

Genetic structure of leaf-beetles populations: microgeographic and sexual differentiation in *Oreina cacaliae* and *O. speciosissima*

M. Rowell-Rahier

Zoologisches Institut der Universität, Rheinsprung 9, 4051 Basel, Switzerland

Key words: Population genetics, microgeographic differentiation, leaf-beetles, Chrysomelinae, Coleoptera

Abstract

We used F -statistics to quantify the population structure of two sympatric species of leaf beetles, *Oreina cacaliae* and *O. speciosissima* (Chrysomelinae, Coleoptera), which share the same microhabitat since they feed on the same herbaceous host plants. We measured genetic differentiation at six allozyme loci 1) among populations separated by relatively small distances (40 to 250 kilometers), 2) within each population, and 3) between sexes within populations. For both species, the populations studied are not panmictic. For each population, heterozygosities are relatively high, but the observed heterozygosities are generally lower than the expected values. Overall, within-population differentiation is high and similar for both species ($F_{is} = 0.326$ for *O. cacaliae* and 0.332 for *O. speciosissima*). Additionally, populations of both species are highly differentiated ($F_{st} = 0.234$ versus 0.051 for *O. speciosissima*). For *O. cacaliae*, F_{is} and F_{st} are greater among females than among males, while for *O. speciosissima*, F_{st} is substantially greater among the males while F_{is} is slightly greater among males. Differences in gene frequency among the sexes were statistically tested using a modified F_{st} with sex as the defining category, and the sexes differed significantly with the exception of one population in *O. cacaliae*. Possible explanations for this difference are discussed.

Introduction

Many animal species live in discrete populations. When movement between populations is restricted, this can result in genetic differentiation among them. Such populations can become genetically differentiated at small spatial scales, either through heterogeneity in selective pressures or through random genetic drift (Wright, 1978). Herbivorous insects commonly live in such isolated populations. Many specialize on patchily distributed host plants, which are separated by

unsuitable habitats. Recent studies indicate that insect herbivores with limited vagility are genetically subdivided among host patches (McCauley & Eanes, 1987; McCauley *et al.*, 1988; Rank, in press). Additionally, genetic divergence has been shown between host races of *Rhagoletis pomonella* (McPheron *et al.*, 1988; Feder *et al.*, 1990a, 1990b). In some cases, genetic differentiation within local populations may lead to genetic divergence in host plant use (Guttman *et al.*, 1981; Feder & Bush, 1989).

Insects are also sometimes genetically subdivi-

vided within populations. Crouau-Roy (1988) documented pervasive deficiencies of heterozygotes within populations of cave-dwelling beetles, which may reflect inbreeding or a Wahlund effect resulting from pooling genetically heterogeneous subpopulations. Yet this within-population subdivision has rarely been reported among insect herbivores, and in one case, the differentiation appeared to reflect sampling a few family groups (Guttman *et al.*, 1989).

Leaf beetles (Chrysomelidae) are food plant specialists which spend most or all of their lives on the same food plants. *Oreina* species specialize on herbaceous perennial plants in the Apiaceae or Asteraceae, both containing numerous toxins, e.g. coumarins, sesquiterpene lactones, alkaloids and others (Hegnauer, 1964 & 1989). Locally isolated populations are found at high densities on host plant patches from middle to high elevations (600–2500 m). Like other leaf beetles, they are notoriously patchy because of the irregular distribution of their host plants and also because of their low dispersion rate. The adults are aposematic, form conspicuous aggregations and, in some species, also show colour polymorphisms. *Oreina* species are chemically defended like many other chrysomelids (Deroe & Pasteels, 1982; Pasteels *et al.*, 1989), however some *Oreina* species produce their defensive secretions autogenously while others derive them from chemicals in the host plant (Pasteels *et al.*, 1988).

In comparison with other well-studied leaf beetles which feed on willows (Rank, in press; McCauley *et al.*, 1988), *Oreina* species are longer lived and exploit 'smaller' host plants, i.e. herbaceous plants rather than trees. Local populations are found in restricted patches from 500 to 1000 m, within which the host plants as well as the beetles are abundant and regularly distributed. Field data on natural populations of a typical *Oreina* species, *O. gloriosa*, showed that local populations reach high densities (5–13 beetles per m²), and that the beetles have low dispersal rates (on average, 0.5 m per day), show high survival over a season (73–96% per week), and can survive for several years in nature (individuals marked in July 1988 were recaptured in June

1990). The species we studied in this paper also disperse little and can be similarly long lived. Field studies of *Oreina cacaliae* showed that the adults moved only a few meters over 6 weeks, and of 26 beetles marked in May 1988, five individuals were recaptured in August 1989, and all of them were recaptured less than 5 meters from the place where they were marked.

There is considerable variation in life history within *Oreina*. The two species reported on in this paper, *Oreina cacaliae* and *O. speciosissima* have similar life histories. Adults are observed copulating during most of the season. The fertilized females are ovoviviparous and give birth to first instar larvae which develop to the fourth instar within 3 to 4 weeks. New adults emerge from the pupa at the end of the summer, and feed for a short time before overwintering.

