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Genetic differentiation among host-associated *Alebra* leafhoppers (Hemiptera: Cicadellidae)

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The limited importance ascribed to sympatric speciation processes via host race formation is partially due to the few cases of host races that have been reported among host populations. This work sheds light on the taxonomy of *Alebra* leafhoppers and examines the possible existence of host races among host-associated populations. The species of this genus show varying degrees of host association with deciduous trees and shrubs and, frequently, host populations of uncertain taxonomic status coexist and occasionally become pests. Allozyme electrophoresis of 21 Greek populations including sympatric, local and geographically distant samples collected on 13 different plant species, show that they represent at least five species: *A. albostriella* Fallén, *A. viridis* (Rey) (*sensu* Gillham), *A. wahlbergi* Boheman and two new species. Of these, one is associated to *Quercus frainetto* and other is specific to *Crataegus* spp. Significant genetic differences among sympatric and local host populations were found only in *A. albostriella*, between populations on Turkey oak, beech and common alder. It is suggested that the last two of these host populations may represent different host races. The results show that both the host plant and geographical distance affect the patterns of differentiation in the genus. The formation of some species seems to have been the result of allopatric speciation events while, for others, their origin can be equally explained either by sympatric or allopatric speciation.
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Introduction

Sympatric speciation is a controversial subject in evolutionary biology (see Mayr, 1963; Futuyma and Mayer, 1980; Paterson, 1981; Via, 2001). One of the reasons for this controversy is that sympatric speciation seems to be an extremely rare phenomenon occurring only in very few groups of taxa, represented chiefly by phytophagous insects (Tauber and Tauber, 1977; Menken, 1981; Wood, 1993; Emelianov *et al*, 1995; Via, 1999; Finchak *et al*, 2000; Craig *et al*, 2001). The limited number of reported cases among organisms with sexual reproduction can be at least partially attributed to the fact that taxa undergoing sympatric speciation events must fulfill very restrictive biological and ecological requirements.

Most sympatric speciation models demand that there is intraspecific genetic variation in traits that differentially affect the fitness of individuals that colonise new habitats or hosts (Dieckman and Doebeli, 1999; Hawthorne and Via, 2001 but see Higashi *et al*, 1999 and Takimoto *et al*, 2000). They assume that selection acting on these traits can prevent genetic exchange between populations (Bush, 1975; Tauber and Tauber, 1977; Diehl and Bush, 1989). In phytophagous insects, this means that host preferences must be genetically determined and mating should occur on the host (Bush, 1975; Diehl and Bush, 1989; Hawthorn and Via, 2001). However, these conditions are very unlikely to be met in most taxa, and are also difficult to prove (Diehl and Bush, 1984). In fact, *Rhagoletis pomonella* is, so far, the only well-documented case of host race formation (Feder *et al*, 1988; Filchak *et al*, 2000).

Wood (1993) proposed a new and less restrictive mechanism of gene flow disruption, for treehoppers of the *Enchenopa binotata* complex. In these treehoppers, which show the unusual biological traits of univoltine life cycles, brief mating periods on the host, and host plant specificity (Wood, 1993), genetic divergence seems to have occurred in sympatry through the seasonal isolation of host populations mediated by differences in host plant phenology (Wood *et al*, 1990; Wood and Keese, 1990; Wood, 1993). Allochronic differences in life history and continuous selection on genetic traits that favour the use of the novel host may allow genetic divergence among coexisting host populations. This conclusion, based on empirical evidence, is also supported by a mathematical model (Butlin, 1990).

However, although the previous hypothesis is feasible under controlled conditions (Wood, 1993), it has been proposed for true biological species of treehoppers, and, for these, we do not know whether their divergence has in fact occurred initially in sympatry. Therefore, careful studies of recently established host races, preferably of closely related taxonomic groups showing a similar biology, are crucial to test this new hypothesis experimentally. *Alebra* leafhoppers, which show host-associated populations of uncertain taxonomic status

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(Drosopoulos and Loukas, 1988; Gillham, 1992), and have similar lifestyles and host specificity, seem a suitable group in which to look for host races. In the present study, the analyses of enzyme polymorphisms in 21 host populations shows that some of these populations have already reached specific status, while others may be interpreted as host races at intermediate stages of speciation.

