. Percentages of PAV4 transmission to barley plants for the 31 *S. avenae* microsatellite patterns (genotypes). Bars are standard errors.

genotype. The 31 genotypes displayed a continuum in PAV4 transmission percentage (Figure 1), and the effect of the genotype was significant ($F = 8.93$, $P < 0.0001$), but only the extreme genotypes were significantly different for their transmission percentage. The analysis of relatedness between the 31 genotypes, based on pairwise genetic distances (D_{AS}) did not show any grouping of the genotypes according to their transmission phenotype (data not shown). Genotype 1 (Sa1, Sa8, Sa41, Sa42, Sa45, Sa47) was collected six times from wheat and barley; two genotypes were collected three times: 16 (Sa16, Sa31, Sa32) from wheat and oat and 6 (Sa6, Sa11, Sa39) from wheat; four other genotypes were collected two times: 15 (Sa15, Sa17) from wheat, 13 (Sa13, Sa18) from wheat, 25 (Sa25, Sa35) from wheat and oat, and 44 (Sa44, Sa53) from barley and wheat. Some of the genotypes that were collected more than once in our sample of 1990 (1, 15 and 16) were also collected in different French regions in the years 1993, 1994, 1995 or 1997 (Table 3). This was also the case for genotypes 4 (four copies in 1993, 1994, 1995), 26 (one copy in 1995), 46 (three copies in 1993 and 1994) and 49 (5 copies in 1994 and 1995). The distribution of the 31 genotypes by transmission classes is roughly bell-shaped and not significantly different from a normal distribution ($\chi^2 = 7.2$; ddl = 5) for $\alpha = 0.05$. However, this distribution is asymmetrical with a maximum (30%) of genotypes in the transmission class 50–62.5%, and with more genotypes (4 vs. 1) in the lower transmission class (<12.5%) than in the higher transmission class (<87.5%).

Comparison of transmission rates of multiple PAV isolates

Figure 2 shows the results of PAV 4 and PAV 13 transmission tests on barley of 10 selected *S. avenae* clones with different microsatellite patterns (genotypes). The mean percentage of transmission

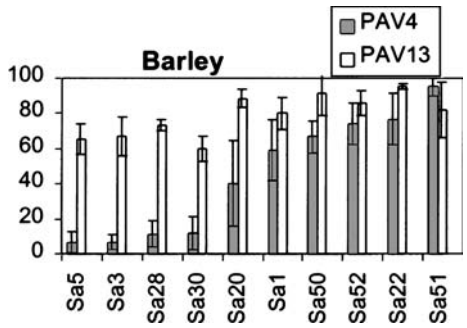

Figure 2. Percentages of PAV4 and PAV13 transmission (ordinate) to barley for 10 *S. avenae* selected clones. Bars are standard errors.

was significantly higher for PAV 13 (78.70%) than for PAV 4 (47.17%). Furthermore, a significant effect of the clone (df = 9, $F = 8.71$, $P < 0.00001$) was noted as well as a significant effect of the isolate (df = 1, $F = 63.05$, $P < 0.00001$) with a weak interaction between them ($F = 2.59$, $P = 0.015$). However, the ranking of the clones does not greatly change with respect to the isolate involved.

The transmission of 14 isolates of PAV collected in France and Canada was tested for Sa1, Sa5, and for the 2 F1 clones derived from the selfing of Sa5, which exhibited extreme values for PAV4 transmission (see below). There is an isolate effect ($F = 7.57$, $P < 0.0001$) and a clone effect ($F = 57.55$, $P < 0.0001$) with no interaction between them ($F = 1.18$, $P = 0.245$). Thus, PAV-Rp115 (22.17%) and PAVfr (23.92%) were significantly less well transmitted than PAVC9 (63.33%) and PAV13 (64.54%), and the ranking of the clones for transmission percentage was independent of the virus isolate (Figure 3).

Comparison of PAV4 transmission rates among F1 clones of S. avenae

Figure 4 shows the boxplots associated with the transmission rates of the F1 offsprings (except for Sa5 × Sa26, because too few clones were obtained in this cross), compared with the transmission rates of the parents. For the three crosses, the mean of the F1 was intermediate between the means of the parents. These results suggest that this character is polygenic, with a large degree of additivity among the genes involved. The mean value for the selfing (Sa5 × Sa5) is higher (23.5%) than the mean of the parent (6.2% in the mean, for seven tests), which suggests that Sa5 is heterozygous for the character PEV, and that some of the genes involved are dominant, partially dominant, or additive. In the crosses the range of transmission rates of the offspring was higher than that of the two parents, and also generally higher than the range of experimental variability of each parent. In the offspring of the Sa5 × Sa5 selfing, the extreme values were 0% and 88%, but most clones had a transmission rate below 40%.