Because a species' population structure results among other things from movement patterns of individuals between subpopulations, sex-based differences in dispersal should be detectable with standard measurements of genetic differentiation. Indeed, Prout (1981) developed a model showing that local departures from Hardy-Weinberg equilibrium can be linked to differential patterns of dispersal between the sexes.

Studies of population structure have generally taken two very different approaches: mark-recapture studies which document actual movement patterns of individuals among subpopulations, and electrophoretic studies which document the overall effect of gene flow on the genetic composition of a population (Slatkin, 1987). The first approach frequently takes the sex of the individuals into account, and the sexes indeed commonly differ in their tendency for dispersal (Shields, 1982). However, few electrophoretic studies have even analyzed the sexes separately, much less attempted to determine how they affect a population's genetic structure (but see Schwartz & Armitage, 1980; Foltz & Hoogland, 1983).

We studied the population structure of *O. cacaliae* and *O. speciosissima*. These species share the same microhabitat since they are oligophagous on the same host plants *Petasites*, *Senecio*

and *Adenostyles* spp. (Asteraceae). They are frequently but not invariably found sympatrically. They are morphologically very similar and probably closely related, since they show low levels of allozyme divergence compared to other *Oreina* species (in prep). We measured genetic differentiation at six allozyme loci among several populations of *O. cacaliae* and *O. speciosissima* separated by from 40 to 250 kilometers. We use *F* statistics to quantify and compare the population structure of these two species. Additionally, we introduce a new technique, based on *F* statistics, to determine whether the sexes differ genetically within populations, which can occur when the sexes have different tendencies to emigrate from their natal patches to new ones (Prout, 1981).

Methods

Collecting sites. *Oreina cacaliae* adults were collected at 4 different sites: the French Vosges (VO, Lieserwassen), the southern French Alps near Glacier Blanc (BV, Les Bons, Vallouise), the Swiss Bernese Alps (BE, Kandersteg) and the Swiss Appenzel (AP, Brülisau). The VO, BE and AP sites are approximately equidistant whereas the BV site is more isolated geographically (see Fig. 1). Additionally the BV site is situated at the edge of a glacier and is thus more isolated from other populations. Both field observations and laboratory-choice tests reveal differences among these *O. cacaliae* populations in host plant use. *Oreina speciosissima* adults were collected at 3 different sites: the Swiss Vauds (VD, La Lecherette), and at the same BE and AP localities as *O. cacaliae* (Fig. 1). The *O. speciosissima* sites are part of a transect through the Alps, the first 2 sites (VD and BE) being separated by 45 km and the second and third sites (BE and AP) by 160 km.

Each sample results from collection along a transect (circa 500 m) through a patch where the host plants are common and evenly distributed. The insect populations are limited to the patch of host plants so that each sample reflects the leaf beetle population found on one host plant patch in the field. To our knowledge the next colonised

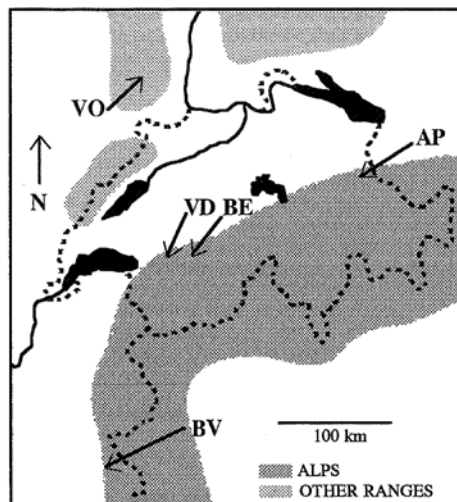


Fig. 1. Map of Switzerland and surrounding countries showing the localities from which beetles were collected.

host plant patch is always minimally 5 km away. Most of the samples are male-biased, probably because the males usually feed on the top portion of the plants and are therefore more likely to be caught. The sex ratios in these species are equal when they are bred in the laboratory (F. Eggenberger pers. com.). With the exception of one population (BE), adult beetles were collected in the early summer: i.e. after the beetles have migrated to their host plants from their overwintering sites in the soil and before the new generation emerges later in the summer.

Allozyme electrophoresis. Each individual beetle was sexed before electrophoresis. The thoracic muscles of each beetle were dissected and homogenized in a buffer solution (Tris at pH 7 with mercaptoethanol), then absorbed onto 4 filter paper wicks. Starch gel electrophoresis was performed on 6 enzyme loci which were polymorphic in both *Oreina* species: *aconitase* (*acon-1*; E.C. 4.2.1.3), *aspartate aminotransferase* (*aat-1*; E.C. 2.6.1.1, stained using a *got* stain recipe), *fumarate hydratase* (*fumh-1*; E.C. 4.2.1.2), *glycerol-3-*

phosphate dehydrogenase (*g3pdh-1*; E.C. 1.1.1.8), glyceraldehyde-3-phosphate dehydrogenase (*gapdh-1*; E.C. 1.2.1.2), and malate dehydrogenase (*mdhp-1*; E.C. 1.1.1.40., also known as *malic enzyme*). The enzyme nomenclature follows the new usage of Murphy *et al.* (1990). All gels were run at 50 mA in refrigerated chambers. Three buffer systems were used: tris citrate (pH 6.7, *acon-1*), a discontinuous tris citrate buffer (TCB, pH 8.0, *fumh-1*, *me-1*), and a discontinuous high pH buffer (Tris Borate EDTA I (Murphy *et al.*, 1990), pH 8.6, *aat-1*, *g3pdh-1*, and *gapdh-1*). For more details on the buffer and staining recipes used, see Eggenberger and Rowell-Rahier (1991).

