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ORIGINAL ARTICLE

Biometrical genetic analysis of luteovirus transmission in the aphid *Schizaphis graminum*

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The aphid *Schizaphis graminum* is an important vector of the viruses that cause barley yellow dwarf disease. We studied the genetic architecture of virus transmission by crossing a vector and a non-vector genotype of *S. graminum*. F1 and F2 hybrids were generated, and a modified line-cross biometrical analysis was performed on transmission phenotype of two of the viruses that cause barley yellow dwarf: *Cereal yellow dwarf virus* (CYDV)-RPV and *Barley yellow dwarf virus* (BYDV)-SGV. Our aims were to (1) determine to what extent differences in transmission ability between vectors and non-vectors is due to net additive or non-additive gene action, (2) estimate the number of loci that determine transmission ability and (3) examine the nature of genetic correlations between transmission of CYDV-RPV and BYDV-SGV. Only additive effects contributed significantly to divergence in transmission of both CYDV-RPV and BYDV-SGV. For each luteovirus, Castle–Wright's estimator for the number of effective factors segregating for transmission phenotype was less than one. Transmission of CYDV-RPV and BYDV-SGV was significantly correlated in the F2 generation, suggesting that there is a partial genetic overlap for transmission of these luteoviruses. Yet, 63% of the F2 genotypes transmitted CYDV-RPV and BYDV-SGV at significantly different rates. Our data suggest that in *S. graminum*, the transmission efficiency of both CYDV-RPV and BYDV-SGV is regulated by a major gene or set of tightly linked genes, and the transmission efficiency of each virus is influenced by a unique set of minor genes.
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Introduction

It is well known that insect populations vary in their ability to transmit viruses to plants and animals (Gubler and Rosen, 1976; Gooding, 1996; Bencharki *et al.*, 2000; Gray *et al.*, 2002; Lazzaro *et al.*, 2004). However, very little is known about the genetic regulation of virus transmission. HH Storey (Storey, 1932) was the first to determine that transmission of *Maize streak geminivirus* to maize (*Zea mays* L.) by leafhoppers was under genetic control. He maintained 'active' and 'inactive' (vector and non-vector) genotypes of leafhoppers (*Cicuadulina mbila* Naude), and crossed these populations to determine that transmission was inherited via a sex-linked, dominant gene. The inheritance of virus vector competence in other leafhopper species has since been investigated. The transmission phenotype of *Beet curly top hybrigeminivirus* was found to be heritable in *Eutettix tenellus* (Baker) (Bennett and Wallace, 1938), and transmission of *Potato yellow dwarf nucleorhabdovirus* (PYDV) is sex linked or autosomal in *Aceratagallia sanguinolenta* (Prov.) (Black, 1943). Populations of *Agallia constricta* (van Duzee) were selectively bred for high and low transmission phenotype of two different viruses, PYDV and wound-tumor virus (now *Clover wound tumor phytoreovirus*) (Nagaraj and Black, 1962). More recently, the role of genetics in the transmission of human diseases by mosquitoes has been intensively investigated. The susceptibility or refractiveness of mosquitoes (*Aedes* spp and *Culicoides* spp) to viral diseases has generally been found to involve one major gene (Gubler and Rosen, 1976; Hardy *et al.*, 1978; Miller and Mitchell, 1991; Tabachnick, 1991). However, in other systems, vector competence is multigenic (Tardieux *et al.*, 1991; Tabachnick, 1994). Several quantitative trait loci influencing transmission of *Dengue-2 flavivirus* (Bosio *et al.*, 2000) and *La Crosse bunyavirus* (Anderson *et al.*, 2005) in the mosquitoes *Aedes aegypti* and *Ochlerotatus* spp have been identified. However, specific genes controlling vector competence in insects have not been cloned. Knowledge of the genes and proteins involved in the transmission process would be useful for the development of novel disease control strategies that target insect–virus interactions.

