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Comparing the spatial genetic structures of the Flavescence dorée phytoplasma and its leafhopper vector *Scaphoideus titanus*

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ABSTRACT

The Nearctic leafhopper *Scaphoideus titanus* Ball is the vector of "Flavescence dorée" phytoplasma (FDp) in European vineyards. We studied the genetic diversity and structure of *S. titanus* populations in France and of the FDp they carried. A total of 621 *S. titanus* individuals, sampled in 24 FDp-infected and uninfected vineyards, were genotyped using seven polymorphic microsatellite loci. The mean observed heterozygosity in *S. titanus* populations was between 0.364 and 0.548. There was evidence of only a low level of population genetic differentiation (mean F_{ST} = 0.027) suggesting that there is long-distance gene flow between *S. titanus* populations. This may be a consequence of the high migration capacity of the vector associated with large effective population size and, at least in part, of passive dispersion over long distances by the transport of grapevine-planting material carrying eggs. For each insect, FDp was detected and typed by nested-PCR followed by RFLP and sequencing of a 674 bp fragment of the FDp *map* gene. Twelve of the 24 populations were found to be infected by FDp, with the percentage of infected individuals varying from 3% to 29%. FDp isolates were classified into two FDp genetic clusters (FD1 and FD2), which differed by 12–13 SNPs. FD1 genotypes were detected in the insect populations at two sites and the FD2 genotypes in the other ten populations. Both FD1 and FD2 genotypes were found to be transmitted by the insect. No significant relationship was found between the genetic structure of these French *S. titanus* populations and the distribution of the various FDp strain types they carried. Nevertheless, overall genetic differentiation between FDp-infected and healthy *S. titanus* "subsamples" was found to be significantly higher than zero. These results suggest that FDp-infected *S. titanus* individuals are more philopatric (disperse less) than healthy *S. titanus*.

1. Introduction

The epidemiology of vector-borne diseases can be strongly influenced by the genetic diversity and population structure of their insect vectors (Manguin et al., 2008). However, our knowledge of the genetic structure of both the pathogens and their vectors remains limited even for systems of medical or agronomical importance (Criscione et al., 2005). For the Plasmodium-mosquito interactions (malaria), information on the scale of mosquito vector dispersal is highly relevant to improving understanding of the epidemiology of the disease and consequently would be valuable for devising effective means of control. Two recent comparative genetic studies of the malaria parasite (*Plasmodium falciparum*) and the vector population structure (*Anopheles spp.*) revealed very little genetic differentiation between populations in this system, a finding that may explain the speed at which antimalarial drug resistance and insecticide resistance spread (Annan et al., 2007; Prugnolle et al., 2008). For agricultural pathosystems, comparative genetic studies between the population structure of plant pathogens and their insect vector were also carried out. For example in Uganda, the cassava mosaic geminiviruses (CMGs), which are transmitted by the whitefly *Bemisia tabaci*, cause major losses to cassava (*Manihot esculenta*) production. Two distinct genotype clusters of *B. tabaci* have been identified and associated with the geographical distribution of cassava mosaic disease epidemics (Legg et al., 2002). In another study, *B. tabaci* genotypes were found to be correlated to the geographical distribution of the cotton leaf curl virus in Pakistan (Simon et al., 2003).

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Flavescence dorée (FD) is a severe grapevine yellows which has been declared a quarantine disease in Europe (Boudon-Padieu, 2002). It is caused by the FD phytoplasma (FDp), a non-cultivatable wall-less bacterium recently suggested to be a *Candidatus* species (Firrao et al., 2004). This bacterium multiplies within the phloem cells of the host plant and is highly pathogenic for several important grapevine cultivars, triggering quick death of the vine stock (Boudon-Padieu, 1996; Osler et al., 2002; Pavan et al., 1997). FDp is transmitted from vine to vine by the phloem-feeding Nearctic leafhopper *Scaphoideus titanus* Ball (Homoptera: Deltocephalinae) (Schvester et al., 1963). Note, however, that there is no evidence of vertical transmission of FDp by trans-ovarial infection (Alma et al., 1997). FDp transmission can also occur in nurseries as a result of grafting of FDp-infected material. Reported for the first time in the late 50s in the vineyards of south-west France (Bonfils and Schvester, 1960), *S. titanus* was probably accidentally introduced into Europe from North America by importing grapevine canes carrying eggs under the bark (Caudwell, 1983). After its introduction, *S. titanus* spread from south-western France towards Italy and the Balkans and also towards Spain and Portugal. *S. titanus* has an univoltine biological cycle and all developmental stages occur on *Vitis vinifera*, the cultivated grape vine species in Europe (Vidano, 1964). Eggs are laid during the summer by mated females into the bark of grapevine wood and the overwintering eggs hatch during the following spring. *S. titanus* specifically transmits FDp with persistence and propagation of the phytoplasma in the insect body. In spite of compulsory protection regulations (large-scale insecticide treatments, eradication of FDp-infected grapevine plants and protection of mother plants in nurseries), FD disease is still spreading epidemically in the south-east and the west of Europe. This spread depends on the occurrence and diffusion of the leafhopper in vine-growing areas (Alma, 2002; Bressan et al., 2006).