Analysis. We tested for departures from Hardy-Weinberg expectations (HWE) at each locus using BIOSYS software (Swofford & Selander, 1981). Before testing, the genotypes were pooled into homozygotes for the most common allele, heterozygotes of the most common allele and one of the other alleles, and heterozygotes and homozygotes of the rarer alleles. This pooling procedure is a conservative test, because it does not detect departures among the rarer alleles (Weir, 1990a). Exact tests were used in significance testing to avoid the problems posed by low expected frequencies in some genotypic classes.

Direct-count heterozygosities were calculated for each population and the average expected heterozygosities were determined as recommended by Nei (1978). To estimate the average F_{is} , the overall inbreeding coefficient for each population, we combined estimates over the loci and calculated a jackknifed mean and variance according to the procedures outlined in Weir and Cockerham (1984) for F statistics (c.f. Holtsford & Ellstrand, 1989). We determined whether the F_i values were significantly different from zero with two-tailed t -tests of the jackknife estimates (cf. McCauley *et al.*, 1988 for F statistics).

To determine whether there was significant between-population differentiation at each locus, we conducted heterogeneity G tests of the genotype frequencies (pooling the allelic classes as described above). We did not conduct the heterogeneity tests on allele frequencies because this test

is appropriate only when subpopulations are in HW equilibrium (Weir, 1990b).

To quantify overall genetic differentiation, we used Weir and Cockerham's (1984) modification of Wright's F statistics (Wright, 1978) where F_{it} is the overall inbreeding coefficient, F_{is} is the within-population component of F_{it} , and F_{st} is the between-population component of F_{it} . To determine the means and standard deviations of F_{is} and F_{st} , we used jackknife over loci and tested the significance of these values with t -tests (two-tailed and one-tailed, respectively). Finally, we tested for genetic differences among the sexes using a modified F_{st} where sex rather than population was the category defining the subpopulations to be compared.

Results

All six loci were highly polymorphic in nearly every population of both *Oreina* species, even though sample sizes are sometimes small when the sexes are considered separately (Tables 1, 2). In many cases in both sexes, there were deficiencies in the observed numbers of heterozygotes relative to the expected values. In the overall sample, the observed heterozygosities are generally lower than the expected values (Table 3). Among the individual loci, all of the significant departures from HWE showed heterozygote deficiencies. All of the jackknifed F_i estimates for the seven populations were positive and significantly different from zero. The overall F_{is} values for these two species also show that on average, these populations are heterozygote deficient (Table 4). F_{is} values at each locus are positive, and the jackknifed estimates of F_{is} are significantly greater than zero. The overall F_{is} values for both species are very similar. Thus, for both species, deviations from random mating appear to be pervasive.

Both *Oreina* species showed significant differentiation among the populations sampled, but the *O. cacaliae* populations were more highly differentiated than the *O. speciosissima* populations. Genotype frequencies are significantly heterogeneous at five loci for *O. cacaliae*, compared to

Table 1. Observed and expected genotype frequencies in local populations of *Oreina cacaliae*. Alleles are numbered according to their anodal migration. *N* = sample size