Aphids are the most successful and numerous insect vectors of plant viruses. Considerable information is known about the biology of aphid–virus interactions and the viral determinants that regulate the specificity of transmission. However, the aphid factors regulating virus transmission are not well studied. Aphids are amenable to genetic investigation and the genetics of several ecologically important phenotypes have been dissected, including alate production (Weisser and Braendle, 2001; Caillaud *et al.*, 2002; Braendle *et al.*,

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2005a, b), insecticide resistance (Rider and Wilde, 1998; Rider *et al*., 1998), virulence on plants (Puterka and Peters, 1989, 1990), host preference (Via, 1991; Hawthorne and Via, 2001; Tosh *et al*., 2004), and life-cycle patterns (Zhang and Zhong, 1990; Dedryver *et al*., 1998). While the intraspecific variation of aphids to transmit a virus has long been known (Bjorling and Ossiannilsson, 1958; Price *et al*., 1971), the genetics of virus transmission is just beginning to be explored (Papura *et al*., 2002; Dedryver *et al*., 2005; Burrows *et al*., 2006). Studies to date have focused on the transmission of the viruses causing barley yellow dwarf disease in cereal crops and wild grass species.

The viruses that cause barley yellow dwarf are members of two genera in the viral family Luteoviridae, *Polerovirus* and *Luteovirus* (Miller and Rasochova, 1997). The icosahedral virions contain a positive sense, single-stranded RNA encapsidated by two proteins: a major 22 kDa coat protein and a minor 72 kDa read-through protein. Both proteins are involved in regulating virus transmission efficiency (Van den heuvel *et al*., 1993; Chay *et al*., 1996; Gildow, 1999; Brault *et al*., 2005). Luteoviruses are phloem limited and transmitted from plant to plant solely by aphids in a circulative, non-propagative manner (Gray and Gildow, 2003). Ingested virions are actively transported across gut epithelial cell cytoplasm in vesicles and released into the hemocoel. To be transmitted into a plant, the virions accumulate at the surface of the accessory salivary glands and are actively transported across the cells and deposited into the salivary duct. The virus does not replicate in any tissue of the aphid.

There is a great deal of specificity among the seven characterized species of virus causing barley yellow dwarf and five well-studied aphid vector species. Although all aphids can ingest any virus while feeding on the phloem of an infected plant, not all viruses are transmitted by all aphid genotypes. Failure to transmit a particular virus is regulated by an inability of the virus to be transported across the gut or salivary tissue. The ability to move through one tissue is independent of movement through the other tissues (Gray and Gildow, 2003). As mentioned, both viral proteins are involved in the transport process, but presumably they must interact with genetically regulated aphid components at each of the independent tissues.

Previous research on the inheritance of luteovirus transmission phenotype in aphids found *Sitobion avenae* transmission of *Barley yellow dwarf virus* (BYDV)-PAV to be a polygenic trait inherited in an additive manner (Papura *et al*., 2002; Dedryver *et al*., 2005). Burrows *et al*. (2006) examined F1 and F2 hybrids derived from two parental genotypes of *Schizaphis graminum* differing in their ability to transmit two viruses, *Cereal yellow dwarf virus* (CYDV)-RPV (*Polerovirus*) and BYDV-SGV (*Luteovirus*). Segregation of the transmission phenotype for both viruses was observed in the F1 and F2 populations, indicating that the transmission phenotype was under genetic control and the parents were heterozygous for genes involved in virus transmission. Additional studies determined that gut and salivary barriers were operating to prevent virus transmission in the non-vector genotypes, and that these barriers were segregating independent of one another (Burrows *et al*., 2006). In the present study, we expand on the work presented in Burrows *et al*. (2006). Data from the same F1 generation and an expanded collection of F2 hybrids are used to perform a biometrical analysis of virus transmission. First, we use a modified version of line-cross analysis (Mather and Jinks, 1982; Kearsey and Pooni, 1996) to reveal the relative contributions of additive, dominance and epistatic effects to genetic differentiation between a vector and a non-vector parental line. We asked whether there is a net difference between the additive effects of the genes in the vector and non-vector parents, whether the genes in the vector parent tend, on average, to be dominant over those of the non-vector parent (and *vice versa*) and whether there are net epistatic interactions between the genes of the vector and non-vector parents. Secondly, we estimated the number of segregating factors responsible for behavioral differences between parental lines using the Castle–Wright estimate incorporating modifications suggested by Cockerham (1986) and Zeng (1992). Last, we examined the nature of the genetic correlation in transmission ability of two luteoviruses, BYDV-SGV and CYDV-RPV.