Table 4 summarises the results of the ANOVAs on the transmission percentages of each F1 offspring and of the parents. There was a significant effect of the clone and a non-significant effect of the replicate, except for Sa5 × Sa1. Residues were distributed normally, and variance homogeneity tests for clone and replicate effects were not significant, with one exception. For crosses involving Sa5 (Sa5 × Sa1, Sa5 × Sa26, Sa5 × Sa5), the

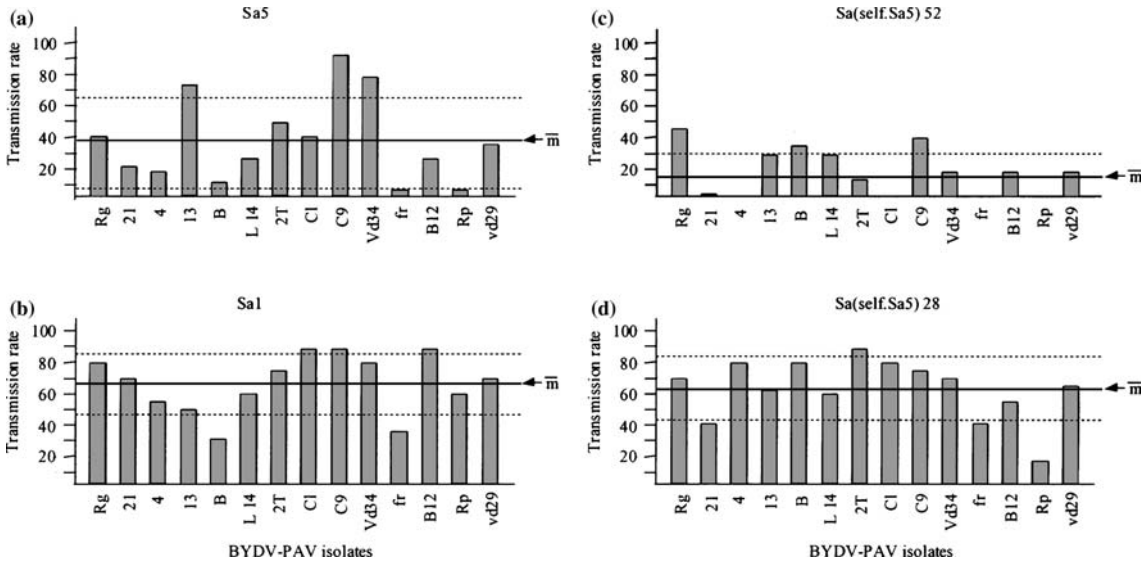

Figure 3. Percentage of transmission of 14 PAV isolates to barley, for 4 *S. avenae* clones. Horizontal lines (—) represent the mean percentage of transmission calculated for the 14 isolates (A) clone Sa5; (B) clone Sal; C and D clones Sa (self.Sa5) 52 and Sa (self.Sa5) 28 from the selfing of clone Sa5.