Natural history of *Alebra* leafhoppers

The genus *Alebra* is widely distributed in the Nearctic and Palaearctic regions (Nast, 1972). In Europe it is represented by six species, which are sympatric over part, or all, of their ranges. Morphological characters in these leafhoppers show little or no diagnostic differences between species. Identification is based only on slight differences in the shape of the male abdominal apodemes, on host association, and on differences in the colour pattern (Gilham, 1991). Intraspecific variation in these characters makes species identification very difficult, and thus the taxonomic status of host-associated populations is often controversial, especially for pest populations (Drosopoulos and Loukas, 1988; Gillham, 1992). In *Alebra* all the developmental stages and oviposition are intimately related to the host and most species have univoltine life cycles but some may complete up to two generations per year (Lauterer, 1986; Drosopoulos *et al*, 1987; Gilham, 1992; Demichelis and Bosco, 1995). They feed on the contents of the leaf mesophyll cells of deciduous trees and shrubs, causing visible damage to leaves, and often becoming pests (Lauterer, 1986; Drosopoulos *et al*, 1987). Some species coexist frequently and even feed together on the leaves of the same tree but the degree of host specificity varies among them. Of the six European species, two are monophagous, one is oligophagous and three are polyphagous on plants of two or more families.

Materials and methods

Twenty-one populations were collected from 12 localities in Greece on 13 host plant species. The locations of the sampling sites are indicated on the map in Figure 1. The populations consisted of male and female adults collected by sweeping the foliage of the different host plants.

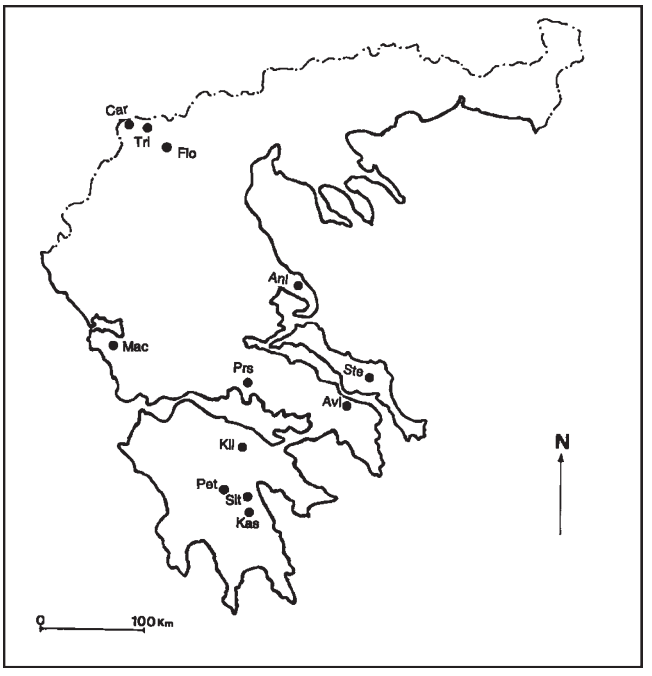

Figure 1 Sample sites of *Alebra* from Greece. Locality abbreviations as in Table 1.

Table 1 Sampling sites of *Alebra* populations from Greece

Populations	*Species codes*	*Collection codes*	*Site codes*	*Host plants*
A. wahlbergi	WAHL	Kastanitsa	KAS	*Castanea sativa*
		Agia Triada	TRI	*Acer opalus*
		Agios Petros	PET	*Castanea sativa*
		Caries	CAR	*Acer campestre*
Alebra on *Q. frainetto*	FRA	Agios Petros	PET	*Quercus frainetto*
A. albostriella	ALB	Kastanitsa	KAS	*Castanea sativa*
		Anilio	ANL	*Castanea sativa*
		Agia Triada	TRI	*Quercus cerris*
		Caries	CAR	*Alnus glutinosa*
		Steni	STE	*Castanea sativa*
		Agia Triada	TRI	*Fagus sylvatica*
A. viridis	VIR	Kastanitsa	KAS	*Castanea sativa*
		Avlona	AVL	*Quercus aegilops*
		Agia Triada	TRI	*Quercus cerris*
		Florina	FLO	*Castanea sativa*
		Machairas	MAC	*Quercus pubescens*
Alebra on *Crataegus*	CRA	Killini Mt	KIL	*Crataegus pycnoloba*
		Agios Petros	PET	*Crataegus monogyna*
		Sitaina	SIT	*Crataegus heldreichii*
		Caries	CAR	*Crataegus orientalis*
		Parnassos Mt	PRS	*Crataegus orientalis*

Material for electrophoresis was stored at –25°C after carefully sorting out parasitised specimens. For each population the specimens were identified on the basis of apodeme shape, colour pattern and host plant association (Gillham, 1992). According to these traits, samples from *Quercus frainetto* and *Crataegus* differ from any known species of *Alebra*, therefore, they were named after their host plant (Table 1). Arbitrarily, three categories of populations were considered: sympatric, local and geographically distant populations. Sympatric populations are those coexisting on the same host, or on different hosts whose branches often intermesh with each other; local populations are those separated by less than 15 km; finally, to the last category belong those populations separated by more than 15 km. According to this the following were analysed: (1) sympatric populations from the same or different host species, (2) local populations from the same or different host species, (3) populations from distant localities from the same or different host species.