	AP		BE		BV		VO	
	Female	Male	Female	Male	Female	Male	Female	Male
<i>acon-1</i>								
<i>N</i>	12	29	3	8	22	20	15	61
11	2 (0.5)	0 (0.1)	0 (0.1)	1 (0.5)	5 (1.6)	1 (0.3)	3 (1.4)	7 (2.8)
12	0 (3.3)	4 (3.1)	1 (0.7)	2 (2.0)	2 (6.3)	3 (2.1)	3 (6.0)	12 (19.2)
13	1 (0.6)	0 (0.6)	0 (0.2)	0 (1.0)	0 (2.4)	0 (2.2)	0 (0.3)	0 (1.3)
22	7 (5.3)	19 (17.5)	1 (1.3)	2 (2.0)	7 (6.0)	3 (3.6)	8 (6.7)	36 (33.2)
23	2 (2.0)	3 (7.0)	1 (0.7)	2 (2.0)	7 (4.7)	8 (7.6)	1 (0.7)	6 (4.4)
33	0 (0.2)	3 (0.7)	0 (0.1)	1 (0.5)	1 (0.9)	5 (4.1)	0 (0.02)	0 (0.2)
<i>g3pdh-1</i>								
<i>N</i>	11	29	5	15	27	26	17	63
11	9 (8.2)	24 (24.2)	1 (1.2)	6 (4.8)	19 (17.9)	16 (16.2)	11 (11.5)	41 (37.3)
12	1 (2.6)	5 (4.6)	3 (2.5)	5 (7.4)	6 (8.2)	9 (8.7)	6 (4.9)	15 (22.3)
22	1 (0.2)	0 (0.2)	1 (1.2)	4 (2.8)	2 (0.9)	1 (1.2)	0 (0.5)	7 (3.3)
<i>fumh-1</i>								
<i>N</i>	12	29	5	15	27	26	17	64
11	–	–	–	–	1 (0.1)	1 (0.1)	0 (0.1)	0 (0.1)
12	–	–	–	–	1 (2.8)	1 (2.8)	2 (1.9)	5 (4.8)
22	9 (9.2)	26 (24.2)	3 (3.2)	9 (7.4)	25 (24.1)	24 (23.1)	15 (15.1)	59 (59.1)
23	3 (2.6)	1 (4.6)	2 (1.6)	3 (6.3)	–	–	–	–
33	0 (0.2)	2 (0.2)	0 (0.2)	3 (1.4)	–	–	–	–
<i>gapdh-1</i>								
<i>N</i>	12	29	4	15	27	26	22	64
11	4 (2.5)	11 (10.0)	1 (1.0)	4 (2.0)	16 (12.0)	11 (9.8)	0 (0.1)	6 (1.9)
12	3 (6.0)	12 (11.7)	2 (2.0)	3 (5.5)	–	–	2 (1.9)	10 (15.3)
13	–	0 (1.1)	–	0 (1.5)	4 (12.0)	10 (12.3)	–	0 (2.9)
22	5 (3.5)	4 (4.1)	1 (1.0)	5 (3.8)	–	–	20 (20.0)	37 (30.9)
23	–	2 (0.8)	–	2 (2.0)	–	–	–	5 (11.8)
33	–	0 (0.03)	–	1 (0.3)	7 (3.0)	5 (3.8)	–	6 (1.1)
<i>aat-1</i>								
<i>N</i>	10	22	5	15	27	26	12	54
11	0 (0.6)	3 (4.1)	0 (0.8)	5 (3.3)	20 (17.1)	21 (19.5)	0 (0.02)	1 (0.7)
12	5 (3.8)	13 (10.8)	4 (2.4)	4 (7.5)	3 (8.8)	3 (6.1)	1 (1.0)	10 (10.7)
22	5 (5.6)	6 (7.1)	1 (1.8)	6 (4.3)	4 (1.1)	2 (0.5)	11 (11.0)	43 (42.7)
<i>mdhp-1</i>								
<i>N</i>	12	29	5	15	16	18	15	60
11	2 (0.5)	0 (0.01)	–	1 (0.07)	–	–	3 (0.8)	2 (0.3)
12	1 (3.9)	1 (1.0)	–	0 (1.7)	–	–	1 (5.4)	5 (8.0)
13	–	–	–	0 (0.13)	–	–	–	0 (0.3)
22	9 (7.5)	28 (28.0)	4 (4.1)	12 (11.3)	8 (5.6)	14 (12.5)	11 (8.8)	50 (47.7)
23	–	–	1 (0.9)	2 (1.7)	3 (7.7)	2 (5.0)	–	2 (3.6)
33	–	–	0 (0.05)	0 (0.07)	5 (2.6)	2 (0.5)	–	1 (0.07)

Table 2. Observed and expected genotype frequencies in local populations of *Oreina speciosissima*. Alleles are numbered according to anodal migration and the same numbers denote alleles shared with *O. cacaliae*. *N* = sample size

	AP		BE		VD	
	Female	Male	Female	Male	Female	Male
<i>acon-1</i>						
<i>N</i>	13	20	3	22	14	27
11	—	0 (0.1)	—	—	—	—
12	—	1 (1.8)	—	—	—	—
13	—	0 (0.1)	—	—	—	—
22	7 (7.7)	17 (16.2)	1 (1.3)	15 (15.6)	10 (9.4)	24 (24.1)
23	6 (4.6)	2 (1.8)	2 (1.3)	7 (5.9)	3 (4.1)	3 (2.8)
33	0 (0.7)	0 (0.1)	0 (0.3)	0 (0.6)	1 (0.4)	0 (0.1)
<i>g3pdh-1</i>						
<i>N</i>	13	20	3	12	14	27
22	5 (6.2)	17 (15.3)	0 (0.3)	9 (9.2)	10 (9.5)	25 (24.1)
23	8 (5.5)	1 (4.4)	2 (1.3)	3 (2.6)	3 (4.1)	1 (2.8)
33	0 (1.2)	2 (0.3)	1 (1.3)	0 (0.2)	1 (0.4)	1 (0.1)
<i>fumh-1</i>						
<i>N</i>	13	20	3	12	14	27
11	0 (0.1)	0 (0.2)	—	—	0 (0.02)	0 (0.01)
12	2 (1.8)	4 (3.6)	—	—	1 (1.0)	1 (1.0)
22	11 (11.1)	16 (16.2)	3 (3.0)	12 (12.0)	13 (13.0)	26 (26.0)
<i>gapdh-1</i>						
<i>N</i>	13	20	3	12	14	27
11	7 (5.6)	19 (19.0)	2 (2.1)	12 (12.0)	9 (7.1)	26 (26.0)
12	3 (5.9)	1 (1.0)	1 (0.8)	—	2 (5.7)	1 (1.0)
22	3 (1.6)	0 (0.2)	0 (0.1)	—	3 (1.1)	0 (0.01)
<i>aat-1</i>						
<i>N</i>	12	20	3	12	13	27
11	1 (1.0)	1 (0.6)	2 (1.3)	3 (1.7)	3 (1.0)	2 (0.3)
12	5 (5.0)	5 (5.8)	0 (1.3)	3 (5.6)	1 (5.1)	2 (5.3)
22	6 (6.0)	14 (13.6)	1 (0.3)	6 (4.7)	9 (6.9)	23 (21.3)
<i>mdhp-1</i>						
<i>N</i>	13	20	2	12	16	27
11	10 (7.7)	16 (12.8)	2 (1.3)	6 (5.3)	10 (9.4)	26 (25.0)
12	0 (4.6)	0 (6.4)	0 (1.3)	3 (5.3)	4 (4.1)	0 (1.9)
22	3 (0.7)	4 (0.8)	0 (0.3)	3 (1.3)	2 (0.4)	1 (0.04)