Materials and methods

Generation of F1 and F2 crosses

Virus-free genotypes of *S. graminum* were maintained parthenogenetically as described by Katsar and Gray (1999). The parental genotype F (Sg-F) (Porter *et al*., 1997) was a gift from John Burd (USDA, ARS, Stillwater, OK, USA) and is an efficient vector of CYDV-RPV and BYDV-SGV (Gray *et al*., 1998). Parental genotype SC (Sg-SC) was collected in South Carolina, and is an inefficient vector (non-vector) of CYDV-RPV and BYDV-SGV (Gray *et al*., 1998).

To induce sexual forms for mating, aphids colonizing caged barley plants were transferred from growth chambers maintained at 20°C (18 h light) to chambers maintained at 15°C (12 h light) or 13°C (11 h light) and observed weekly for males and sexual females. To produce F1 hybrids, virgin female Sg-SC were placed in cages with male Sg-F. Sg-SC does not produce males, and thus a reciprocal cross was not possible. To generate the F2 cross, F1 genotypes were placed in the same environmental conditions as the parents to induce sexual aphids. Resulting virgin females and males were mated randomly. No matings of the same genotype were permitted. We chose this method because the parental genotypes used for our crosses are drawn from natural populations, and are thus likely to be heterozygous for most loci (Lynch and Walsh, 1998). Eggs from the parental and F1 crosses were collected as described by Via (1992) and stored for 3 months at 0–4°C. To induce hatching, eggs were placed in dishes containing moist filter paper and fresh barley tissue and incubated at 20°C. First instar nymphs that hatched and survived were individually transferred to barley plants and allowed to develop parthenogenetically reproducing genotypes that were maintained as described previously (Katsar and Gray, 1999).

Virus transmission

Transmission assays were performed essentially as described by Gray *et al*. (2002). Briefly, aphids were placed on source material, which had been infected

approximately 5 weeks previously. Aphids were allowed to feed for a 48 h acquisition access period and then transferred at a rate of five aphids per plant to 16 individual oat plants (*Avena byzantina* K. Koch cv. Coastblack). Four oat plants were inoculated with aphids, that had fed on healthy oat plants (five aphids per plant) as a negative control. Aphids were allowed to feed on oat plants for a 5-day inoculation access period before being fumigated. Symptoms were allowed to develop in the greenhouse for 3–5 weeks. Plants with weak symptoms were tested for the presence of virus using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as described previously (Gray *et al*., 1998). All tissue used as source material was tested using DAS-ELISA to confirm virus infection. Transmission tests for each F1 genotype were repeated 3–5 times over a 12-month period. Transmission tests for each F2 genotype were repeated 2–5 times from January to June 2004 and January to August 2005. Sg-F and Sg-SC were included in each experiment as positive and negative controls, respectively.

Statistical analysis

All data were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). Transmission phenotypes of the F2 generation were first subjected to an analysis of variance, for BYDV-SGV and CYDV-RPV separately, to determine which characters showed significant genetic variation (segregation) (PROC MIXED). 'F2 genotype' was considered as a random factor. For visualizing the data, means of each level of fixed effects (parents Sg-F and Sg-SC, F1 and F2 genotypes) were calculated in PROC MIXED as least-squares means (LSMEANS statement).

We tested the generation means for goodness-of-fit to genetic models incorporating additive or dominant effects using the joint scaling test of Mather and Jinks (1982) as described by Kearsey and Pooni (1996). The joint scaling test has been shown to be applicable to non-homozygous lines (as Sg-F and Sg-SC are) as long as mating between close relatives did not occur (Lynch and Walsh, 1998). Some generations (e.g. F2) have much larger variance among individuals than others due to genetic segregation, and there may be large differences in family sizes. This heterogeneity within the generation makes the accuracy of the means unequal. The joint scaling test adjusts for this by weighting the means differently in the regression analysis (Kearsey and Pooni, 1996). The weighted regression analysis and the χ^2 test were implemented using PROC REG.