Horizontal starch gel electrophoresis was performed for PGM (phosphoglucomutase, EC 2.7.5.1), 6-PGD (6-phosphogluconate dehydrogenase, EC 1.1.1.44), MPI (mannose phosphate isomerase, 5.3.1.8), PHI (phosphohexose isomerase, 5.3.1.9) and TO (tetrazolium oxidase, 1.15.1.1). The standard electrophoretic procedures employed and the assays are given in Loukas and Krimbas (1980) and Loukas and Vergini (1986). A number that represented the distance moved relative to that of the most common band, which was characterised as 1.00, was defined for each allozyme band obtained. Of all loci studied, *6-PGD* was the only one that did not show sufficient enzyme activity in samples of *A. wahlbergi* from Caries and Agia Triada. Although they had a common allele with the same mobility to that of *A. wahlbergi* from Kastanitsa, it was not possible to screen all the alleles.

Average heterozygosity per locus ($\overline{H}$), percentage of polymorphic loci (0.99 criterion) and mean number of alleles per locus (Ne) was calculated for each population. χ^2-tests of heterogeneity of allele frequencies for each locus among all populations were also performed. Allele classes were pooled when necessary to minimise the number of cells with expected numbers less than five but in some cases, when pooling was not possible, if the expected frequency was less than 5, Fisher's exact test was performed. The significance levels for multiple comparisons were corrected following the Bonferroni procedure. The significance level, $\alpha = 0.01$, is obtained after dividing the significance level of 0.05 by the number of loci. Significance levels are shown as follows: $0.001 < P < 0.01$ (*), $0.0001 < P < 0.001$ (**) and $0.0001 > P$ (***). Nei's (1978) unbiased genetic distance (*D*) was used with the UPGMA clustering algorithm to create a phenetic tree (Figure 2) and, in this analysis, the samples of *A. wahlbergi* from Caries and Agia Triada were considered to be monomorphic for *6-PGD*. Except for the heterogeneity tests, all analysis were performed with BIOSYS-1 (Swofford and Selander, 1989).

Results

Interspecific genetic differentiation

To identify the species of *Alebra*, allele frequencies at each locus were compared among all possible pairs of populations. Significant differences obtained were due to three causes: (a) quantitative differences in allele frequencies, (b) quantitative and qualitative differences, because one or more alleles with considerable frequencies in one population were absent from another, and (c) qualitative differences, when there are no alleles in common. Of these, causes b and c are the most diagnostic for the recognition of species because they indicate very low or no gene flow. As a result, in the 21 populations analysed, at least five species were recognised by significant differences at one or more loci.

Thus, the population on *Quercus frainetto* is identified as a different species because it has no alleles in common with other populations for at least one locus (cause c) except for the population of *A. viridis* from Avlona. But even in this case, both populations differ significantly at the loci *MPI* ($\chi^2_{(2)} = 25.76$ ***), *TO* ($\chi^2_{(2)} = 194.08$ ***), *PHI* ($\chi^2_{(1)} = 182.51$ ***) and *6-PGD* ($\chi^2_{(1)} = 31.97$***) due to cause b. The *Crataegus* populations are recognised by differences mainly at the loci *TO*, *MPI*, *6-PGD* and *PGM*. Finally, of the remaining 15 populations, four were identified as *A. wahlbergi*, five as *A. viridis*, and six as *A. albostriella*. These species differ from each other in two loci at least. The loci *PHI* and *TO* separate *A. albostriella* from *A. wahlbergi* while *A. wahlbergi* is separated from *A. viridis* by differences at the loci *TO* and *MPI*. Among *A. albostriella* and *A. viridis*, the two closest species, seven of the 30 pairwise comparisons have no common alleles at the loci *PHI* and *MPI*, the remaining differ in two or more loci due to cause b. The phenetic tree elaborated using Nei's *D* clearly clusters the populations associated with *Crataegus* in a different lineage, while in the other lineage are the remaining species of the genus, of which *A. viridis* and *A. albostriella* are the closest (Figure 3).