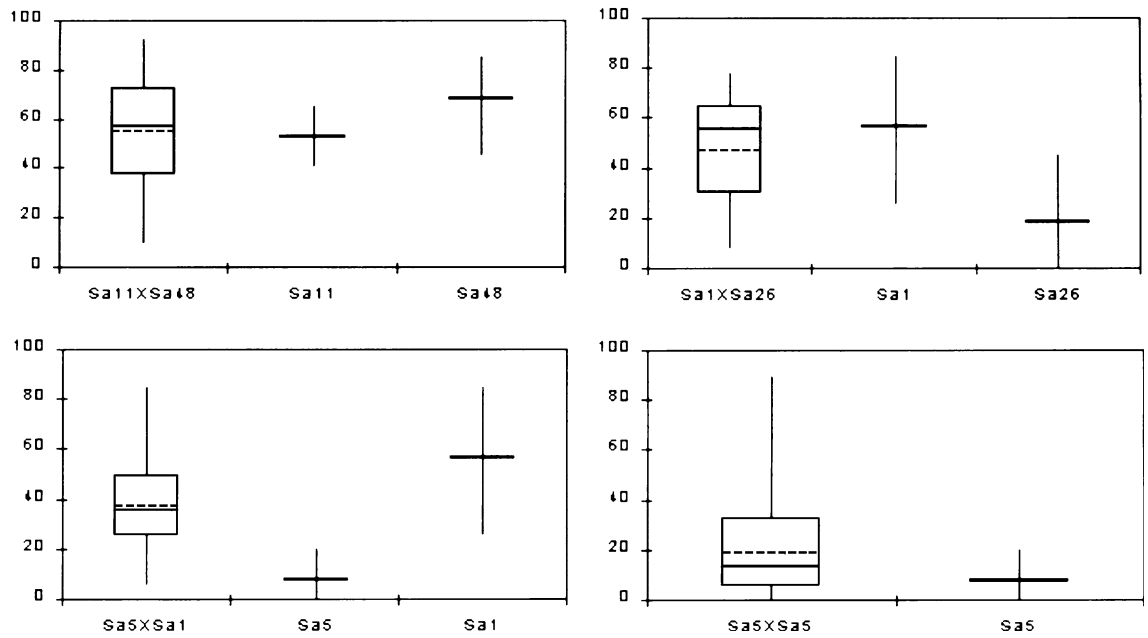

Figure 4. Boxplots (1st quartile-3rd quartile) associated with the transmission rates of four F1 offsprings of *S. avenae* (—: median; - - - - mean) and mean transmission rates of the parents. On the boxplots, upper and lower vertical bars represents the mean transmission values of the highest and the lowest transmitting clone in the offspring. On the parent plot, a horizontal line represents the mean and bars represent standard deviation of the mean.

mean's comparison tests (Duncan) showed many cases of transgression for higher transmission percentage, i.e. some F1 clones had a significantly higher transmission percentage than their better transmitting parent. Thus, for Sa5 × Sa1, two clones had a higher transmission percentage (71.7% and 83.3%) than Sa1 (56.25%), for Sa5 × Sa26, there was one case of transgression (58.8% vs. 18.7% for the parent Sa26), and for the selfing of the PEV Sa5, there were 20 cases of transgression (from 23.3% to 89%) compared to their unique parent (6.2%). For the Sa1 × Sa26 F1 cross, no transgression was observed, and for the Sa48 × Sa11 F1 cross, transgressions in both directions occurred: one offspring had a transmission percentage significantly higher (92.5%) than the better parent (68.3%) and three had transmission percentages significantly lower (11.2%, 10.5% and 9%) than the worse parent (51.7%). This demonstrates a large genetic variability between *S. avenae* clones for transmission performances.

Table 4. Analysis of variance, normality test and variance homogeneity tests for clone and replicate effects on PAV 4 transmission for the six crosses

Crosses	*n*	mean	r^2	Clone $P > F$	Replicate $P > F$	Normality $P > W^*$	Variance homogeneity: clone effect (Levene)
Sa1 × Sa26	16	46.75	0.972	0.0001	0.8473	0.4961	0.5625
Sa1 × Sa5	20	37.35	0.948	0.0001	0.0171	0.2357	0.0297
Sa48 × Sa11	10	54.27	0.978	0.0001	0.6888	0.2282	0.2991
Sa5 × Sa26	5	34.97	0.674	0.0371	0.6937	0.6106	0.2230
Sa5 × Sa5	44	23.51	0.912	0.0001	0.0757	0.1074	0.0001

W^*: W of Shapiro-Wilk.

Figure 3 also shows the mean transmission rate for the 14 isolates (see above) of two F1 clones from the selfing of Sa5, which exhibited the extreme values for PAV4 transmission. Sa (self.Sa5)52 had a mean transmission percentage of 18.34%, not significantly different from the mean transmission percentage of its parent Sa5 (31.22%). Conversely, Sa(self.Sa5)28 had a mean transmission percentage of 63.12%.

Figure 5 shows the regression of the mean F1 value on the midparent value, for the 4 above crosses and for the Sa5 × Sa26 cross (5 offsprings only): the coefficient of determination (r^2) is equal to 0.94 and the slope of the regression line is b = 0.5163, which can be considered as equal to the narrow-sense heritability h^2 of this character (Hartl and Clark, 1989). The good alignment of the points suggests (i) that additivity is important for this character, or (ii) that dominant and recessive factors are equally distributed in the parents.

Discussion

Range of PAV4 transmission variation in natural S. avenae *populations*

All clones of *S. avenae* were able to transmit PAV4, but with a wide range of inter-clonal variation, from less than 5% to more than 90% of transmission. Moreover, a continuum in transmission percentages was observed when only clones exhibiting different microsatellite patterns

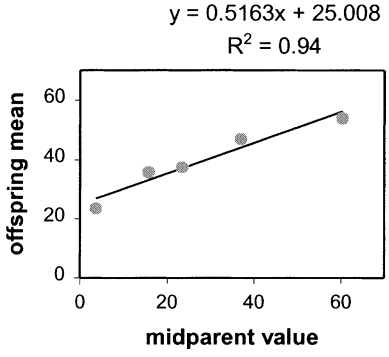

Figure 5. Regression of the mean offspring values on the midparent values for PAV4 transmission, for the four crosses (Sa5 × Sa26, Sa48 × Sa11, Sa5 × Sa1, Sa1 × Sa26, and for the selfing (Sa5 × Sa5). R^2 is the coefficient of determination.