The minimum number of segregating factors involved in genetic divergence in virus transmission between Sg-F and Sg-SC parents was estimated using the method first developed by Castle and Wright (Castle, 1921) and then modified by Lande (Lande, 1981) for use with non-homozygous populations. We use here the method to estimate n_e suggested by Cockerham (1986) that corrects for sampling variances in the estimates of parental populations. As described in Lynch and Walsh (1998), after computing n_e using the Cockerham equation (Cockerham, 1986), we substituted this estimate in an expression suggested by Zeng (Zeng *et al*., 1990; Zeng, 1992) that takes into account possible linkage and inequality of allelic effects. In this estimate, the haploid number of chromosomes in *S. graminum* is 4 ($2n = 8$) (Mandrioli *et al*., 1999).

Genetic correlations between transmission of BYDV-SGV and CYDV-RPV were calculated in the F2 generation as Pearson's product–moment correlations of clone means (Via, 1991) using PROC CORR. If the same loci influence transmission of BYDV-SGV and CYDV-RPV, then cross-environment correlations in F2 are expected to be significantly different from zero and positive.

Results

Development of F1 and F2 generations

As reported previously (Burrows *et al*., 2006), a cross between a female inefficient vector genotype, Sg-SC, and a male vector genotype, Sg-F, produced 13 F1 hybrid genotypes. The 13 F1 genotypes were reared under the environmental conditions that induced sexual forms in the parents. Six of the F1 genotypes (F1–1, F1–4, F1–5, F1–9, F1–11 and F1–12) produced males, and 10 of the F1 genotypes (F1–1, F1–2, F1–3, F1–4, F1–5, F1–7, F1–9, F1–10, F1–11 and F1–12) produced females. Genotypes F1–6, F1–8 and F1–13 did not produce any males or females. Males and females were intermated to avoid inbreeding, producing an F2 generation. Multiple random crosses resulted in 1859 eggs. Three hundred and fifty-four of these eggs hatched, and 96 individual F2 fundatrices formed parthenogenetic colonies.

Transmission phenotypes of the F2 hybrid generation

In the F2 generation, transmission efficiency ranged from 0 to 100% for both CYDV-RPV and BYDV-SGV (Figure 1). Significant genetic variation for transmission phenotype was seen in the F2 generation for both CYDV-RPV and BYDV-SGV (CYDV-RPV: $F_{93,255} = 6.0$, $P < 0.001$; BYDV-SGV: $F_{93,254} = 9.1$, $P < 0.001$). Transmission efficiency of the positive control, Sg-F, was stable throughout the experiment (CYDV-RPV: $76 \pm 3\%$; BYDV-SGV: $70 \pm 3\%$). Transmission of either CYDV-RPV or BYDV-SGV by the negative control, Sg-SC, was rare ($0 \pm 0\%$ for both).

Line-cross analysis

The mean transmission efficiency of the F1 and F2 genotypes was intermediate between that of the parents (Table 1), indicating no directional dominance of either parent on transmission phenotype. If vector and non-vector aphids diverge primarily in genes with additive effects, then hybrid means for all generations should fall along the dotted lines joining the observed parental means in Figure 2. The extent to which the hybrid means are displaced from this line is proportional to the degree of dominance. In the presence of epistasis, the displacement for the F1 hybrids and the F2 hybrids is comparable (Lynch and Walsh, 1998). For both CYDV-RPV and BYDV-SGV transmission, hybrid means fall along the line (Figures 2a and b), suggesting that these characters are almost completely additive, and this was confirmed with the joint scaling test (Table 2).