only three loci for *O. speciosissima*. Moreover, the jackknifed estimate of F_{st} is also considerably greater in *O. cacaliae* than in *O. speciosissima*.

When the sexes are analyzed separately, both females and males show the same pattern of high F_{is} and F_{st} values as in the overall analysis (Table 5). For *O. cacaliae*, F_{is} is significantly greater ($t = 2.5$; $df = 10$, $P < 0.05$) among females than

among males, while for *O. speciosissima*, F_{is} is slightly greater (but not significantly) among males. In *O. cacaliae* and in *O. speciosissima*, F_{st} are not significantly different between sexes (Table 5). Thus, *O. cacaliae* females show greater within-population differentiation while in *O. speciosissima* the reverse could be the case.

To determine whether the sexes differ geneti-

Table 3. Observed heterozygosities (Ho), expected heterozygosities (He), and fixation indices (Fi) for six polymorphic loci in *O. cacialtae* and *O. speciosissima*. Significance of the deviations from HWE was determined by exact tests. The average Fi's represent jackknifed estimates of the individual Fi's and their significance was determined by *t*-tests (see text)

Species	<i>O. cacialtae</i>				<i>O. speciosissima</i>		
	AP	BE	BV	VO	AP	BE	VD
<i>acon-1</i>							
Ho	0.24	0.55	0.48	0.29	0.24	0.36	0.15
He	0.41	0.60	0.63	0.42	0.27	0.30	0.18
Fi	0.42	0.14	0.25	0.32	-0.12	-0.20	0.18
P				*			
<i>g3pdh-1</i>							
Ho	0.15	0.40	0.28	0.26	0.27	0.33	0.10
He	0.18	0.49	0.32	0.34	0.32	0.36	0.18
Fi	0.18	0.22	0.12	0.24	0.15	0.10	0.46
P				*			*
<i>fumh-1</i>							
Ho	0.10	0.25	0.04	0.09	0.18	-	0.05
He	0.18	0.40	0.11	0.08	0.16	-	0.05
Fi	0.46	0.40	0.65	-0.04	-0.08	-	-0.01
P	*		**				
<i>gapdh-1</i>							
Ho	0.42	0.37	0.26	0.20	0.12	0.12	0.07
He	0.52	0.58	0.46	0.39	0.26	0.12	0.20
Fi	0.20	0.39	0.43	0.50	0.54	-0.03	0.63
P			**	***	*		***
<i>aat-1</i>							
Ho	0.56	0.40	0.11	0.17	0.31	0.20	0.07
He	0.47	0.50	0.28	0.18	0.34	0.49	0.27
Fi	-0.18	0.22	0.60	0.07	0.10	0.61	0.73
P			***			*	***
<i>mdhp-1</i>							
Ho	0.05	0.15	0.15	0.11	0.18	0.27	0.07
He	0.14	0.23	0.40	0.24	0.44	0.44	0.16
Fi	0.65	0.36	0.64	0.55	0.49	0.42	0.54
P	**		***	***	***		*
Average							
Ho	0.26	0.35	0.22	0.19	0.22	0.21	0.09
He	0.31	0.48	0.37	0.28	0.30	0.29	0.17
Fi	0.20	0.27	0.40	0.34	0.24	0.28	0.52
s.e.	0.06	0.02	0.04	0.03	0.05	0.08	0.04
P	*	***	***	***	**	*	***

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ for exact test of HWE.

cally within populations, we developed a modified F statistic, which we call F_{gt} , where g denotes gender. Like F_{st} , F_{gt} measures genetic differenti-

ation among subpopulations, but in this case, the subpopulations are defined by the sexes rather than by spatially separated populations. We cal-

