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POPULATION STRUCTURE OF A HERBIVOROUS INSECT AND ITS HOST PLANT ON A MICROGEOGRAPHIC SCALE

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Parasites typically live in a patchy environment. This environment is often heterogeneous, in the sense that parasites may encounter hosts with different degrees of suitability for the parasite's development and reproduction. Therefore, knowledge of the gene flow of both the parasite and the host would be valuable to infer the heterogeneity of the environment of the parasite (i.e., the heterogeneity of the host populations) and the potential for parasites to become locally adapted.

The study of the genetic structure in host-parasite interactions has been used, so far, to attest the existence of host races, and therefore, only the genetic structure of the parasite is well studied. One well-known example is the existence of sympatric host races in the apple maggot fly *Rhagoletis pomonella*, which has been demonstrated by allozyme electrophoresis (Feder et al. 1988; McPheron et al. 1988).

The only study, to our knowledge, in which the genetic structure of populations of both the

host and the parasite has been investigated, concerns flukes of the species *Fascioloides magna* and their definitive host, the white-tailed deer *Odocoileus virginianus* (Mulvey et al. 1991). Mulvey et al. (1991) have shown that (1) gene flow was of the same order in both hosts and parasites, (2) both host and parasite populations presented weak though significant spatial subdivision, and (3) there was no congruence between genetic distances of parasite populations to that of deer populations, and the genetic distances were not correlated with geographic distance.

Population subdivision is the outcome of a variety of factors, including selection on dispersal rate, selective forces in a heterogeneous environment, and genetic drift. Assessing the degree of interconnection between the populations of phytophagous insects and populations of their host plants may provide us with evidence of local adaptations and the potential of the herbivores to coevolve with their hosts. Despite the importance of these processes in the understanding of the evolutionary relationships between plants and insects, we are not aware of a single study that compares the population structure of a phytophagous insect and its host plant.

In this paper we investigate the population structure of an insect herbivore, the weevil *Lari-*

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TABLE 1. Nei's genetic distances between pairs of *Larinus cynarae* populations.

Population	V6	V3	V5	V4	V2	V1	V8	CM
V3	.002							
V5	.001	.001						
V4	.004	.002	.002					
V2	.002	.002	.003	.007				
V1	.010	.009	.012	.015	.006			
V8	.001	.000	.000	.003	.001	.008		
CM	.004	.002	.002	.000	.007	.012	.002	
CS	.006	.003	.003	.000	.010	.016	.004	.000

nus cynarae (Col.: Curculionidae), and its host, the thistle *Onopordum illyricum* (Asteraceae: Cardueae). This weevil has a geographic range restricted to the Mediterranean area, from Portugal to Greece. It is a seed predator on three genera of Cynaroidea thistles, but in the region where our study was conducted, that is, south-central France, *L. cynarae* shows a clear preference for *O. illyricum*. We sampled populations of both the weevils and the thistles at different geographic scales, ranging from 50 m to 100 km. If the weevils are able to track their environment, the thistles, in an adaptive way, then the weevil population subdivision should follow the subdivision of thistle populations. We tested the hypothesis that the weevil's genetic structure matches that of the thistles.

MATERIALS AND METHODS

The weevil *Larinus cynarae* is a univoltine, sexually reproducing, phytophagous insect. Adults mate during late spring and early summer. Females lay their eggs between the bracts of thistle flower heads during the same period. Each female lays a limited number of eggs (about 30), which are distributed over different flower heads. Larvae develop within the flower heads and feed on the developing seeds. The development from egg to adult lasts about 6 wk. After completing development, the adults overwinter and appear again the following spring.

The thistle *Onopordum illyricum*, is a sexually reproducing monocarpic perennial plant, producing a relatively large number of flower heads and many seeds (Young and Evans 1969). The flowers are insect pollinated and the seeds are wind dispersed. Germination of seedlings takes place largely in autumn, and reproduction is size dependent. This thistle is considered a major weed in Australia, where a biological control program is underway against it (Briese 1989).

The collecting design was hierarchical within

two regions of southern France, Viols-en-Laval and Saint Martin-de-Crau. We studied seven populations of *O. illyricum* around Viols-en-Laval, north of Montpellier. These populations were separated by 3 to 5 km from the nearest *O. illyricum* populations of comparable size. We collected adult weevils during the reproductive season, together with tissues samples of one of the first two true leaves of seedlings from all of these sites for allozyme analysis. The second region, located at Saint Martin-de-Crau (about 100 km east of the other sites) was also sampled. In this region we collected weevils from two different populations (CM and CS), about 150 m apart, whereas thistles were sampled in only one population (CS).