We estimated the number of genes involved in the transmission phenotype for both CYDV-RPV and BYDV-SGV. Biometrical estimates using the method suggested by Cockerham (1986) were low: 0.58 ± 0.08 for CYDV-RPV and 0.60 ± 0.11 for BYDV-SGV. In *S. graminum*, using equation 9.27 of Lynch and Walsh (1998) with possible

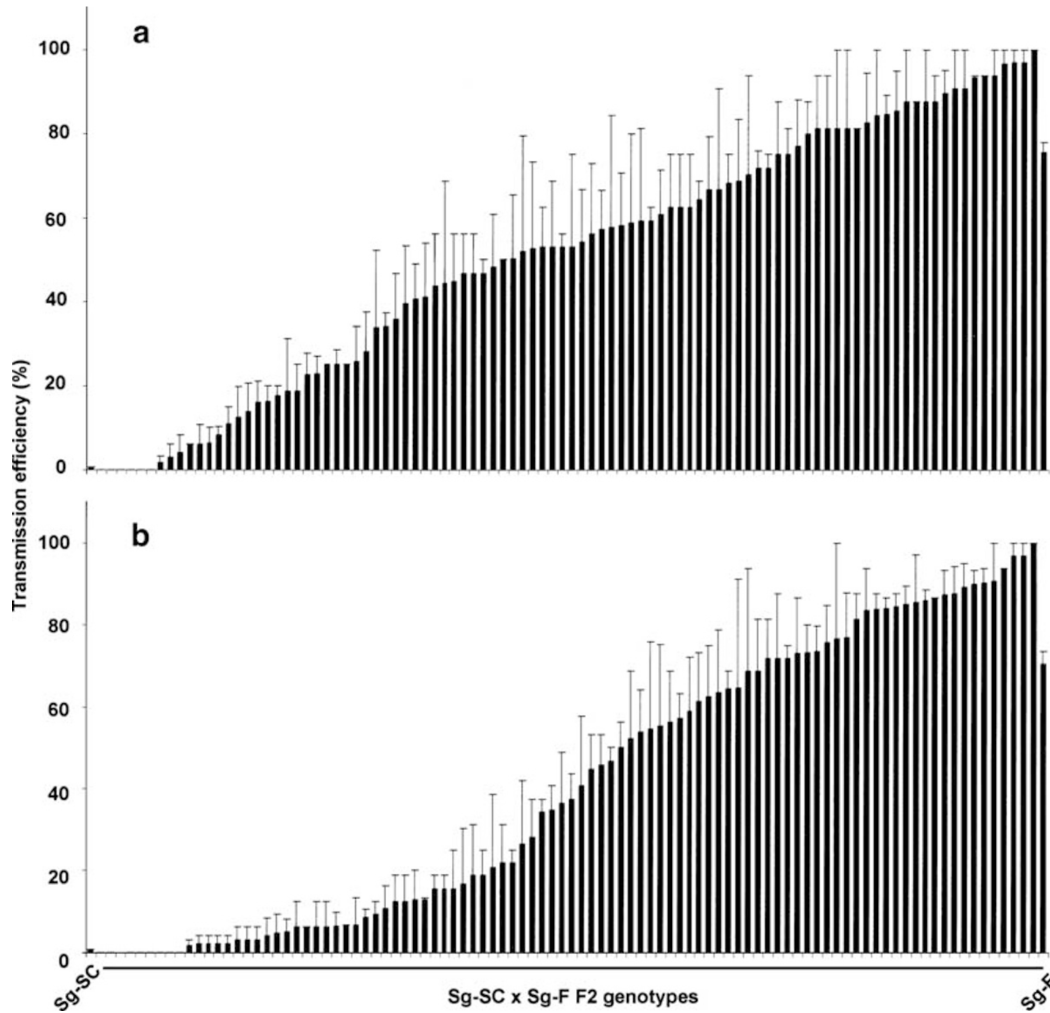

Figure 1 Transmission efficiency of CYDV-RPV (**a**) and BYDV-SGV (**b**) for 96 F2 hybrid clones of a cross between a non-vector (Sg-SC) and a vector (Sg-F) genotype of *S. graminum*. The mean transmission efficiency of Sg-SC and Sg-F are on the left and right-hand sides of the *x* axis, respectively. F2 genotypes are arranged along the *x* axis in order of increasing transmission efficiency. Each bar represents the mean of two to five experiments plus the standard error of the mean.