Table 4. F -statistics analysis for *O. cacaliae* and *O. speciosissima*. For both species, among-population heterogeneities in genotype frequencies were tested by G -tests (*O. cacaliae* d.f. 6, *O. speciosissima* d.f. = 4). Significances of the jackknifed F statistics were determined by t -tests

Locus	<i>O. cacaliae</i>			<i>O. speciosissima</i>		
	F_{is}	F_{st}	G	F_{is}	F_{st}	G
<i>acon-1</i>	0.303	0.062	20.1**	-0.052	0.000	5.2
<i>g3pdh-1</i>	0.194	0.059	15.8*	0.235	0.016	6.0
<i>fum-1</i>	0.356	0.100	18.2**	-0.063	0.032	6.5*
<i>gapdh-1</i>	0.399	0.330	141.9***	0.523	-0.012	3.1
<i>aat-1</i>	0.178	0.457	119.7***	0.466	0.056	12.5*
<i>mdhp-1</i>	0.575	0.074	9.1	0.484	0.131	14.2**
Average over loci						
mean	0.326***	0.234***		0.332**	0.051**	
s.e.	0.021	0.035		0.039	0.011	

Table 5. Jackknifed estimates of F_{is} and F_{st} for the sexes in *O. cacaliae* and *O. speciosissima*. The asterisks indicate significant differences from zero

	Females		Males	
	F_{is}	F_{st}	F_{is}	F_{st}
<i>O. cacaliae</i>				
mean	0.390***	0.272***	0.292***	0.220***
s.e.	0.035	0.052	0.016	0.033
<i>O. speciosissima</i>				
mean	0.389**	-0.012	0.433***	0.058**
s.e.	0.068	0.013	0.061	0.014

culated this sex-based F_{gt} within each population in both species and for the pooled populations (omitting the BE population because there were very few females in this sample and because the beetles were collected substantially later there than in the other populations). The resulting F_{gt} values indicated consistent significant differences among the sexes in *O. speciosissima* and *O. cacaliae*; only in the VO sample in *O. cacaliae* were the differences not significant (Table 6).

Discussion

Variation between populations. The population structure of these 2 *Oreina* species is probably

Table 6. F statistics analysis of sex differences in *O. cacaliae* and *O. speciosissima*. In Significances of the jackknifed F_{gt} values were determined by one-tailed t -tests

Species	F_{gt}	S.E.	P
<i>O. cacaliae</i>			
AP	0.021	0.007	0.015
BV	0.021	0.009	0.033
VO	0.012	0.008	0.095
overall	0.011	0.005	0.035
<i>O. speciosissima</i>			
AP	0.062	0.022	0.018
VD	0.097	0.020	0.002
overall	0.085	0.022	0.005

similar to a stepping stone model: the beetles live in isolated patches and most individuals breed within the population. The host plant patches are not continuously distributed in space and a very small proportion of dispersers move to the next nearest patch as suggested for other beetles (e.g. Smith-King, 1987, for Melyridae beetles on rock outcrops, and McCauley & Eanes, 1987, for a herbivorous milkweed beetle). This is supported by the fact that in both species studied there is considerable genetic divergence between populations only 40 to 250 km distant from each other. The overall F_{st} values are relatively high for insects at that spatial scale, especially the value for *O. cacaliae* (0.234). Presently, we can not exclude

differential selection, linked for example to microclimate, latitude or altitude differences, across the distribution range of *O. cacaliae*. McCauley and Eanes's (1987) review of F_{st} estimates for different flighted insects across a broader spatial scale showed values ranging from 0.01 to 0.15 for beetles, including a value of 0.068 for the leaf beetle *Leptinotarsa decemlineata*. In a study of willow leaf beetles (*Plagioderma versicolora*) McCauley *et al.* (1988) found variance between regions to be 0.057. Feder and Bush (1989) showed that hawthorn and apple flies (*Rhagoletis pomonella*) display inter population differentiation between numerous populations in the Midwest USA (F_{st} 0.0626 for the hawthorn race and 0.0125 for the apple race). Also, a latitudinal allele frequency cline is present between population of *R. pomonella* separated by the same distance as our *O. cacaliae* (Feder, pers. comm.). Additionally Guttman and Weight (1989) found high values (0.10 to 0.39) for membracid species specialized on different host trees. Here we found equally high F_{st} values for specialized herbivorous insects with limited dispersal capabilities, based on a small geographic scale.

Several studies have already shown microgeographic genetic differentiation between populations of insect feeding on different host plants: e.g. Feder and Bush (1989) for flies (*Rhagoletis pomonella*) on apple and hawthorn; Guttman *et al.* (1989) for a membracid (*Enchenopa binotata*) among several North American tree species. Guttman *et al.* (1989) argue that on the smaller and more sporadic host trees selection should promote vagility and therefore a lower F_{st} value, whereas the large and longer-lived trees should function as islands and favor stable populations with higher genetic diversity. However, these examples stem from insects feeding on trees or shrubs in which each individual host plant represents a more persistent resource than *Oreina*'s herbaceous host plants. Our study of microgeographic differentiation in leaf beetles is based both on a patchy limited resource and on herbivores with very low vagility. Because of these two factors, the high F_{st} values compared to those found in the literature are not unexpected.