Electrophoretic Techniques

Weevils.—Three highly polymorphic loci were analyzed by horizontal starch gel electrophoresis, a fourth one presenting only a single heterozygote. These loci were (abbreviations and buffer systems given in parentheses): phosphoglucose isomerase (*PGI*, EC 5.3.1.9, LiOH 8.3), glutamate oxaloacetate transaminase (*GOT*, EC 2.6.1.1, LiOH 8.3), isocitrate dehydrogenase (*IDH*, EC 1.1.1.42, TC 8.7/TC 8.0), and malic enzyme (*ME*, EC 1.1.1.40, TC 6.3). The techniques used are described in Pasteur et al. (1987), except for the buffer system for *IDH*, which is described in Herbst (1986).

Thistles.—Only two polymorphic loci, leucine aminopeptidase (*Lap*; EC 3.4.11.1) and glutathione reductase (*Gr*; EC 1.6.4.2), giving repeatable results have been identified. Both loci were scored on 8% vertical polyacrylamide gels (Laemmli 1970). Staining techniques are described in Pasteur et al. (1987).

Data Analysis

The data were first analyzed by the program BIOSYS-1 (Swofford and Selander 1981), to ob-

TABLE 2. Hierarchical F -statistics per locus and over all loci for *Larinus cynarae* (CI, 95% confidence interval). Loci names are followed by mean sample size per population.

Locus	θ_1	θ_2
<i>PGI</i> (31.55)	0.0156	0.0244
<i>GOT</i> (31.44)	0.0066	-0.0055
<i>IDH</i> (31.44)	0.0450	0.0381
<i>ME</i> (31.75)	-0.0056	-0.0023
All loci	0.0153	0.0133
CI	0.0065 0.0437	-0.0055 0.0371

tain allelic frequencies, test panmixia by Fisher's exact test, and to provide Nei's (Nei 1972) genetic distances. To study population subdivision, we estimated Wright's (Wright 1978) fixation indices. Because the collecting design is hierarchical, we estimated correlations for pairs of alleles between individuals within populations (θ_1), and correlations for pairs of alleles between populations within regions (θ_2) following Weir and Cockerham (1984). Thus, θ_2 is a measure of differentiation between regions, whereas θ_1 is a measure of differentiation of populations within regions. These statistics were calculated for each locus, and were then combined to give estimates over all loci. To obtain 95% confidence intervals (CI) on estimates over all loci, we performed 1000 bootstrap samples over loci.

RESULTS

Weevils.—Of 26 χ^2 tests performed for the hypothesis of random mating in weevil populations, only 1 was significant at the 5% level (locus *GOT* in population V6). This is the proportion expected by chance. Nei's distances between pairs of populations were extremely low, most of them (with only four exceptions) lying below 0.01 (table 1). Genetic distances between pairs of populations were not correlated with geographic dis-

TABLE 3. Nei's genetic distances between pairs of *Onopordum illyricum* populations.

Popu- lation	V1	V6	V2	V3	V4	V5	V8
V6	.021						
V2	.065	.022					
V3	.005	.035	.070				
V4	.006	.045	.098	.004			
V5	.008	.037	.080	.010	.006		
V8	.099	.169	.191	.081	.073	.054	
CM	.156	.269	.324	.134	.112	.099	.015

TABLE 4. Hierarchical F -statistics per locus and over all loci for *Onopordum illyricum* (CI, 95% confidence interval). Loci names are followed by mean sample size per population.

Locus	θ_1	θ_2
<i>LAP</i> (36.75)	0.2279	0.1815
<i>GR</i> (40.63)	0.1517	0.0888
All loci	0.1887	0.1339
CI	0.1517 0.2279	0.0888 0.1815

tance ($r = -0.02$, $P > 0.05$). Hierarchical F -statistics analysis showed that, at all levels, weevil populations were not subdivided (table 2). Differentiation between regions (θ_2) was not significantly different from 0, whereas differentiation between populations within regions (θ_1) was extremely low.