Table 1 Transmission efficiency of CYDV-RPV and BYDV-SGV for the parents, F1 and F2 hybrids

	$n_{genotypes}$	*Sample size* (n)		*Transmission efficiency (%)*	
		CYDV-RPV	*BYDV-SGV*	*CYDV-RPV*	*BYDV-SGV*
P1 (Sg-SC)	1	49	47	0	0
P2 (Sg-F)	1	52	50	78	73
F1	13	50	39	48	42
F2	96	255	253	48	39

Abbreviations: BYDV = *Barley yellow dwarf virus*; CYDV = *Cereal yellow dwarf virus*.
Transmission efficiency was calculated as the number of plants that became infected out of the total number of plants inoculated with five viruliferous aphids per oat plant. Sixteen plants were included in each trial.

linkage (*c*) equal to 3/8 and the inequality of allelic effects (C_α) equal to 1, we find an n_e of 0.06 for BYDV-SGV transmission and of 0.04 for CYDV-RPV transmission.

The relationship between CYDV-RPV and BYDV-SGV transmission was investigated by calculating the r_{cm} of Via (1991). The transmission of CYDV-RPV and BYDV-SGV were significantly correlated in both the F1 ($r_{cm} = 0.59$, $P < 0.001$; Burrows *et al*., 2006) and F2 ($r_{cm} = 0.40$, $P < 0.001$; Figure 3) generations, suggesting that there is a partial genetic overlap between transmission of CYDV-RPV and BYDV-SGV. The majority of F1 and F2 genotypes transmitted CYDV-RPV and BYDV-SGV at significantly different rates (F1, six of 13 genotypes; F2, 60 of 96 genotypes), showing that some genes uniquely influence transmission of either CYDV-RPV or BYDV-SGV. Additionally, several F2 genotypes transmitted more efficiently than the vector parent

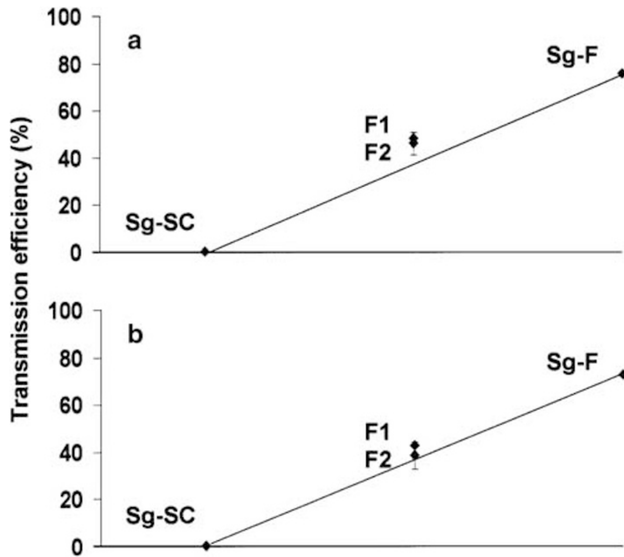

Figure 2 Mean transmission efficiency of CYDV-RPV (**a**) and BYDV-SGV (**b**) for the parents (Sg-SC and Sg-F), F1 and F2 hybrid genotypes. The solid line represents the weighted least-squares estimate of a simple additive model. Standard error bars are included for each mean.

Table 2 Parameter estimates for models incorporating additive and dominant effects

	m	a	d	aa	χ^2
Transmission of RPV					
y = m+xa	0.540	−0.530			0.56^{NS}
y = m+xa+xd	0.541	−0.530	0.007		0.52
y = m+xa+xaa	0.536	−0.543		0.011	0.52
Transmission of SGV					
y = m+xa	0.476	−0.475			1.41^{NS}
y = m+xa+xd	0.474	−0.491	−0.017		1.28
y = m+xa+xaa	0.457	−0.523		0.066	0.55

Abbreviations: m, mean (average phenotype of the two parents); a, additive genetic component of the expected mean; d, dominance genetic component of the expected mean; aa, additive by additive genetic component of the expected mean; NS = non-significant.
See Kearsey and Pooni (1996, pp 19–33) for details.