Estimates of genetic variability indicate that these species differ also in their genetic structure (Table 2).

The species with the greatest genetic variability were *A. albostriella*, *A. viridis* and *Alebra* from *Q. frainetto*. Unlike these, all populations on *Crataegus* show a remarkably low genetic variability. The small sample size of these populations does not seem to be responsible for these differences since other species with similar sample sizes showed greater *D* values (compare population on *Crataegus* from Killini *vs A. viridis* from *Agia Triada* and those on *Crataegus* from Parnassos *vs A. wahlbergi* from Agios Petros).

Intraspecific patterns of genetic variation

Within species, significant genetic differences exist between populations associated with different host plant species. Such differences were observed not only among geographically distant populations, but also among local and even among sympatric populations from different hosts. First, among sympatric populations, significant differences were recorded for *A. albostriella* (Table 3). Populations on *Fagus sylvatica* and *Quercus cerris* from Agia Triada differ because the allele $MPI^{0.77}$, with a frequency of 0.619 in the *F. sylvatica* population was absent from that on *Q. cerris* ($\chi^2_{(1)} = 40.49$ ***). Other significant differences in allele frequencies were registered at the locus *PGM* ($\chi^2_{(2)} = 32.34$ ***) (Figure 2). These populations differ also in the number of polymorphic loci and the number of alleles per locus (Table 2).

Second, among local populations from different hosts, genetic differences were recorded only for *A. albostriella* and *A. wahlbergi* but not for *Alebra* on *Crataegus*. In *A.*

Table 2 Loci, allele frequencies, sample sizes (*N*), unbiased heterozygosities ($\overline{H}$), mean number of alleles per locus (*Ne*), percentage of polymorphic loci ($P_{0.99}$) and the standard errors (s.e.) in the populations studied

Locus	*WAHL kas*	*WAHL tri*	*WAHL pet*	*WAHL car*	*FRA pet*	*ALB kas*	*ALB anl*	*ALB tri*	*ALB car*	*ALB ste*	*ALB tri*	*VIR kas*	*VIR avl*	*VIR tri*	*VIR flo*	*VIR mac*	*CRA kil*	*CRA pet*	*CRA sit*	*CRA car*	*CRA prs*
PGM																					
(*N*)	11	26	8	14	57	43	15	28	18	53	50	24	53	46	22	8	48	27	36	12	15
0.61	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.72	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.022	0.000	0.000	0.167	0.000	0.083	0.000	0.000
0.75	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.110	0.000	0.019	0.000	0.000	0.000	0.083	0.074	0.000	0.000	0.000
0.80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.062	0.000	0.000	0.000	0.000	0.000
0.085	0.000	0.039	0.000	0.000	0.237	0.139	0.100	0.161	0.000	0.161	0.040	0.104	0.613	0.630	0.273	0.000	0.000	0.000	0.000	0.000	0.000
0.89	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.729	0.907	0.820	1.000	1.000
1.00	1.000	0.942	1.000	0.893	0.711	0.849	0.867	0.589	0.806	0.821	0.810	0.792	0.359	0.304	0.727	0.938	0.021	0.019	0.097	0.000	0.000