Comparison between species. *O. cacaliae*'s among-population differentiation which is higher than the one of *O. speciosissima* probably reflects a real difference between them. It could be due to differences in gene flow or in breeding structure. *O. cacaliae* males are long lived. Males from one generation can potentially mate with females from the next one and thus favor inbreeding. In contrast, each generation of *O. speciosissima* seems to be more temporally distinct (M. Rowell-Rahier, unpubl.). It is also possible that *O. speciosissima* individuals migrate more frequently among populations than *O. cacaliae* individuals. Alternatively, selective pressures could be different for both species. Further investigations are necessary to differentiate between these possibilities.

Variation within populations. Overall, heterozygosities were relatively high for the two species, even compared with insects in general, which tend to be more polymorphic than other animals (Hartl & Clark, 1989). For both species, the populations studied are not panmictic and deviate from Hardy Weinberg proportions: they have deficiencies in heterozygotes. Fixation indices (F_i) within populations are similar in both species and for both populations where the species occur sympatrically. A possible explanation would be the existence of null alleles in the populations. However, the likelihood of null alleles at all six allozyme loci in both species is very low, but can presently not be ruled out. For communities of subterranean Coleoptera which live in cracks in the rocks or the deepest layer of soil, Crouau-Roy (1988) demonstrated important structuring within local populations, with F_{is} ranging from 0.450 to 0.432. She suggested that these high F_{is} values arise from inbreeding. Our estimates for herbivorous leaf beetles are almost equally high: 0.326 for *O. cacaliae* and 0.332 for *O. speciosissima*, but in the absence of further information about the breeding structure of the leaf beetles, we cannot distinguish between effects of inbreeding and within-population differentiation caused by the pooling of genetically heterogeneous samples. Inbreeding due to the limited dispersal capability of the leaf beetles should affect all loci roughly equivalently and

this seems to be the case at least for *O. cacaliae*. Fine-grained genetic analysis within a patch is planned to confirm this.

For *O. speciosissima*, the VD population has a markedly lower heterozygosity than the other 2 populations. Agricultural practice might have resulted in complete isolation of this patch from its nearest neighbour. There is no morphological evidence, or difference in host plant use, suggesting that *O. speciosissima* population VD could be in a hybrid zone between two species.

Within-population differences between sexes. The fact that F_{gt} values were statistically significant within populations of *O. speciosissima* and *O. cacaliae* indicates that the sexes disperse differently in both species. Of course such differences must be reestablished every generation: our samples were collected in the early summer after the beetles have migrated to their host plants following overwintering and before the new generation emerges later in the summer, and only then can the consequence of the different dispersal patterns among the sexes be observed.

Our results suggest that differential levels of dispersal between the sexes are sufficient to influence F_{is} but not F_{st} , probably because dispersal in either sex is very low. Indeed, for *O. cacaliae*, the results suggest that, within a population, males are less differentiated than the females and F_{is} is greater for females. Because the samples were collected early in the year, before most matings take place, they consist of individuals which have dispersed but have not completed their mating. Thus in this case, the differences between the sexes may represent greater dispersal among the males than among the females. In *O. cacaliae*, the life span of the males is probably longer than that of the females, giving them more time to move. This is true in the laboratory where females die after their reproductive period, but the males survive into a second season. The longer lifespan of the males in *O. cacaliae* may result in lower levels of differentiation among the males. Little is known about sex-specific dispersal in leaf beetles, but in an exhaustive review of the evolution of flightlessness in insects, Roff (1990) found

in some cases (18%), males were the flightless sex.

In conclusion, this study shows that leaf beetles are strongly genetically differentiated on a small spatial scale. Both low dispersal and inbreeding may contributed to this. Additionally, the genetic data suggests that sex-specific differences in dispersal and/or survival are possible. To our knowledge, this was never documented in insects before. These results should encourage population genetic studies of other herbivores with low mobility that feed on patchy resources such as herbaceous plants.

Acknowledgements

I warmly thank Jacques Pasteels for collecting several of the beetles samples and for sharing his enthusiasm. I also thank H. Rowell and S. Stearns for reading and improving the manuscript, N. Rank and T. Prout for helpful discussions and statistical advices, and T. Holsford for making his program available.

References

- Crouau-Roy, B., 1988. Genetic structure of cave-dwelling beetles populations: significant deficiencies of heterozygotes. *Heredity* 60: 321–327.
- Deroe, C. & J. M. Pasteels, 1982. Distribution of adult defense glands in Chrysomelids (Coleoptera: Chrysomelidae) and its significance in the evolution of defense mechanisms within the family. *J. Chem. Ecology* 8: 67–82.
- Eggenberger, F. & M. Rowell-Rahier, 1991. Chemical defence and genetic variation. Interpopulational study of *Oreina gloriosa* (Coleoptera: Chrysomelidae). *Naturwissenschaften* 78: 317–320.
- Feder, J. L. & G. L. Bush, 1989. Gene frequency clines for host races of *Rhagoletis pomonella* in the midwestern United States. *Heredity* 63: 245–266.
- Feder, J. L., C. A. Chilcote & G. L. Bush, 1990a. The geographic pattern of genetic differentiation between sympatric host races of the apple maggot fly *Rhagoletis pomonella* in the eastern United States and Canada. *Evolution* 44: 570–594.
- Feder, J. L., C. A. Chilcote & G. L. Bush, 1990b. Regional, local and microgeographic allele frequency variation between apple and hawthorn populations of *Rhagoletis pomonella* in western Michigan. *Evolution* 44: 595–608.