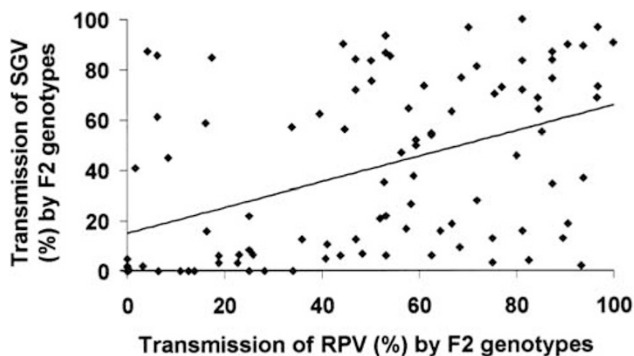

Figure 3 Genetic correlations between transmission efficiency of CYDV-RPV and BYDV-SGV for F2 hybrid genotypes of a cross between *S. graminum* genotypes Sg-SC and Sg-F. The line represents the Pearson's product-moment correlation of clone means (Via, 1991).

(Sg-F), indicating Sg-F was not homozygous for all genes facilitating transmission and there is transgressive segregation in the F2 genotypes (Figure 1).

Discussion

In this study, we examined the genetic basis of luteovirus transmission in *S. graminum*. Our main goal was to determine which of two alternative genetic models was more plausible: (a) a few major genes or (b) many genes, each with a small influence on the phenotype. If the difference in transmission ability between parents Sg-F and Sg-SC is caused by a one to a few major segregating factors, then a search for molecular markers tightly linked to these loci is likely to be successful, and the eventual molecular characterization of these loci using a positional cloning approach is feasible.

The biometrical estimates we found are strikingly low (less than 1) and suggest that few loci explain differences in transmission ability between parents Sg-F and Sg-SC. It is tempting to speculate that a major gene influencing virus transmission segregates in our F2 generation. The biometrical estimation we used rests on several assumptions that, if not met, lead to an underestimation of the 'true' number of loci segregating in hybrid generations. It assumes unlinked loci, equality of allelic effects, additive gene action and that all the genes with a positive influence on the traits are sorted into one parental line and all those with negative influence into the other parental line (Lynch and Walsh, 1998). Although we were able to test for the adequacy of the additive gene action model, examining whether other assumptions of this method are met in our study is more difficult. Following the modification of the Castle–Wright method recommended by Lynch and Walsh (1998), most of the potential violations of these assumptions are taken into account in c and C_{α}. Yet, our estimates of the number of loci associated with transmission phenotype remain approximations. Our goal, however, was not to provide definite estimates of gene number for CYDV-RPV and BYDV-SGV transmission, but to place some bounds on the number of genes involved in transmission. From our results, it is clear that one major aphid gene (or a set of tightly linked genes) controls virus transmission. This is the one we detected with our estimate n_e. This one major aphid gene (or a set of tightly linked genes) likely explains the significant positive genetic correlation observed between CYDV-RPV and BYDV-SGV transmission in both the F1 and F2 generation. Yet, other genes, contributing less to differences in transmission phenotype between Sg-F and Sg-SC, and harder to detect with our biometrical estimates, also contribute to the variation in transmission phenotype. These loci will affect either CYDV-RPV or BYDV-SGV transmission and explain why many F2 genotypes can efficiently transmit one virus and poorly transmit the other virus. The availability of a fine-scale genetic map and the possibility of mapping BYDV-SGV and CYDV-RPV transmission onto this linkage map would provide much better estimates of the gene number and their individual effects.

From this and previous studies (Papura *et al.*, 2002; Dedryver *et al.*, 2005), several trends have emerged. First, the transmission of luteoviruses is multigenic, and inheritance is generally additive in manner. Second, there is transgressive segregation of hybrid genotypes,