1.04	0.000	0.000	0.000	0.107	0.017	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.13	0.000	0.019	0.000	0.000	0.017	0.012	0.033	0.232	0.194	0.009	0.010	0.104	0.009	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.33	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PHI																					
(*N*)	11	26	8	13	57	46	15	28	18	39	51	16	44	46	51	55	51	26	39	12	10
0.62	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.73	0.091	0.000	0.500	0.000	0.000	0.630	0.933	0.911	1.000	0.974	0.941	0.344	0.000	0.000	0.010	0.000	0.010	0.000	0.000	0.000	0.000
1.00	0.909	1.000	0.500	0.885	0.000	0.370	0.067	0.089	0.000	0.000	0.029	0.656	0.943	1.000	0.980	0.955	0.990	0.981	1.000	1.000	0.950
1.15	0.000	0.000	0.000	0.115	1.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.011	0.000	0.010	0.045	0.000	0.019	0.000	0.000	0.050
1.31	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TO																					
(*N*)	19	31	13	18	48	42	15	24	18	41	44	24	51	42	49	9	45	25	36	16	4
1.00	0.026	0.000	0.000	0.000	0.000	0.524	1.000	1.000	1.000	1.000	1.000	1.000	0.990	1.000	1.000	1.000	1.000	1.000	0.944	0.000	0.000
2.90	0.974	1.000	1.000	1.000	0.448	0.476	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.056	1.000	1.000
3.75	0.000	0.000	0.000	0.000	0.552	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MPI																					
(*N*)	11	27	8	12	36	37	15	27	9	28	42	31	42	45	45	56	46	27	39	12	5
0.67	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.77	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.946	0.619	0.000	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.84	0.000	0.037	0.000	0.000	0.000	0.189	0.003	0.000	0.500	0.018	0.000	0.000	0.036	0.000	0.000	0.000	1.000	1.000	1.000	0.000	1.000
1.00	1.000	0.963	1.000	1.000	0.722	0.811	0.991	0.852	0.333	0.018	0.345	0.355	0.345	0.000	0.045	0.071	0.000	0.000	0.000	1.000	0.000
1.08	0.000	0.000	0.000	0.000	0.278	0.000	0.003	0.074	0.167	0.000	0.036	0.081	0.524	0.722	0.722	0.643	0.000	0.000	0.000	0.000	0.000
1.21	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.000	0.000	0.000	0.564	0.000	0.278	0.233	0.286	0.000	0.000	0.000	0.000	0.000
6-PGD																					
(*N*)	11	0	5	0	42	22	15	24	18	15	18	15	26	42	33	49	42	30	43	15	10
0.86	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.97	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.983	1.000	1.000	1.000
1.00	0.955	0.000	0.700	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.972	1.000	1.000	1.000	1.000	0.990	0.000	0.017	0.000	0.000	0.000
1.29	0.045	0.000	0.300	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000
Ne ± s.e.	1.6 ± 0.24	–	1.4 ± 0.24	–	2.4 ± 0.93	2.0 ± 0.32	2.2 ± 0.58	2.2 ± 0.58	1.6 ± 0.40	2.4 ± 0.68	3.0 ± 0.84	2.0 ± 0.45	2.8 ± 0.58	2.0 ± 0.77	2.0 ± 0.45	2.0 ± 0.32	1.8 ± 0.58	1.8 ± 0.37	1.6 ± 0.40	1.0 ± 0.00	1.2 ± 0.20
$P_{0.99}$	60.0	–	40.0	–	60.0	80.0	60.0	60.0	40.0	60.0	80.0	80.0	80.0	40.0	60.0	80.0	40.0	60.0	40.0	0.00	20.0
N ± s.e.	0.063 ± 0.032	–	0.200 ± 0.123	–	0.270 ± 0.111	0.310 ± 0.090	0.113 ± 0.050	203 ± 0.108	0.194 ± 0.129	0.092 ± 0.056	0.201 ± 0.094	0.277 ± 0.117	247 ± 0.127	0.184 ± 0.114	0.175 ± 0.099	0.147 ± 0.092	0.092 ± 0.087	0.049 ± 0.032	0.085 ± 0.061	0.000 ± 0.000	0.020 ± 0.020

