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# Are there several biotypes of *Cacopsylla pruni*?

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## Abstract

The population structure of *Cacopsylla pruni*, the insect vector of '*Candidatus* Phytoplasma prunorum', was studied from a sampling in 12 localities in western and southern France and in northern Spain. All specimens were collected on *Prunus spinosa*. More than 300 individuals were genotyped at nine microsatellite loci. Two complementary approaches (Fst, Bayesian clustering) were used to analyse the data and gave congruent results. They show the existence of two strongly differentiated populations (provisionally called A and B). Both populations occurred sympatrically in most sites studied, but the first one was strongly dominant in the Massif Central, and the second one in western France. Despite both populations overlapped over a large geographic area, hybrids seem to be very rare or absent.

**Key words:** Psyllidae, '*Candidatus* Phytoplasma prunorum' microsatellites, phytoplasma, European stone fruit yellows, genetic structure.

## Introduction

The psyllid *Cacopsylla pruni* (Scopoli) is the vector of '*Candidatus* Phytoplasma prunorum' (Seemüller and Schneider, 2004) inducing ESFY disease which is widely spread in south-eastern France on apricot (*Prunus armeniaca*) and Japanese plum (*P. salicina*) (Carraro *et al*., 1998). Some issues about the biology of the vector remain unclear and prevent a complete understanding of the epidemiology of the disease. *C. pruni* is a univoltine species which reproduces on *Prunus* sp. (mainly blackthorn: *P. spinosa)* in plains, and overwinters on conifers at an altitude of 700-1300 m before returning to the plains (Thébaud, 2005). Thus psyllids would be capable of migrations over a range of at least several tens kilometres.

Recent studies on the transmission processes suggest that the infected overwintering reimmigrants are the most efficient vectors of the phytoplasma (Thébaud, 2005). But questions remain about the distances and trajectory of the migratory flights, and consequently about the phytoplasma dispersion. To clarify these questions, microsatellites in combination with assignments tests and a Bayesian-clustering approach were used to analyse the genetic structure of *C. pruni* across a range of samples of western and southern France and northern Spain.

## Materials and methods

Nine localities covering south-eastern France were selected for this study (figure 1): [2] Prades, [3] Torreilles, [4] Haut-Languedoc, [5] Prades-le-Lez, [6] Larzac, [7] La Tieule, [8] Vesseaux, [9] Coursegoules, [10] Romette. Three other localities out of this area were analysed: [1] Tordera, [11] Bellac and [12] Montgamé. 30 females per locality were genotyped except for [10] and [11] where only 7 and 5 females respectively have been sampled. All psyllids were sampled in March and April from 2002 till 2007.

DNA was extracted and then amplified using the protocol described in Sauvion *et al*., (unpublished). Each individual was screened using 9 microsatellite loci. After amplification, fragment sizes were determined on a MegaBace 1000 automated sequencer GE Healthcare. Screening for polymorphism and allele scoring was performed using the Genetic Profiler software version 1.5.

Genetic diversity within each locality was quantified by the number of alleles per locus, the observed and expected heterozygoses using FSTAT 2.9.3 (Goudet, 2001). For each locus and each locality, deviation from Hardy-Weinberg equilibrium, genetic linkage disequilibrium and differences in alleles frequencies were analysed using GENEPOP 3.4 (Raymond and Rousset, 1995). In addition, pairwise estimates of *Fst* were computed following Weir and Cockerham (1984) to quantify levels of differentiation between localities. Finally, we used the model-based clustering method (STRUCTURE) described by Pritchard *et al*. (2000) to infer population structure and assign individuals to populations by using multilocus genotype data.

## Results

The number of alleles per locus was high for each locus (10 to 40). Nei's genetic diversity index varied between 0.443 and 0.957. Observed heterozygosis varied from 0.283 to 0.798 (mean = 0.509); these values were always slightly too extremely lower than the expected ones. The data showed significant deficits in heterozygotes for most of the localities and loci. Four loci out of 9 were in disequilibrium in all sampling sites, and only one was at equilibrium in most of them. No linkage disequilibrium has been observed in the majority (31 *vs.* 36) of pair of loci.

*Fst* values and results of the Bayesian analysis were in agreement. Populations from localities [2], [5], [6], [7] and [8] were fairly structured ($Fst < 0.01$), and markedly different ($Fst > 0.05$) from the localities [1], [4]

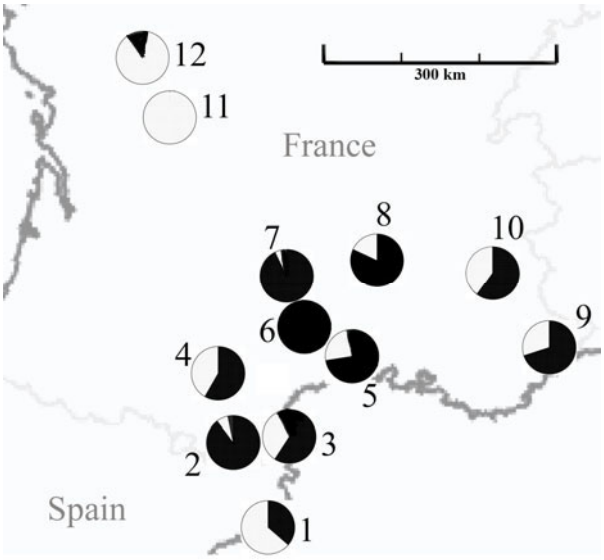


**Figure 1.** Distribution of sample locations used in the study. The graph represents the ratio of Group A (black) and B (white) at each locality.

and [12]. These last ones were rather related among each other (*Fst* < 0.03), particularly [1] and [4]. The populations [3] and [9] were almost identical (*Fst* = 0.003) and seemed to be intermediate; they were markedly different (Fst > 0.05) from the locality [12], but not from the other localities (Fst < 0.03). The highest difference (*Fst* = 0.158) was observed between populations from localities [6] and [12].

The optimal number of populations estimated by STRUCTURE was two (figure 1). Most of the individuals (290 *vs.* 301) were assigned with a very high probability (p > 0.95) to one of both populations. The assignation was partially correlated to their geographic origin. All individuals from locality [6], and most of the individuals from the localities [2], [7] and [8], were assigned to Group A (figure 1). On the contrary, the individuals from the localities [11] and [12] were almost all assigned to the Group B. In the other localities individuals belonging to both groups co-occurred. The multilocus *Fst* between both populations was 0.1737, which indicates a high level of intraspecific differentiation.

## Discussion

Our results demonstrate the existence of two strongly differentiated groups of populations in the area of our study, occurring sympatrically in most sites studied, the first one being dominant in the south-eastern France. Despite a wide range overlap between these two populations, hybrids seem very rare or absent.

We cannot exclude that two different species co-exist and that *C. pruni* as presently defined on the base of morphological characters is a species complex. However, one could also hypothesize the presence of different biotypes, and that individuals of one biotype could fecundate individuals of the other but hybrids are rare because of biological, behavioural, or ecological incompatibility.

Interbreeding of individuals of both populations should be done to verify this hypothesis. Possible morphological differences should also be investigated.

Whatever the taxonomic status of both populations, it will be important to know more about biological traits of both groups of *C. pruni* and to investigate their relative efficiency as vectors of '*Ca*. P. prunorum'. This information could be essential for a better understanding of the epidemiology of ESFY and to study the population structure of the pathogen in the light of the genetic structure observed within *C. pruni*.

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