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Research Note

Confirmation of random mating and indication for gene flow in the grapevine dieback fungus, *Eutypa lata*

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Summary: RAPD markers were used to analyze the genetic structure in an *Eutypa lata* population from a single vineyard located in Charente, France. The high level of genotypic diversity and the lack of genetic disequilibrium between RAPD loci strongly suggest that the population originated from random mating. Its genetic structure was similar to that previously observed within a population from a vineyard located in Hérault, France. No genetic differentiation was found between the two populations separated by 390 km. Ascospore-mediated gene flow would unify *E. lata* populations into a panmictic population at least at the geographic scale studied.

Key words: Eutypa lata, population genetics.

Introduction: The means by which fungi reproduce have marked effects on the genetic structure of their populations (MILGROOM 1996). In the ascomycete fungus *Eutypa lata* (Pers:Fr.) Tul & C. Tul. (anamorph *Libertella blepharis* A.L. Smith) sexual reproduction is present (CARTER 1991). The population from a single vineyard situated at Villeneuve-les-Maguelone, Hérault, France ("Villeneuve population") was characterized by a large pathogenic diversity, a maximum level of genotypic diversity and a random association between RAPD loci (PEROS *et al.* 1997). These findings could be explained by random mating and an exclusive role of ascospores in disease propagation. However, because population structure can vary within the same species (MILGROOM 1996) it is necessary to analyze several populations sampled in different geographical areas before a conclusion can be drawn on the population genetics of *Eutypa lata*.

Another important factor influencing the genetic structure of populations is the occurrence of a gene flow (LEUNG *et al.* 1993). When there is no restriction of gene flow, i.e. a sufficient number of individuals migrating between populations, a low level of genetic differentiation may be expected. The measure of genetic differentiation is based on the comparison of allelic frequencies at independent loci. Progeny analysis in *E. lata* showed that RAPD markers generally had a Mendelian segregation 1:1 as expected in a haploid random mating fungus (PEROS and BERGER, unpubl.).

Here we report the genetic structure of a population from a vineyard situated at Angeac-Champagne, Charente, France ("Angeac population"). In addition, we tested the differentiation between the "Angeac population" and the "Villeneuve population" previously analyzed (PEROS *et al.* 1997), the single vineyards from which the two populations originated were separated by 390 km.

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Material and methods: Forty-five isolates were obtained in July 1996 each from a different vine in a vineyard located at Angeac-Champagne, Charente, France. This vineyard, 60 x 30 m in size, was planted in 1964 with the *Vitis vinifera* cultivar Ugni blanc grafted to 41 B. In this vineyard the sexual stage of *E. lata* was easily observed on several vines. Isolation from wood was made according to LARIGNON and DUBOS (1997).

DNA extraction and amplification were performed as described by PEROS *et al.* (1996, 1997). All isolates were tested using six 10-mer primers: A10, A15, B02, C20, D18, E03 (Bioprobe, Montreuil, France). Analysis was repeated twice from extraction to electrophoresis of amplification products. Calculation of Jaccard's distance and gene diversity was performed as already described (PEROS *et al.* 1997). The significance of gametic disequilibrium among pairs of RAPD loci was tested with Fisher's exact test at $P<0.05$ using the GENEPOP software (RAYMOND and ROUSSET 1995). The Bonferroni sequential test procedure was used to overcome the problem of an excess of significant tests due to multiple comparisons (RICE 1989). To study the genetic differentiation between the "Angeac population" and the "Villeneuve population", the significance of an unbiased estimator of Fst (WEIR and COCKERHAM 1984) was tested at $P<0.05$ for each locus and across all loci for a total of 14 RAPD loci using the GENEPOP software.

Results and Discussion: Using 6 primers, 18 RAPD markers (Table) were accurately scored to study the genetic relatedness between the isolates of the "Angeac population". Among the 45 isolates there were 44 different pat-

Table

Frequency of positive allele and Nei's measure of gene diversity at RAPD loci within a population of *Eutypa lata* from a single vineyard in Charente

RAPD locus[a]	Frequency of positive allele	Gene diversity
A10-1240	0.386	0.474
A15-1060	0.432	0.491
A15-910	0.477	0.499
A15-490	0.023	0.044
A15-360	0.250	0.375
B02-1660	0.705	0.416
B02-860	0.159	0.268
B02-830	0.750	0.375
C20-2220	0.818	0.298
C20-1410	0.841	0.268
C20-850	0.591	0.483
C20-800	0.205	0.325
D18-1340	0.091	0.165
D18-720	0.114	0.201
D18-330	0.136	0.336
E03-1120	0.091	0.165
E03-570	0.341	0.449
E03-420	0.636	0.463

[a] Code includes name of the primer followed by the length of the amplicon in base pairs.

terns. The observed distribution of marker differences clearly illustrated the random assortment of the RAPDs markers (Figure). As outlined by MILGROOM *et al.* (1992), such distribution of differences is not consistent with diversity generated by mutations within clonal lineages but indicates random mating.