Thistles.—Half (8/16) of the tests for departure from random mating were significant. The F_{IS} calculated across all loci and populations was equal to 0.32 (CI: 0.29 to 0.34). This large deficit of heterozygotes can be attributed to partial selfing.

The populations of thistles were much more subdivided than the populations of weevils. Nei's genetic distances (table 3) lie above 0.01, with only four exceptions. The populations from Viols were well differentiated from the Crau population, with the exception of site V8, which seemed to be more related to the Crau population. Genetic distances between pairs of populations were positively correlated with geographic distance ($r = 0.56$, $P < 0.01$). This correlation with geographic distance was no longer significant when we considered only the populations from Viols ($r = 0.22$, $P > 0.05$). Hierarchical F -statistics analysis showed that thistle populations were significantly differentiated at all levels (table 4). The between populations within regions component (θ_1), however, reflects differences only between Viols populations because only one thistle population was sampled at Saint Martin-de-Crau.

Genetic distances between insect populations were not correlated with the genetic distances between the corresponding thistle populations whatever the geographical scale, either when all populations were considered (fig. 1, top), or when only Viols populations (fig. 1, bottom) were considered ($P > 0.05$ in both cases).

DISCUSSION

The weevil populations studied were not subdivided over a large area (south-central France),

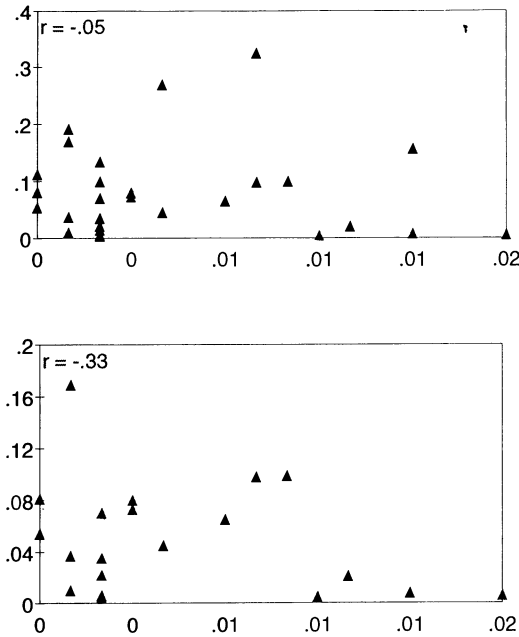


FIG. 1. Correlation of Nei's genetic distance between pairs of insect populations (ordinate axis) and that of the corresponding thistle populations (abscissa axis), when all populations from Viols are considered (top) and when only populations from Viols are considered (bottom). Regression coefficients are $r = -0.05$ and $r = -0.33$, respectively, both NS ($P > 0.05$).

since differentiation measures between groups of populations 100 km apart were not significantly different from zero. In contrast, the thistle populations were much more subdivided, considerable differentiation occurring between populations 100 km apart, but also between populations separated by only 100 m. Genetic distance between insect populations was not correlated with geographical distance, in contrast to thistle populations. The genetic distance between insect populations was also not correlated with the genetic distance of the corresponding thistle populations.

This discrepancy between the population structures of the herbivores and their hosts can be explained if we take into account the ecological context in which these organisms live. The habitat of weevils, that is, a thistle population, is ephemeral. Thistle populations are frequently disturbed, by both frequent fires in this region and human activities. When a thistle population is destroyed, adult weevils have to move to adjacent patches. Local "extinction" of thistles

therefore, does not result in the death of adult weevils, but rather in increased migration between weevil populations. Such large scale migration events, combined with occasional migration between sites, homogenize the weevil populations (Sheppard et al. 1992). Thus weevils are constrained to develop efficient dispersal mechanisms to survive. Adult weevils were seen to fly into adjacent patches, several individuals covering more than 500 m.

Following disturbance, thistle populations will either become extinct or show temporary disappearance followed by recruitment from a seed bank. Recolonization following extinction is likely to be from adjacent patches. Whitlock and McCauley (1990), in a theoretical investigation of the effects of local extinctions on the genetic differentiation among populations, showed that local extinctions will increase genetic differentiation if the number of effective colonizers is small relative to the number of effective migrants between extant populations, and if the probability of common origin of the colonizers is large. Furthermore, these authors showed that inbreeding within populations may even increase the genetic differentiation between populations. All three processes are likely to occur in the formation of new thistle populations. Effective migration can happen through either the seeds or the pollen. Colonization, however, can be successful only through the seeds, so that probably the number of effective migrants is larger than the number of effective colonizers. The patches of suitable habitat are surrounded by unsuitable evergreen oak forest (garrigue) so that any seeds that immigrate are likely to do so from only one direction, and therefore probably from a single adjacent population. The large F_{is} values indicate that inbreeding is significant in this plant, which would also tend to increase the differentiation among populations. Following a disturbance, recruitment from the seed bank, that is, the existing gene pool, will maintain the genetical identity of the patch, and therefore the genetic differentiation among patches.