- Foltz, D. W. & J. L. Hoogland, 1983. Genetic evidence of outbreeding in the Black-tailed Prairie Dog (*Cynomys ludovicianus*). *Evolution* 37: 273–281.
- Guttman, S. I. & L. A. Weigt, 1989. Macrogeographic genetic variation in the *Enchenopa binotata* Complex (Homoptera: Membracidae). *Ann. Entomol. Soc. America* 82: 156–165.
- Guttman, S. I., T. Wilson & L. A. Weigt, 1989. Microgeographic genetic variation in the *Enchenopa binotata* complex (Homoptera: Membracidae). *Ann. Entomol. Soc. America* 82: 225–231.
- Guttman, S. I., T. K. Wood & A. A. Karlin, 1981. Genetic differentiation along host plant lines in the sympatric *Enchenopa binotata* Say complex (Homoptera: Membracidae). *Evolution* 35: 205–217.
- Hartl, D. L. & A. G. Clark, 1989. Principles of population genetics. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.
- Hegnauer, R., 1964. Chemotaxonomie der Pflanzen. Vol 3. Birkhäuser, Basel, Switzerland.
- Hegnauer, R., 1989. Chemotaxonomie der Pflanzen. Vol 8. Birkhäuser, Basel, Switzerland.
- Holtsford, T. P. & N. C. Ellstrand, 1989. Variation in outcrossing rate and population genetic structure of *Clarkia tembloriensis* (Onagraceae). *Theor. Appl. Genet.* 78: 480–488.
- McCauley, D. E. & W. F. Eanes, 1987. Hierarchical population structure analysis of the milkweed beetle, *Tetraopes tetraophthalmus* (Foster). *Heredity* 58: 193–201.
- McCauley, D. E., M. J. Wade, F. J. Breden & M. Wohlman, 1988. Spatial and temporal variation in group relatedness: Evidence from the imported willow leaf beetle. *Evolution* 42: 184–192.
- McPheron, B. A., D. C. Smith & S. H. Berlocher, 1988. Genetic differences between host races of *Rhagoletis pomonella*. *Nature* 336: 64–67.
- Murphy, R. W., J. W. Sites, D. G. Buth & C. H. Haufler, 1990. Proteins I: isozyme electrophoresis. Pages 45–126. In D. M. Hillis and C. Moritz, editors. *Molecular systematics*. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.
- Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.
- Pasteels, J. M., M. Rowell-Rahier, J. C. Braekman, D. Daloz & S. Duffey, 1989. Evolution of exocrine chemical defense in leaf beetles (Coleoptera: Chrysomelidae). *Experientia* 45: 295–300.
- Pasteels, J. M., M. Rowell-Rahier, T. Randoux, J. C. Braekman & D. Daloz, 1988. Pyrrolizidine alkaloids of probable host-plant origin in the pronotal and elytral secretion of the leaf beetle *Oreina cacaliae*. *Entomol. exp. appl.* 49: 55–58.
- Prout, T., 1981. A note on the island model with sex dependent migration. *Theor. Appl. Genet.* 59: 327–332.
- Rank, N. (In press). A hierarchical analysis of genetic differentiation: The willow leaf beetle (*Chrysomela aeneicollis*) in the Eastern Sierra Nevada. *Evolution*.
- Roff, D. A., 1990. The evolution of flightlessness in insects. *Ecol. Monographs* 60: 389–421.
- Schwartz, O. A. & K. B. Armitage, 1980. Genetic variation in social mammals: the marmot model. *Science* 207: 665–667.
- Shields, W. M., 1982. Philopatry, Inbreeding, and the Evolution of Sex. State University of New York Press, Albany, New York.
- Slatkin, M., 1987. Gene flow and the geographic structure of natural populations. *Science* 236: 787–792.
- Smith-King, P., 1987. Macro- and microgeographic structure of a spatially subdivided beetle species in nature. *Evolution* 41: 401–416.
- Swofford, D. L. & R. B. Selander, 1981. BIOSYS-1. A Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* 72: 281–283.
- Weir, B. S., 1990a. Genetic analysis. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.
- Weir, B. S., 1990b. Intraspecific differentiation. Pages 373–410. In D. M. Hillis and C. Moritz, editors. *Molecular systematics*. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.
- Weir, B. S. & C. C. Cockerham, 1984. Estimating F statistics for the analysis of population structure. *Evolution* 44: 1358–1370.
- Wright, S., 1978. *Evolution and the genetics of populations*. Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago, Illinois, USA.