that is, a number of F1 and F2 genotypes were found to transmit virus at a higher level of efficiency than that of the vector parent. Third, individual genotypes do not exhibit the same phenotype for transmission of all Luteoviridae. Taken together, we can conclude that transmission is a multigenic, non-dominant trait. There are few genes involved in transmission phenotype, some of which are shared and others which are isolate-specific. We can only speculate on the function of these genes at this time. One hypothesis is that the genes involved in transmission include receptors. A search for proteins that bind to virus using far-Western blots has been partly successful, and several proteins have been identified (Li *et al*., 2001; Seddas *et al*., 2004). However, the lack of genomic data for the aphid has limited the identification of many potentially influential proteins, and the lack of a reverse genetic system in the aphid has limited our investigations into their functionality. The adaptive immune system could also be involved in regulating virus transmission by recognition and response to non-self-particles. Little is known about immunity in aphids, and little is known about insect immune responses to viruses in general. Responses to bacterial, fungal and protozoal pathogens include melanization, phagocytosis and cellular encapsulation by hemocytes, as well as secretion of antimicrobial peptides (Leclerc and Reichhart, 2004; Levashina, 2004; Loker, 2004). However, the response to viral pathogens is considered to be distinct, as *Drosophila* genes involved in anti-bacterial and anti-fungal immunity are not induced by virus pathogens (Sabatier *et al*., 2003; Leclerc and Reichhart, 2004). An encapsulation immune response to a baculovirus was described in *Helicoverpa zea* (Trudeau *et al*., 2001). Other responses to viral pathogens could include apoptosis (Narayanan, 1998) or RNA interference (RNAi). RNAi is a sequence-specific targeting of pathogen RNA, which is initially induced by a double-stranded RNA, and has been found to be active against viruses in mosquitoes (Keene *et al*., 2004), *Drosophila* (Li *et al*., 2002) and shrimp (Robalino *et al*., 2004, 2005). An RNAi response to luteoviruses in aphids is considered unlikely since luteoviruses do not uncoat or replicate in the aphid host. In addition to immune responses, there could be conformational changes in the structural proteins of luteoviruses due to pH or other biochemical changes in the aphid. It could be postulated that any of these alterations to the virus could be isolate-specific, thus substantiating the results of our genetic analysis.

It has been postulated that symbionin, a protein produced in copious amounts in endosymbionts such as *Buchnera* in aphids (van den Heuvel *et al*., 1994) and *Bemisia tabaci* in whiteflies (Morin *et al*., 1999), is involved in stabilizing the virus in the aphid hemocoel and thus facilitating transmission (van den Heuvel *et al*., 1994). Symbionin is a homolog of the heat-shock protein GroEL of *Escherichia coli*, which is a member of the chaperonin 60 family of proteins (Morioka and Ishikawa, 1998). The N-terminal domain of PLRV binds symbionin from vector and non-vector aphids, as well as GroEL from *E. coli* (van den Heuvel *et al*., 1997). Aphids treated with antibiotics to eliminate endosymbionts are less efficient vectors of PLRV (van den Heuvel *et al*., 1994), which has led to the postulate that symbionin facilitates virion stability in the hemolymph and ultimate transmission by the aphid (van den Heuvel *et al*., 1994). Endosymbionts have been found to influence resistance to parasitic wasps (Oliver *et al*., 2005) and transovarial transmission of rice dwarf virus (Nasu, 1965). *Buchnera* is inherited through the maternal line. The facilitation of transmission by association with *Buchnera* is contradicted by the evidence in this study that CYDV-RPV transmission is facilitated by genes or factors inherited from the male (vector) parent. A reciprocal cross between a vector and a non-vector of PAV and RPV indicated some influence of maternal inheritance, but transmission phenotype could not be solely explained by this (Gray *et al*., 2006). In addition, we found that the hindgut and accessory salivary gland barriers to transmission were genetically controlled and separated in F2 hybrid genotypes, providing additional evidence for genetic control of luteovirus transmission in *S. graminum*, rather than endosymbiont influence. Although endosymbionts may play a role in stabilizing virus particles, we do not believe they determine vector competence in our system.

Our conclusions about the genetic architecture of virus transmission in *S. graminum* are based on a single cross. Our genetic analysis is therefore informative only about the alleles present in the two original parents, but it does not provide us with information about alleles present in the whole aphid population. More genes and more complex interactions among them may be detected as we add more parental genotypes to this study. Yet, our results provide an inroad to the molecular characterization of a gene having a major effect on transmission phenotypes in *S. graminum*. Although this may seem a monumental enterprise, ongoing improvements in genomic technologies are bringing the realization of this goal, in non-model organisms such as aphids, within reach.

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