Information about the genetic diversity and structure of both vector and phytoplasma populations would undoubtedly contribute to a better understanding of the role of host-mediated dispersal in FD epidemics. A first evaluation of the genetic variability and structure of *S. titanus* populations worldwide, using random amplified polymorphic (RAPD) DNA markers, has been reported (Bertin et al., 2007): European *S. titanus* populations are less diverse and structured than American populations. The low genetic variability in Europe was interpreted as a consequence of the recent introduction of *S. titanus*, whereas the lack of genetic structure was considered to result from the transport of grapevine canes and grafts carrying eggs to vineyards across Europe. multi locus sequence typing (MLST) has been used to study FDp isolates collected from French and Italian vineyards so as to describe the genetic diversity of phytoplasma strains and to trace their propagation in vineyards (Arnaud et al., 2007). The study involved analysing the sequences of house-keeping genes (*map, secY* and *uvrB*) in the genome of this non-cultivatable bacterium. These genes were selected because they display variability at the subspecies level. The study showed the existence of two genetic clusters of phytoplasma strains in the French vine host; these clusters present differences in molecular variability, prevalence and distribution at national scale (Arnaud et al., 2007). Strains from the cluster FD1 displayed some genetic variability and were responsible for 17% of the cases of the disease; they were preferentially found in south-west France. Strains of the cluster FD2 that showed no genetic variability were responsible for 83% of cases and were evenly distributed across vineyards in France.

Focusing on diffusion by the way of the insect, there are several possible explanations of the difference in geographical distribution of the FDp strains in French vineyards: first, coevolutionary interaction occurring between FDp and its insect vector could lead to a phenomenon of dispersion of phytoplasma strains by genetically and geographically differentiated insect populations. However, the association between this Nearctic leafhopper and the FDp pathogen has been demonstrated to be of recent origin. FD is indeed considered to be an emergent plant disease in Europe resulting from a recent association between a widely cultivated plant (*V. vinifera*), a local native phytopathogen and a newly introduced insect vector (Angelini et al., 2004; Arnaud et al., 2007). Therefore, a coevolutionary process between FDp and *S. titanus* seems unlikely, so two alternative possibilities need to be considered. First, assuming that *S. titanus* presents a spatial population genetic structure (that may be the result of a low migration rate, independent introductions into France, or adaptation to *V. vinifera* varieties), then the geographical distribution of different FDp strains in the French vineyards would be explained by the co-dispersion of the FDp by the genetically differentiated vector populations. Second, phytoplasma strains present significant differences of transmissibility (in the plant or in the insect); the limited spatial diffusion of FD1 strains in south-west France would then result from a lower transmission rate than that of FD2 strains. Were this the case, we would expect there to be no correlation between the genetic structure of FDp and that of *S. titanus*.

In order to determine the contribution of the leafhopper vector in shaping FDp population structure, we (1) assessed genetic diversity and structure of *S. titanus* populations in southern France, (2) characterised the prevalence and the genetic diversity of FDp strains found in *S. titanus* populations, (3) evaluated the ability of the insect to transmit the different FDp strains, and (4) evaluated the level of genetic differentiation between healthy and infected *S. titanus* populations. Using a large *S. titanus* sample ($N = 746$), we determined the prevalence of FDp in 24 vine plots in southern France. The FDp strains were characterised by RFLP and sequence typing of the *map* gene as previously described (Arnaud et al., 2007). A subsample of *S. titanus* individuals ($N = 621$) was further characterised using seven recently isolated co-dominant microsatellite markers that have proven to be highly polymorphic (Papura et al., 2006; Papura et al., 2007).

2. Materials and methods

2.1. Insect sampling

The *S. titanus* sample ($N = 746$) was collected from 24 vine plots in the south of France between 2004 and 2006 (as L5 larval stage in July and as adults in August) (Fig. 1, Table 1). Insects were randomly sampled from each vine plot using a vacuum insect net collector (D-vac). Samples collected from the same vine plots during the same period were considered as a single 'population'. For each population we tried to collect insects with the same development stage (larva or adult) and on the same number of symptomatic and non symptomatic plants. Ten of the 24 populations were sampled on plots declared to be FDp-infected by the regional plant protection services (SRPV): Casseuil, St. Sulpice et Cameyrac, St. Aulaye, La Force, Aire sur Adour, Lasseube, Pardaillan, Peyrière, Seyches and Auzeville. Forty percent *titanus* individuals collected from these FDp-infected populations were kept alive on vine leaves in insect-proof cages for subsequent transmission experiments.

2.2. Plant sampling

Plant samples were collected from FDp-infected vine plots by taking leaves with petioles of symptomatic vines from which insects had been aspired. Samples were kept for a maximum of one week at 4 °C until DNA extraction for phytoplasma genotyping.