(genotypes) are considered. Before the present work, cereal aphid inter-clonal variation in BYDV-PAV transmission was studied with a lower number of clones, and there were some contradictory conclusions. Transmission variation among 21 *R. padi* clones (range of 27–93%) was moderate in one study (Guo et al., 1997b), and low (80–98%) among 20 clones in another study (Sadeghi et al., 1997b). Concerning *S. avenae*, using a methodology different from ours (1 aphid / plant instead of 3, experiments at 14 °C instead of 20), Guo et al. (1997b) found a range of 0–76% for transmission among 21 clones collected in 4 French regions, but, unfortunately, their experiment was not replicated. Among 82 *M. persicae* clones transmitting 'beet yellows viruses' (a persistent luteovirus: BMYV or a semi-persistent closterovirus: BYV, or both, see Introduction) Björling and Ossiannilsson (1958), found a range of transmission from 0–10% to 70–80% with a median at 40–50%. Bourdin et al. (1998) also found a continuum in transmission percentages of a PLRV isolate for 17 *Myzus* clones. For stylet-borne viruses, Lupoli et al. (1992) observed a 13–46% range of transmission abilities of a *Papaya ringspot virus* (PRSV, a Potyvirus) isolate in a collection of 72 *Aphis gossypii* field-collected clones.

Our results obtained with a large sample of clones collected locally demonstrate the large intraspecific variation of PAV transmission in the aphid *S. avenae*. Such a variation explains why this species has been considered to be a more or less good PAV vector, depending on the characteristics of the clones considered in the experiments. By evaluating our field-collected *S. avenae* clones not only for virus transmission but also with molecular tools, we have been able to draw a distinction between inter and intra genotypic components of the transmission variability.

Mechanisms and genetic control of vectoring ability

In order to explore the possible role of differences of aphid feeding behaviour in PAV transmission variation, we have compared two contrasting clones, a PEV clone (Sa5) and a HEV clone (Sa1) by electropenetrography (EPG) using the methodology described by Prado and Tjallingii (1994). Differences between both clones for length and number of salivation (E1) and phloem feeding (E2) phases were

not significant (results not shown), indicating that feeding behaviour was probably not involved in transmission differences, at least for these two clones. In the aphid, the virus must cross from the hindgut into the hemocoel, then survive in the hemocoel, join the salivary glands and penetrate into the accessory salivary glands to be egested: thus, it is likely that several barriers and therefore several genetic loci are responsible for vector competence. In some cases (*Potato leafroll virus*, PLRV transmitted by *M. persicae*), gut membrane seems to regulate transmission efficiency (Rouzé-Jouan et al., 2001). For our *S. avenae* clones, it was shown (Papura, 2001) that the RNA of PAV4 could be detected by RT-PCR in the hemolymph of a PEV as well as of a HEV clone (Sa (self.Sa5)52 and Sa (self.Sa5)28), suggesting that viruses are acquired similarly by the two clones and that the molecular determinants of transmission may be situated in the hemolymph and/or in the salivary glands. The GroEL protein (symbionin) produced in the hemolymph by the aphid bacterial symbiont *Buchnera* was shown to bind *in vitro* with several luteoviruses (van den Heuvel et al., 1994) and is supposed to act as a chaperonin, but its binding capacity is not correlated with transmission efficiency of the different species tested (van den Heuvel et al., 1997), suggesting that, if GroEL is involved in transmission, it does not play a role in vector specificity. At an intraspecific level, molecular variation in some *Buchnera* genes was detected between *R. padi* clones (Simon et al., 1996) and between biotypes of the pea aphid *Acyrthosiphon pisum* (Simon et al., 2003), but until now, there is no evidence that these molecular variations are correlated with qualitative or quantitative variation in symbionin production (Simon, pers. comm.). Moreover, the GroEL gene was shown to be monomorphic in *Buchnera* from *A. pisum* (Simon et al., 2003). In *S. avenae*, two proteins have been isolated from head tissues and identified as potential receptors for BYDV-MAV by Li et al. (2001). Similarly, a protein from *S. graminum* and from *S. avenae*, situated in the membrane of the accessory salivary glands, binds with a Chinese variant of BYDV-MAV and is probably involved in virus transmission (Wang and Zhou, 2003). In both cases no comparison was done for these proteins between clones of the same aphid species.