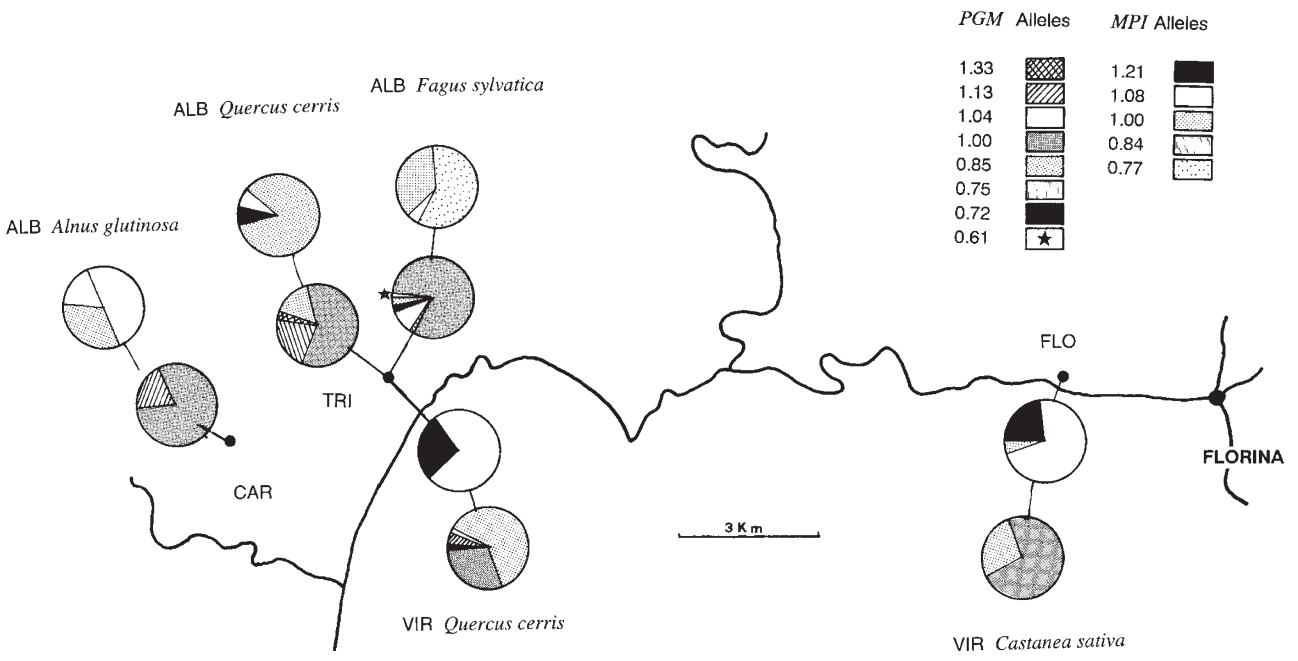

Figure 2 Allelic variation at *PGM* and *MPI* loci in populations of *Alebra albostriella and A. viridis* at the localities Agia Triada, Caries and Florina. The pie charts above and below represent the loci *MPI* and *PGM* respectively. Populations and sampling locality details are as in Table 1.

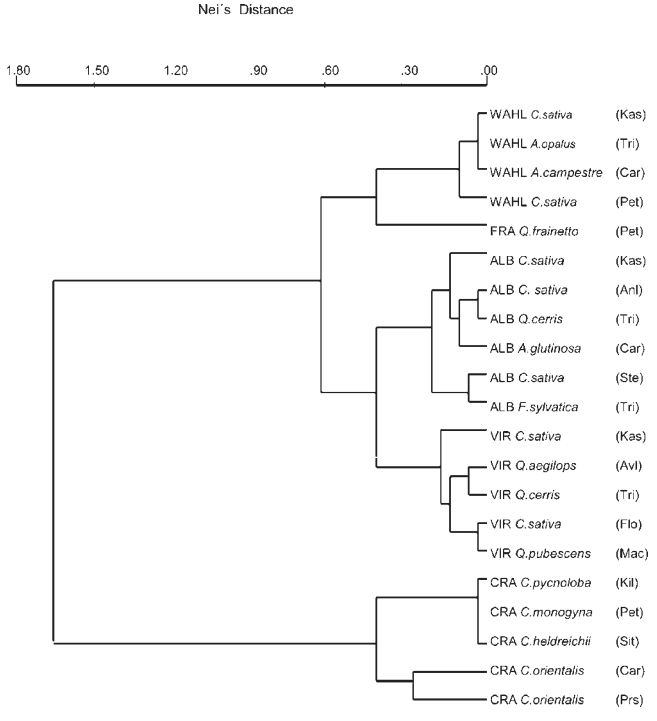

Figure 3 Cluster analysis performed with the unweighted pair group method based on Nei's (1978) coefficient of unbiased genetic distance.

albostriella, populations on *Alnus glutinosa* show significant differences from those on *Fagus sylvatica* and *Quercus cerris* at the loci *MPI* and *PGM*. At the locus *MPI*, the differences between them were qualitative ($\chi^2_{(1)}$ = 20.32 *** and $\chi^2_{(1)}$ = 18.09 *** respectively) while, at the locus *PGM*, they were due to differences in allele frequencies but only among *A. glutinosa* and *F. sylvatica* ($\chi^2_{(2)}$ = 17.53 **). These comparisons suggest that populations on *A. glutinosa* and on *Q. cerris* separated by about 15 km are more similar to each other than are the populations on *F. sylvatica* and on *Q. cerris* occurring sympatrically. In contrast to *A. albostriella*, smaller genetic differences were found among local samples of *A. wahlbergi*, the only species in which local samples were examined. Thus, for this last species, local samples from Agios Petros and Kastanitsa differ in allele frequencies at the locus *PHI* (*P* = 0.006).

Finally, among geographically distant populations, great genetic differences denote the existence of geographic patterns of genetic variation in four of the five species studied. Thus, within the same species, distant populations differ more than close populations collected on the same host species (within *A. albostriella* compare samples on chestnuts from Kastanitsa with those from Anilio and from Steni). On the other hand, the degree of geographic isolation seems also important in explaining the genetic differences. In *A. viridis* and *A. albostriella*, populations of Kastanitsa from south of Greece differ greatly in gene frequencies from the rest of the populations, possibly as the result of geographic isolation. A similar explanation may be applied to the allele differences observed among samples on *Crataegus* collected in the south, centre and north of Greece.