Throughout this paper we have been concerned only with the population structure of neutral markers. The interactions between hosts and their parasites, however, are dependent on the evolution of loci such as those coding for resistance of the hosts to the parasites and those determining host choice by the parasites and performance on different hosts. The population

structure of neutral markers does not necessarily correspond to that of selected loci. The correlation between estimates of population differentiation for neutral markers and that for selected loci will depend on the migration and selection coefficients as well as on the recombination rate between the markers and selected loci. We currently lack theoretical predictions of this correlation for a host-parasite model.

The recombination rate between markers and selected loci is probably very important for the evolution of this correlation. Empirical studies found associations between resistance to pathogens and allozymic loci in predominantly selfing plants (resistance of *Synchytrium decipiens* in *Amphicarpea bracteata*, Parker 1988; resistance to *Erysiphe graminis hordei* in *Hordeum spontaneum*, Nevo et al. 1984; resistance to *Puccinia striiformis* in *Triticum dicoccoides*, Nevo et al. 1986). Burdon and Roelfs (1985) showed associations between virulence genes and allozymes in asexual populations of *Puccinia graminis*, a pathogen of wheat, while such associations were absent in sexual populations of the same pathogen.

The correlation between markers and selected loci in outcrossing organisms will depend on the magnitude of the migration and selection coefficients. In our system, the migration rate between weevil populations is so large that selection for performance on different hosts or host-choice loci would have to be unrealistically large to overcome the effects of migration. We lack, however, general predictions on this correlation for less extreme situations, as well as empirical studies that would compare the population structure of hosts and parasites in different ecological contexts (e.g., a case where the host-plant populations are less patchy and ephemeral than those of *O. illyricum*).

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REPRODUCTIVE RELATIONSHIPS AMONG TEN SPECIES OF THE *DROSOPHILA REPLETA* GROUP FROM SOUTH AMERICA AND THE WEST INDIES

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In a comprehensive review of the genus *Drosophila*, Bock (1984) listed 266 cases of interspecific hybridization in the laboratory and also 8 reported cases of hybridization in nature. These data show that interspecific hybridization in this genus is not at all a rare phenomenon, at least under the artificial conditions of the laboratory. But this review also shows clearly that the production of viable hybrids is restricted to the most closely related species. When large species groups have been subdivided into subgroups using classical taxonomic criteria, crosses have in most cases been accomplished only within subgroups, and in some cases only within complexes within subgroups. This makes sense because the ability to produce hybrids must require that the two species have similar genetic constitutions. In this study, however, we will show that frequent successful interspecific crosses occur among several species of the *Drosophila repleta* group that are distantly related according to their current phylogenetic classification.

The phylogenetic relationships among the species of the *D. repleta* species group have been traced back by a combination of morphological and cytological analyses (Wasserman 1982 and many earlier references). Among the six main subgroups, the most complex evolutionary situation is that of the *mulleri* subgroup, which comprises 40 species grouped into five species complexes plus four miscellaneous forms. The *stalker* complex comprises two species inhabiting Florida and the West Indies: *D. stalker* and *D. richardsoni* (Wasserman 1982; Vilela 1983). The *mulleri* complex, the largest of the *mulleri* subgroup, has been further subdivided into six clusters of closely related species. Two of the six clusters are endemic to South America (except for the colonizing species *D. buzzatii*, which is subcosmopolitan). The *martensis* cluster consists of four species found in the deserts of northern Colombia and Venezuela: *D. martensis*, *D. uniseta*, *D. starmeri*, and *D. venezolana* (Wasserman 1982; Wasserman et al. 1983). Another four species have been grouped into the *buzzatii* cluster: The Brazilian *D. serido* and *D. borborema* (Sene et al. 1982), and *D. buzzatii* and *D. koepferae*, which are found chiefly in Argentina and

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