Recently, the comparison of 2D proteinograms of HEV and PEV clones from the progeny of our Sa5 selfing revealed differences in only a small number of spots (12 out of 2150). Indeed, only four proteins that could not be related to GroEL according to their biochemical characteristics were qualitatively or quantitatively different for comparisons of two PEV clones and two HEV clones (Papura et al., 2002). Two of these protein spots were only distinguished by their isoelectric point and may be the consequence of expression of biallelic loci. These results suggest that the transmission character is oligo/polygenic, supporting our genetic hypothesis. When several isolates were compared, (PAV4 and PAV13 for 10 clones, 14 isolates for four clones), a strong isolate effect was found and the rank of clones in transmission rate did not fundamentally change with the isolate, whereas general levels of transmission changed, meaning that the roles in transmission performances of the genotype of the vector and of the isolate are at least partly independent.

Our analysis of F1 progenies has demonstrated the genetic basis of vectoring ability of an aphid and the relatively high heritability of this character, which is consistent with recent results on the transmission of BYDV-PAV by *S. graminum* (Gray and Gildow, 2003). Assuming that the determinism of the characters PEV or HEV is oligo/polygenic, we can hypothesise that (i) Sa5 is heterozygous for some genes regulating transmission, (ii) poor transmission is dominant, and (iii) Sa1 or Sa26 differ from Sa5 for some transmission genes. Codominance and interactions between genes involved in the determinism of transmission can explain the variability observed among the progenies. Nevertheless, further assessment of PEV and/or HEV genetic determinism will probably be difficult because of the difficulties in carrying out a complete diallel assay with aphids. These difficulties include: (1) necessity to select transmission variants producing synchronously a large quantity of males and mating females with fecund reciprocal crosses, (2) poor hatching success of the eggs, and above all, (3) high inbreeding depression in F2.

Is S. avenae *variability for PAV transmission of epidemiological interest?*

Even on a small geographic scale (50 km^2), our *S. avenae* clones displayed a wide range in BYDV-PAV4 transmission. This is consistent with other observations showing that *S. avenae* populations

exhibit a high genetic variability, even at field scale (Simon et al., 1999). In many cases, one or few predominant genotypes constitute a large part of the populations for a given year or season (15% for one genotype in our sample, but up to 50% for two genotypes in Haack et al. (2000)), but the turn-over of these genotypes appears to be very rapid. Thus, out of the 31 genotypes collected in 1990, only four were recovered in 1997 using a similar field sampling procedure (Haack et al., 2000). So, as for other traits, the potentiality exists for a variation in time in the mean PAV transmission rate for a *S. avenae* population, depending both on the possibility of emergence of new transmission variants and on their selection.

No assessment of mutation rates during the parthenogenetic reproduction of aphids is available (Wilson et al., 2003), nor has the genetic determinism of transmission been extensively investigated. Here, we have demonstrated that it is possible to generate variability for transmission by recombination. Because of the probable complex determinism of this character, transgressions are not rare among the F1 progenies, which exhibit lower or/and higher capacities of transmission than their parents taken individually. Consequently, this must lead to a Gaussian distribution of transmission and to production of extreme variants at a relatively high frequency in the regions were *S. avenae* achieves a sexual phase i.e. Northern (Newton and Dixon, 1988) and Eastern Europe (Papura et al., 2003), and, to some extent, in regions of France with cold winters (Dedryver et al., 2001).

The emergence of new variants for transmission (particularly extreme ones) is thus possible, but their selection in a complex agricultural environment is unlikely. The bell-shaped distribution of transmission rates across our field-collected genotypes of *S. avenae*, as well as the linearity of the regression of the mean offspring on the mean parents, are consistent with the hypothesis of an absence of selection for this character and a panmictic mating system, and may suggest that lethal genes are not linked to important genes involved in transmission. Assuming the fact that BYDV transmission is neutral for the vectors, genes involved in transmission may be linked to a character with a strong effect on the fitness of the clone, and would thus be selected indirectly (hitchhiking). Even if the probability of such an event seems low, it should be taken into consideration and some of our results suggest that PAV4 transmission performances depend significantly on the type of life-cycle of the *S. avenae* clones and on the plant species on which they were collected, although these finding require confirmation by further experiments. Interestingly, an effect of virus transmission phenotype by the host plant has been described recently. Thus, Gray et al. (2002) reported that, in *S. graminum*, virus transmission and host adaptation are correlated (and probably genetically linked) traits (biotypes adapted to agricultural crops were less efficient vectors of four BYDV species, than biotypes adapted to wild grasses). They suggested that, in *S. graminum*, the fitness cost of adapting to a new crop may have come at the expense of virus transmission.

However, *S. avenae*/BYDV-PAV epidemiological relationships are characterised by interactions between numerous host plants, and vector and virus populations exhibiting different degrees of host specialisation (Weber 1985: De Barro et al., 1995; Mastari et al., 1998; Haack et al., 2000; Sunnucks et al., 1998; Vialatte, unpublished). In such a complex ecological situation, the multiple and contradictory selection pressures exerted by each component on the others may lead to the maintenance of a Gaussian repartition of transmission performances in a *S. avenae* population when it is sampled on a sufficiently large scale (landscape or small region).