Discussion

Species identification

The main problems in the identification of species of the genus *Alebra* are the lack of good diagnostic characters, the fact most species are associated with more than one host, and that different species often share and even coexist on the same plant. As a result of this, we are con-

Table 3 χ^2 heterogeneity tests between populations of *Alebra albostriella* for all loci studied

Species and site codes	*MPI*	*TO*	*6-PGD*	*PHI*	*PGM*
Sympatric samples					
TRIa–TRIb	1***	NS	NS	NS	***
Local samples on different hosts					
CAR–TRIb	1***	NS	NS	NS	**
TRIa–CAR	1***	NS	NS	NS	NS
Distant samples (125 km) on the same host					
ANL–STE	1***	NS	NS	NS	NS
Distant samples (330–350 km) on different hosts					
STE–TRIb	***	NS	NS	NS	**
ANL–TRIb	1***	NS	NS	NS	NS
TRIa–STE	1***	NS	NS	NS	*
CAR–STE	1***	NS	NS	NS	**
TRI–ANL	NS	NS	NS	NS	NS
ANL-CAR	1**	NS	NS	NS	NS
Geographically isolated samples (175–300km) on the same host					
KAS–STE	1***	1***	NS	1***	NS
KAS–ANL	NS	1*	NS	NS	NS
Geographically isolated samples (400km) on different hosts					
KAS–TRIa	1**	1***	NS	**	***
KAS–CAR	**	1***	NS	1***	NS
KAS–TRIb	1***	1***	NS	***	*

Samples of Agia Triada from *Q. cerris* and *F. sylvatica* are referred to as TRIa and TRIb respectively. 1 = indicates allelomorphs with considerable frequency in one population absent in another. The statistically significant cases are presented as: $0.001 < P < 0.01$ (*), $0.0001 < P < 0.001$ (**) and $0.0001 \geq P$ (***).

Table 4 Host plant species of European *Alebra* obtained by the rearing of nymphs on the host plant species

Species	*Plant family*	*Host plant*	*No. of plants*	*Literature*
A. wahlbergi	Betulaceae	*Betula*	2	Claridge *et al*, 1981
		Alnus	1	Claridge *et al*, 1981
	Corylaceae	*Carpinus*	1	Claridge *et al*, 1981
	Aceraceae	*Acer*	2	Claridge *et al*, 1981
	Ulmaceae	*Ulmus*	2	Claridge *et al*, 1981
	Fagaceae	*Castanea*	1	Claridge *et al*, 1981
		Quercus	2	Vidano *et al*, 1987a
	Rosaceae	*Prunus*	1	Claridge *et al*, 1981
		Sorbus	1	Claridge *et al*, 1981
	Tiliaceae	*Tilia*	1	Claridge *et al*, 1981
	Hippocastaneaceae	*Aesculus*	1	Claridge *et al*, 1981
A. coryli	Corylaceae	*Corylus*	1	Claridge *et al*, 1981
A. albostriella	Betulaceae	*Alnus*	1	Vidano *et al*, 1981, 1987b
	Fagaceae	*Quercus*	4	Vidano *et al*, 1987a; Gillham, 1991
		Castanea	1	Vidano *et al*, 1987a
Alebra on *Q. frainetto*	Fagaceae	*Quercus*	1	unpublished data
A. viridis	Fagaceae	*Quercus*	2	Gillham, 1991, 1992
		Castanea	1	Gillham, 1992
A sorbi	Rosaceae	*Sorbus*	1	Dworakowska, 1993 (*)
A. neglecta	Corylaceae	*Carpinus*	1	Arzone *et al*, 1987
Alebra on *Crataegus*	Rosaceae	*Crataegus*	4	unpublished data

The asterisk (*) refers to the presence of adults only.

fronted with host-associated populations of uncertain specific status. This study shows that the 21 populations analysed represent at least five species. Three of these species are morphologically similar to *A. wahlbergi*, *A. viridis* and *A. albostriella* and the other two, morphologically different from the previous ones, are new species, one of which is associated with *Q. frainetto* and the other with species of *Crataegus*. The large differences in allele frequencies seen in this last species could support the hypothesis that this is a species complex of two or more

species associated with *Crataegus*. However, the lack of morphological differences along with the geographic isolation among populations, possibly caused by the patchy distribution of host plants (Christensen, 1994), suggest that there is only one species.

