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Genetic structure of *Scaphoideus titanus* populations and genetic diversity of the epidemic strains of "flavescence dorée" phytoplasma: the situation in France

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Abstract

The genetic diversity and structure of the *Scaphoideus titanus* leafhopper populations and of the "flavescence dorée" phytoplasma (FDp) they carry, was studied in parallel in southern French vineyards. Seven polymorphic microsatellite loci were used to genotype *S. titanus* individuals sampled in 24 healthy or FDp-infected vineyards. For each insect, FDp detection and typing was performed by amplifying and sequencing the FDp *map* gene. A low level of population genetic differentiation ($F_{ST} \leq 0.114$) was found in *S. titanus* French populations. It suggests that long-distance gene flows occur due to migration between populations. In addition, it can be hypothesized that passive transport of grapevine planting material carrying eggs could play a role in homogenizing *S. titanus* population genetic structure. No significant correlation was found between the genetic structure of the French *S. titanus* populations and the distribution of the different strain types they carry.

Key words: grapevine disease, leafhopper, vector, dispersion, microsatellites, molecular typing, *Mollicutes*.

Introduction

Scaphoideus titanus Ball is a Nearctic leafhopper accidentally introduced into Europe before the 1950s (Vidano, 1966). It is the vector of FDp (Schvester *et al*., 1961), the causal agent of a grapevine quarantine disease in France and Europe (Boudon-Padieu, 2002). In spite of mandatory grapevine plants protection regulations, FD disease is still spreading in the South of France and has detected in northerly vineyards of Bourgogne, and Champagne. Knowledge of genetic diversity of both *S. titanus* populations and FD strains they can transmit will facilitate the understanding of the history of European vineyard colonization and dispersal ability of this insect in relation to the spread of the disease. A Multi Locus Sequence Typing study on FDp collected from different French vineyards has shown the existence of two strain clusters in the vine plants (Arnaud *et al.,* 2007): the cluster FD1 displaying some variability and representing 17% of the disease cases, with a specific location in the south west, and the non variable cluster FD2 representing 83% of the cases and distributed in every infected vineyard of France. Our objectives consist in (1) describing *S. titanus* genetic structure in southern French vineyards and (2) the FDp strains genetic diversity found in *S. titanus* and (3) evaluating the possible existence of a correlation between populations of the insects and the FDp strain type they carry.

Materials and methods

S. titanus was sampled in FDp-infected or healthy vineyards from 24 different sites covering the southern half of France (figure 1). Total DNA was extracted from each leafhopper (N=613) using the salting-out method (Sunnucks *et al*., 1996). Each individual was then genotyped using 7 microsatellite loci as described in Papura *et al.*, (2006). PCR products were sized on a Beckman Coulter Ceq8000 automated sequencer. FDp detection in each insect and FDp typing was performed by nested PCR followed by sequencing of the gene *map* as described (Arnaud *et al.,* 2007). Genepop version 3.7 (Raymond and Rousset, 1995) was used to analyse the population structure assessed by FST values and to test for departures from Hardy–Weinberg equilibrium and correlation between genetic distance and geographical distance. Neighbor-joining trees of populations were constructed using Cavalli-Sforza and Edwards chord distance. The trees were reconstructed using POPULATION version 1.2.28 (Langella, 2002).

Results

A high polymorphism was found by analysing the seven *S. titanus* microsatellite loci with a mean number of 13 alleles per locus for all populations and a mean number of 7 alleles per population for all loci. For all the 24 populations, observed heterozygosity (H_O) ranged from 0.364 to 0.548 and was lower than expected heterozygosity (H_E) across all loci. Test of heterozygote deficiency was significant for all populations and all loci ($P<0.001$). This was reflected in positive and significant multilocus F_{IS} values. A low level of population differentiation was found with the pair wise mean F_{ST} = 0.027. The highest pair wise F_{ST} = 0.114 was found between BOU and ASA samples (390 km apart). Nevertheless, genetic distances (F_{ST}) among pairs of populations were not significantly correlated with geographical distance between sample sites (Mantel test, P=0.057). All populations (11) collected from infected vineyards

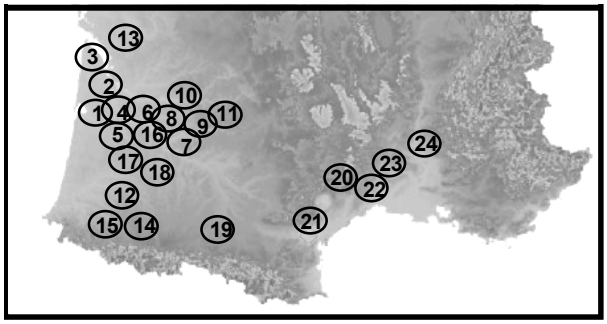

Figure 1. Location of the 24 *S. titanus* populations sampled in southern France.

were found FDp positive with a rate of infected individuals ranging from 3% to 41%. Two and nine populations were found infected by strains of the FD1 and FD2 cluster respectively. No significant correlation was found between the genetic structure of *S. titanus* populations and the distribution of FD1 and FD2 strain type they carry (figure 2). However, a low differentiation was found between healthy and FDp-infected *S. titanus* populations.

Discussion

S. titanus French populations were very weakly differentiated. It suggests that migration between populations is probably common in this species. In addition, it can be hypothesized that transportation of grapevine canes and grafts carrying eggs owing to commercial exchanges could play a role in homogenizing *S. titanus* population genetic structure. The consistent trend of heterozygote deficit observed through all *S. titanus*

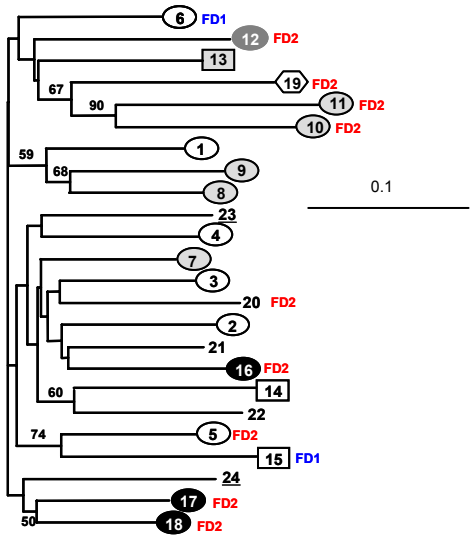

Figure 2. Neighbor-joining tree of the 24 *S. titanus* populations. Each symbol represents a different department of origin. FD1, FD2: populations found infected by FD1 or FD2 respectively.

French populations could be argued by a Wahlund effect. In this case, inbreeding could be the result of larval aggregation at the vine field scale which has already been described by Lessio and Alma (2006). The relative proportion of FD1 and FD2 types found in *S. titanus* is in agreement with the ones found in plants from French infected vineyards (Arnaud *et al.,* 2007). The low differentiation of *S. titanus* populations carrying FD clusters from non-carrying FD clusters needs to be confirmed by a temporal analysis of the *S. titanus* populations from the same sites.

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