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References

Björling K and Ossiannilsson F (1958) Investigations on individual variations in the virus transmitting ability of different aphid species. Handlingar II 14: 1–13.

Black WC, DuTeau N, Puterka G, Nechols J and Pettorini J (1992) Use of random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphism in aphids (Homoptera: Aphididae). Bulletin of Entomological Research 82: 151–159.

Blackman RL (1971) Variation in the photoperiodic response within natural populations of *Myzus persicae* Sulz. Bulletin of Entomological Research 60: 533–546.

Bourdin D, Rouzé J, Tanguy S and Robert Y (1998) Variation among lines of *Myzus persicae* and *Myzus nicotianae* in the transmission of a poorly and a highly aphid-transmissible isolate of potato leafroll luteovirus (PLRV). Plant Pathology 47: 794–800.

Bournoville R, Simon J-C, Badenhausser I, Girousse C, Guilloux T and André S (2000) Lines of pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae) distinguished using genetic markers, differ in their damaging effect on a resistant alfalfa cultivar. Bulletin of Entomological Research 90: 33–39.

Caillaud CM, Dedryver C-A, Di Pietro J-P, Simon J-C, Fima F and Chaubet B (1995) Clonal variability in the response of *Sitobion avenae* (Homoptera: Aphididae) to resistant and susceptible wheat. Bulletin of Entomological Research 85: 189–195.

Carvalho GR, Maclean N, Wratten SD, Carter RE and Thurston JP (1991) Differentiation of aphid lines using DNA fingerprinting from individual aphids. Proceedings of the Royal Society London B 243: 109–114.

D'Arcy CJ and Mayo M (1997) Proposal for changes in luteovirus taxonomy and nomenclature. Archives of Virology 142: 1285–1287.

De Barro PJ, Sheratt TN, Brookes CP, David O and Maclean N (1995) Spatial and temporal genetic variation in British field populations of the grain aphid *Sitobion avenae* (F.) (Hemiptera: Aphididae) studied using RAPD-PCR. Proceedings of the Royal Society London B 268: 2291–2299.

Dedryver C-A, Bos C and Wegorek P (1990) Deux exemples de variabilité du polymorphisme chez *Sitobion avenae* F. (Homoptera, Aphididae). In: Ferron P, Missonnier J and Mauchamp B (eds) Régulation des cycles saisonniers chez les invertébrés (pp. 133–136) INRA, Paris, France.

Dedryver C-A, Le Gallic J-F, Gauthier J-P and Simon J-C (1998) Life cycle of the cereal aphid *Sitobion avenae* F.: Polymorphism and comparison of life history traits associated with sexuality. Ecological Entomology 23: 123–132.

Dedryver C-A, Hullé M, Le Gallic J-F, Caillaud MC and Simon J-C (2001) Coexistence in space and time of sexual and asexual populations of the cereal aphid *Sitobion avenae*. Oecologia 128: 379–388.

Fenton B, Woodford JAT and Malloch G (1998) Analysis of clonal diversity of the peach-potato aphid, *Myzus persicae* (Sulzer), in Scotland, UK and evidence for the existence of a predominant line. Molecular Ecology 7: 1475–1487.

Fuller SJ, Chavigny P, Lapchin L and Vanlerberghe-Masutti F (1999) Variation in clonal diversity in glasshouse infestations of the aphid, *Aphis gossypii* Glover in southern France. Molecular Ecology 8: 1867–1877.

Gray S and Gildow FE (2003) Luteovirus-aphid interactions. Annual Review of Phytopathology 41: 539–566.

Gray SM, Smith DM, Barbierri L and Burd J (2002) Virus transmission phenotype is correlated with host adaptation among genetically diverse populations of the aphid *Schizaphis graminum*. Phytopathology 92: 970–975.

Guo JQ, Moreau JP and Lapierre H (1996) Variability among aphid clones of *Rhopalosiphum padi* L. and *Sitobion avenae* F. (Homoptera: Aphididae) in transmission of three PAV isolates of barley yellow dwarf viruses. Canadian Entomologist 128: 209–217.

Guo JQ, Lapierre H and Moreau JP (1997a) Vectoring ability of aphid clones of *Rhopalosiphum padi* (L.) and *Sitobion avenae* (Fabr.) and their capacity to retain barley yellow dwarf virus. Annals of Applied Biology 131: 179–188.

Guo JQ, Lapierre H. and Moreau JP (1997b) Clonal variations and virus regulation by aphids in transmission of a French PAV-type isolate of Barley Yellow Dwarf Virus. Plant Disease 81: 570–575.