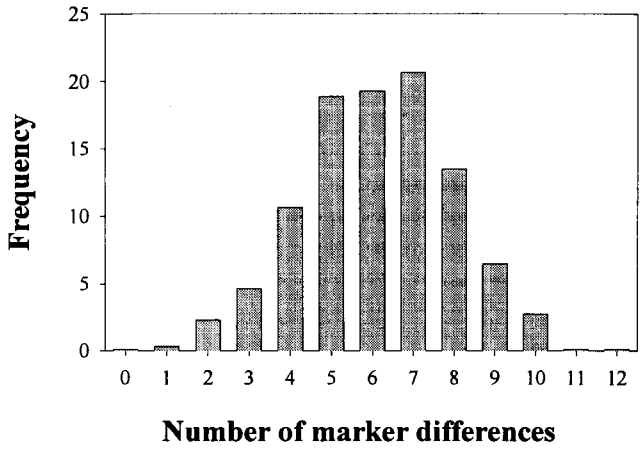

Figure: Distribution of the number of marker differences in 153 pairwise comparisons of RAPD patterns obtained in a *Eutypa lata* population (n=45) from a single vineyard in Charente. There is a total of 18 RAPD markers.

Measures of gametic disequilibrium within the "Angeac population" provided further evidence for random mating. Out of 153 tests for gametic disequilibrium between pairs of the 18 RAPD markers, there were only 6 (3.9 %) that were significant at P=0.05 and none value was significant after the application of the Bonferroni procedure. Only one pair of markers was in gametic disequilibrium for the "Villeneuve population" but these markers were found to be alleles at the same locus after Southern analysis (PEROS *et al.* 1997).

The Jaccard's distance varied from 0.00 to 0.909 with an average value of 0.601 for 18 RAPD markers within the "Angeac population". Similar values were obtained for the "Villeneuve population" using 20 RAPD markers (PEROS *et al.* 1997). The mean gene diversity for the 18 RAPD markers was 0.333 in the "Angeac population" (Table). Based on the 14 RAPD markers scored in both studies, the gene diversity averaged similar values: 0.337 for the "Angeac population" and 0.348 for the "Villeneuve population".

The genetic structure of *E. lata* populations appeared to be very similar in the two areas with large climatic and viticultural differences. The more humid climate of Angeac-Champagne favours the formation of perithecial stromata, however, in both populations infection was probably initiated by diverse outside sources of ascospores. Ugni blanc is nearly the sole variety cultivated in Charente whereas many cultivars are grown in Hérault. The host variability would have no effect on the genetic structure of *E. lata* populations.

The frequency of RAPD markers in the "Angeac population" (Table) was found to be similar to that previously observed in the "Villeneuve population" (PEROS *et al.* 1997). This resulted in very low and nonsignificant Fst values ranging from -0.009 to +0.019 among the 14 putative RAPD loci scored in both studies. The very low overall estimate across all markers (+0.001, P=0.659) indicated no genetic differentiation between the two populations separated by 390 km. This suggests the existence of a gene flow between the two populations. The mechanism for this gene flow would be the aerial transport of ascospores which could propagate the disease on rather long distances (RAMOS *et al.* 1975). In addition, there are no large discontinuities between grapevine areas in France and *E. lata* may reproduce on numerous other perennials (CARTER 1991). Ascospore-mediated gene flow would unify *E. lata* populations into a panmictic population at least at this geographic scale.

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CARTER, M. V.; 1991: The status of *Eutypa lata* as a pathogen. C.A.B. Int., Int. Mycol. Inst., Phytopathol. Pap. No. 31.

LARIGNON, P.; DUBOS, B.; 1997: Fungi associated with esca disease in grapevine. Europ. J. Plant Pathol. **103**, 147-157.

LEUNG, H.; NELSON, R. J.; LEACH, J. E.; 1993: Population structure of plant pathogenic fungi and bacteria. Adv. Plant Pathol. **10**, 157-205.

MILGROOM, M. G.; 1996: Recombination and the multilocus structure of fungal populations. Ann. Rev. Phytopathol. **34**, 457-477.

- -; LIPARI, S. E.; POWELL, W. A.; 1992: DNA fingerprinting and analysis of population structure in the chestnut blight fungus, *Cryphonectria parasitica*. Genetics **131**, 297-306.

PÉROS, J.-P.; BERGER, G.; LAHOGUE, F.; 1997: Variation in pathogenicity and genetic structure in the *Eutypa lata* population of a single vineyard. Phytopathology **87**, 799-806.

- -; THIS, P.; CONFURON, Y.; CHACON, H.; 1996: Comparison by isozyme and RAPD analysis of some isolates of the grapevine dieback fungus *Eutypa lata*. Amer. J. Enol. Viticult. **47**, 49-56.

RAMOS, D. E.; MOLLER, W. J.; ENGLISH, H.; 1975: Production and dispersal of ascospores of *Eutypa armeniacae* in California. Phytopathology **65**, 1364-1371.

RAYMOND, M.; ROUSSET, F.; 1995: GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. J. Hered. **86**, 248-249.

RICE, W. R.; 1989: Analyzing tables of statistical tests. Evolution **43**, 223-225.

WEIR, B. S.; COCKERHAM, C. C.; 1984: Estimating F-statistics for the analysis of population structure. Evolution **38**, 1359-1370.