Host-associated variation

Another important question is how to explain the genetic differences between populations of *A. albostriella* coexisting on *Q. cerris* and on *F. sylvatica* in Agia Triada. The restriction of rare alleles to each of these populations and other host-associated differences (see below (v)) indicate low gene flow. Even though this study was based on a small number of loci, the facts given below suggest that the *F. sylvatica* population may be a host race rather than a sibling species. (i) Populations are in true sympatry; branches of these trees are often in contact. (ii) In Greece these populations are sympatric in at least part of their range. Populations on *Fagus* coexist with others on oaks and chestnuts in three other distant localities (personal observation). Other samples collected on these hosts in Italy (Vidano and Arzone, 1987b) suggest that they may extend over a greater geographical area. (iii) The genetic distance among them ($D = 0.099$) is five times smaller than among sympatric samples of *A. albostriella* and *A. viridis* from the same locality ($D = 0.497$) but is similar to D in host races of other phytophagous species of insects (Zwölfer and Romstöck-Völkl, 1991). (iv) The genetic differences among them were smaller than between samples from different locations but from the same plant, such as those from Steni and Anilio, which were both on chestnuts (note that comparisons with samples more distant that these cannot be taken into account because this species shows geographic patters of allelic variation). (v) These populations are morphologically indistinguishable in the only two structures which show species-specific differences (Gillham, 1991): aedeagus shape and male abdominal apodemes (unpublished data). However, females from *Fagus* from this and other two localities were always consistently smaller, differing also in colouration. (vi) Karyotypes did not show any variation in the number or the shape of chromosomes, which could prevent the formation of viable zygotes (personal observation).

Other data on biology and ecology of *A. albostriella* also suggest that differences in host plant preference and phenology may limit gene flow among host populations of this species. In the first place, this species has a short and mainly univoltine life cycle (Drosopoulos *et al*, 1987; Demichelis and Bosco, 1995). Besides, since mating and oviposition occurs on the host (Gillham, 1992; Demichelis and Bosco, 1995), the shorter lifespan of males may restrict drastically the mating period. In addition, the eggs in arboreal leafhoppers are inserted in the bark of host stems and embedded in water prior to development (Claridge and Reynolds, 1972). Therefore, changes in the water contents of host plants may cause different life history timings of the nymphs and adults. Finally, the differences in sex ratios found among populations on *Fagus* and on *Q. cerris* (personal observation) indicate that differences in phenology are possible. All these facts suggest that disruption of gene flow, through allochronic mating isolation, may occur among specimens from *F. sylvatica* and *Q. cerris*, as in their close relatives of the *Enchenopa binotata* complex (Wood *et al*, 1990; Wood and Keese, 1990).

Additional support comes from the fact that the distribution of host plants (Polunin, 1989) and at least some *Alebra* species (Nast, 1972) are not allopatric, although secondary contact cannot be excluded. The high values of genetic variability in these samples ($D = 0.203$ and $D = 0.201$) do not support either that colonisation has occurred as a result of founder events. On the other hand, the hosts *F. sylvatica* and *Q. cerris*, are not only native to the Balkans (Polunin, 1989) but also were present here during the last glacial period, when this region was a refuge for most species of oaks (Dumolin-Lapegue *et al*, 1997) and beech (Comps *et al*, 2001). The coexistence of these two hosts in this reduced area over a sufficiently long period of time could have made the occurrence of a host shift possible. Therefore, if host races exist, the Balkans will be one of the most likely regions to find them.

Evolutionary biology

The association of a phytophagous species with a particular plant reduces inter- and intraspecific contacts. Therefore, colonisation of a new host, especially of a new genus or family, can be the first step towards the origin of a new species (Futuyma, 1991). The outcome of this study, and host plant records of European *Alebra*, suggest that they are the result of shifts within and/or between plants of four main families (Table 4). Although the current data set is not sufficient to address whether species of *Alebra* have originated by allopatric or sympatric speciation through host shifts, the biology, and the degree of host association suggest both modes of speciation may be equally plausible. It is very likely that the population on *F. sylvatica* may represent one host race differentiating in Fagaceae and another in the *Betulaceae*, on *Alnus glutinosa*. However, to consider these to be true host races, it is necessary to prove that reproductive isolation is incomplete (Diehl and Bush, 1984). The lack of allozyme differences among populations on different hosts is believed to demonstrate the limited importance of sympatric speciation via host race formation (Mitter and Futuyma, 1983). Therefore, this study represents potential evidence for the role of host shifts in genetic differentiation and ultimately in speciation, but more studies remain to be done to understand how these shifts may have occurred.

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