Haack L, Simon J-C, Gauthier J-P, Plantegenest M and Dedryver C-A (2000) Evidence for predominant clones in a cyclically parthenogenetic organism provided by combined demographic and genetic analyses. Molecular Ecology 9: 2055–2066.

Haack L, Leclercq-Le Quillec F, Henry M, Albert H and Dedryver C-A (2002) Vector dynamics and BYD disease progress on barley and maize: Two contrasting cases and consequences for control. In: Henry M and McNab A (eds) Barley Yellow Dwarf Disease: Recent Advances and Future Strategies, (pp. 40–43) CIMMYT, Mexico.

Habekuss A, Leistner HU and Schliephake E (1999) Characterization of *Rhopalosiphum padi* genotypes differing in the geographical origin by transmission efficiency of Barley yellow dwarf viruses and molecular markers. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 106: 437–443.

Hartl DL and Clark AG (1989) Principles of Population Genetics, 2nd edn. Sinauer Sunderland, Massachussets, USA.

Jurik M, Mucha V and Valenta V (1980) Intraspecies variability in transmission efficiency of stylet-borne viruses by the pea aphid (*Acyrthosiphon pisum*). Acta virologica 24: 351–357.

Leclercq-Le Quillec F, Tanguy S and Dedryver C-A (1995) Aerial flow of barley yellow dwarf viruses and of their vectors in western France. Annals of Applied Biology 126: 75–90.

Li C, Cox-Foster D, Gray SM and Gildow F (2001) Vector specificity of Barley yellow dwarf virus (BYDV) transmission: Identification of potential cellular receptors binding BYDV-MAV in the aphid, *Sitobion avenae*. Virology 286: 125–133.

Lister RM and Ranieri R (1995) Distribution and economic importance of barley yellow dwarf. In: D'Arcy CJ and Burnett PA (eds) Barley Yellow Dwarf: 40 years of Progress. (pp. 29–54) The American Phytopathological Society, St Paul, Minesota, USA..

Lucio-Zavaleta E, Smith DM and Gray SM (2001) Variation in transmission efficiency among barley yellow dwarf virus – RMV isolates and clones of the normally inefficient aphid vector, *Rhopalosiphum padi*. Phytopathology 91: 792–796.

Lupoli R, Labonne G and Yvon M (1992) Variability in the transmission efficiency of potyviruses by different clones of *Aphis gossypii*. Entomologia Experimentalis et Applicata 65: 291–300.

Mastari J, Lapierre H and Dessens JT (1998) Asymmetrical distribution of barley yellow dwarf virus PAV variants between host plant species. Phytopathology 88: 818–821.

Matthews REF (1991) Plant Virology, 3rd edn. Academic Press, New-York, USA.

Newton C and Dixon AFG (1988) A preliminary study of variation and inheritance of life-history traits and the occurrence of hybrid vigour in *Sitobion avenae* (F.) (Hemiptera: Aphididae). Bulletin of Entomological Research 78: 75–83.

Papura D (2001) Épidémiologie de la Jaunisse Nanisante de l'Orge en Roumanie et recherche de marqueurs protéiques du caractère vecteur chez *Sitobion avenae*. Thèse de doctorat, ENSA de Rennes, Rennes, France, 160 pp..

Papura D, Jacquot E, Dedryver C-A, Luche S, Riault G, Bossis M and Rabilloud T (2002) Two-dimensional electrophoresis of proteins discriminates aphid clones of *Sitobion avenae* differing in BYDV-PAV transmission. Archives of Virology 147: 1881–1898.

Papura D, Simon J-C, Halkett F, Delmotte F, Le Gallic J-F and Dedryver C-A (2003) Predominance of sexual reproduction in Romanian populations of the aphid *Sitobion avenae* inferred from phenotypic and genetic structure. Heredity 90: 397–404.

Prado E and Tjallingii WF (1994) Aphid activities during sieve element punctures. Entomologia Experimentalis et Applicata 72: 157–165.

Robert Y and Maury Y (1970) Capacités vectrices comparées de plusieurs souches *de Myzus persicae* Sulzer, *Aulacorthum solani* Kaltenbach et *Macrosiphum euphorbiae* Thomas dans l'étude de la transmission de l'enroulement de la pomme de terre. Potato Research 13: 199–209.

Rochow WF (1960) Specialisation among greenbugs in the transmission of barley dwarf virus. Phytopathology 50: 881–884.

Rochow WF (1969) Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59: 1580–1589.

Rochow WF and Eastop VF (1966) Variation within *Rhopalosiphum padi* and transmission of barley yellow dwarf virus by clones of four aphid species. Virology 30: 286–296.

Rouzé-Jouan J, Terradot L, Pasquer F, Tanguy S and Giblot Ducray-Bourdin D (2001) The passage of *Potato leafroll virus* through *Myzus persicae* gut membrane regulates transmission efficiency. Journal of General Virology 82: 17–23.

Sadeghi E, Dedryver C-A and Gauthier J-P (1997a) Role of acquisition and inoculation time in the expression of clonal variation for BYDV-PAV transmission in the aphid species *Rhopalosiphum padi*. Plant Pathology 46: 502–508.

Sadeghi E, Dedryver C-A, Riault G and Gauthier J-P (1997b) Variation in transmission of two BYDV-MAV isolates by multiple clones of *Rhopalosiphum padi* L. European Journal of Plant Pathology 103: 515–519.

Sadeghi E, Dedryver C-A, Tanguy S and Riault G (1995) Intraspecific variations for transmission of BYDV-PAV and -MAV isolates by the aphids *Sitobion avenae* and *Rhopalosiphum padi*. Agronomie 15: 504–505.

Saksena KN, Singh SR and Sill WH (1964) Transmission of barley yellow dwarf virus by four biotypes of the corn leaf aphid, *Rhopalosiphum maidis*. Journal of Economic Entomology 57: 569–571.

SAS Institute Inc., *SAS/STAT User's guide, Version 8*: SAS Institute Inc, 1999. Cary, North Carolina, USA.

Simon J-C, Blackman R and Le Gallic J-F (1991) Local variability in the life cycle of the bird cherry-oat aphid, *Rhopalosiphum padi* (Homoptera: Aphididae) in western France. Bulletin of Entomological Research 81: 315–322.

Simon J-C, Rispe C and Sunnucks P (2002) Ecology and evolution of sex in aphids. Trends in Ecology and Evolution 17: 34–39.

Simon J-C, Martinez-Torres D, Latorre A, Moya A and Hebert PDN (1996) Molecular characterization of cyclic and obligate parthenogens in the aphid *Rhopalosiphum padi* L. Proceedings of the Royal Society of London B 263: 481–486.

Simon J-C, Baumann S, Sunnucks P, Hebert PDN, Pierre J-S, Le Gallic J-F and Dedryver C-A (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 J-C, Carré S, Boutin M, Prunier-Leterme N, Sabater-Muñoz B, Latorre A and Bournoville R (2003) Host-based divergence in populations of the pea aphid: Insight from nuclear markers and the prevalence of facultative symbionts. Proceedings of the Royal Society of London B 270: 1703–1712.

Singh MN, Khurana SM and Nagaich B (1983) Evidence on heredity variations in the virus transmission efficiency of aphid clones. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 90: 345–351.

Sohi SS and Swenson KG (1964) Pea aphid biotypes differing in bean yellow mosaic virus transmission. Entomologia Experimentalis et Applicata 7: 9–14.

Sunnucks P, Chisholm D, Turak E and Hales DF (1998) Evolution of an ecological trait in parthenogenetic *Sitobion aphids*. Heredity 81: 638–647.

Thottappilly G, Tsai JH and Bath JE (1972) Differential aphid transmission of two bean yellow mosaic virus strains and comparative transmission by biotypes and stages of the pea aphid. Annals of the Entomological Society of America 65: 912–915.

Torrance L, Pead MT, Larkins AP and Butcher GW (1986) Characterization of monoclonal antibodies to a U.K. isolate of barley yellow dwarf virus. Journal of General Virology 67: 549–556.

Van den Heuvel JFJM, Verbeek M and van der Wilk F (1994) Endosymbiotic bacteria associated with circulative transmission of potato leafroll virus by *Myzus persicae*. Journal of General Virology 75: 2559–2565.

Van den Heuvel JFJM, Bruyère A, Hogenhout SA, Ziegler-Graff V, Brault V, Verbeek M, van der Wilk F and Richard K (1997) The N-terminal region of the luteovirus read-through domain determines virus binding to *Buchnera* GroEL and is essential for virus persistence in the aphid. Journal of Virology 71: 7258–7265.

Wang X and Zhou G (2003) Identification of a protein associated with circulative transmission of Barley yellow dwarf virus from cereal aphid, *Schizaphis graminum* and *Sitobion avenae*. Chinese Science Bulletin 48: 2083–2087.

Weber G (1985) On the ecological genetics of *Sitobion avenae* (F.) (Hemiptera, Aphididae). Zeitschrift für Angewandte Entomologie 100: 100–110.

Wilson ACC, Sunnucks P and Hales DF (2003) Heritable genetic variation and potential for adaptive evolution in asexual aphids (Aphidoidea). Biological Journal of the Linnean Society 79: 115–135