

POPULATION STRUCTURE AND LEVELS OF GENE FLOW IN THE  
MEDITERRANEAN LAND SNAIL *ALBINARIA CORRUGATA*  
(PULMONATA: CLAUSILIIDAE)

MENNO SCHILTHUIZEN AND MARCEL LOMBAERTS

Systematic Zoology Group, Institute of Evolutionary and Ecological Sciences, University of Leiden,  
P.O. Box 9516, NL-2300 RA Leiden, The Netherlands

**Abstract.**—The amount of gene flow among local populations partly determines the relative importance of genetic drift and natural selection in the differentiation of such populations. Land snails, because of their limited powers for dispersal, may be particularly likely to show such differentiation. In this study, we directly estimate gene flow in *Albinaria corrugata*, a sedentary, rock-dwelling gastropod from Crete, by mark-recapture studies. In the same area, 23 samples were taken and studied electrophoretically for six polymorphic enzyme loci. The field studies indicate that the population structure corresponds closely to the stepping-stone model: demes are present on limestone boulders that are a few meters apart, and dispersal takes place mainly between adjacent demes. Average deme size ( $N$ ) is estimated at 29 breeding individuals and the proportion of migrants per generation at 0.195 ( $Nm = 5.7$ ). We find no reason to assume long-distance dispersal, apart from dispersal along occasional stretches of suitable habitat. Genetic subdivision of the population, as derived from  $F_{ST}$  values, corresponds to the direct estimate only at the lowest spatial level (distance between sample sites  $< 10$  m), where values for  $Nm$  of 5.4 and 17.6 were obtained. In contrast, at the larger spatial scales,  $F_{ST}$  values give gene-flow estimates that are incompatible with the expected amount of gene flow at these scales. We explain these discrepancies by arguing that gene flow is in fact extremely limited, making correct estimates of  $Nm$  from  $F_{ST}$  impossible at the larger spatial scales. In view of these low levels of gene flow, it is concluded that both genetic drift and natural selection may play important roles in the genetic differentiation of this species, even at the lowest spatial scales.

**Key words.**—*Albinaria*, allozymes, dispersal, gene flow, genetic distance, land snail, population structure.

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Species rarely consist of a single panmictic population. Because of limited abilities for dispersal and habitat fragmentation, most species consist of subpopulations, among which genetic exchange is reduced. Because of random genetic drift, this may lead to genetic differentiation within a species. Sufficiently strong natural selection, however, may produce similar patterns in the face of gene flow. When studying the geographic variability of a species, an accurate estimate of the level of gene flow is necessary for determining the relative importance of drift and selection as differentiating forces.

Their proverbial low abilities for dispersal make land snails prone to the effects of population subdivision. The Mediterranean pulmonate genus *Albinaria* is well known for both its speciosity and the polytypy of its species (Gittenberger 1992; Mylonas 1992; Schilthuisen et al. 1993). The present study aims at estimating the levels of gene flow in a representative *Albinaria* species and making predictions about the spatial scale at which substantial differentiation caused by genetic drift may occur.

Gene flow may be estimated directly or indirectly (Slatkin 1985b). Direct approaches usually involve the marking and recapturing of individuals, whereas indirect estimates can be obtained from a measure of the genetic similarity of populations. Many groups of land snails are suitable subjects for both types of approach: they are sedentary, conspicuous, and occur in high population densities, making both direct and indirect measurement of gene flow possible. Several studies on gene flow in terrestrial snails have been carried out, using either direct (e.g., Baur 1988; Cowie 1984; Cameron and Williamson 1977; Woodruff 1978; Murray and Clarke 1984) or indirect methods (e.g., Selander and Kaufman 1975; Selander and Hudson 1976; Arter 1990; Boato 1988). It is striking, however, that very few authors (for instance Johnson et al. 1988) report on the simultaneous use of both direct and indirect methods in a single study.

In this study, we present the results of direct and indirect estimates of gene flow, measured simultaneously in the same area. This allows us to directly compare results from both types of



FIG. 1. An overview of the study area: Mount Yúhtas in Crete. Aggregations of *Albinaria corrugata* are present on the isolated limestone boulders.

approach and to interpret the results from the indirect method with the aid of data on the actual population dynamics of the animals in the field.

#### MATERIALS AND METHODS

*The Species.* — A representative *Albinaria* was chosen, *Albinaria corrugata corrugata* from the island of Crete (Greece). This subspecies has relatively large, smooth, white shells, which are easy to mark (see below). It occurs in large numbers and, unlike certain other Cretan species, it aestivates conspicuously on rock surfaces, which facilitates capture and recapture during summer. The active season, in which feeding and reproduction take place, starts at the onset of the winter rains, around mid-October, and ends when the dry summer starts, in May (Gittenberger and coworkers unpubl. data). Anatomical studies (Schilthuisen, unpubl. data) suggest that subadult snails have full-grown shells (with reflected peristome) already in spring, whereas their reproductive systems mature during summer. The mating system of this or other *Albinaria* species is not exactly known, but the frequent field ob-

servations of many, simultaneously copulating pairs (Schilthuisen, unpubl. data) suggest that outcrossing is the rule.

*Study Area.* — Both field experiments and sampling were carried out on and near Mount Yúhtas, some 12 km south of Iráklion (the capital of Crete). The terrain is barren, devoid of any large vegetation. Only small, tough shrubs ("garigue") cover the soil. Scattered in between are larger or smaller blocks of limestone (fig. 1). These boulders, which are covered in microvegetation such as lichens, algae and mosses, are the preferred habitat of *Albinaria* (Kemperman et al. 1992).

*Field Experiments.* — Two grids, sites I and II, each consisting of 100  $1 \times 1$  m squares, were established on September 9, 1991 and October 24, 1991, respectively. The two sites were approximately 1.5 km apart. At each site, the distributions of rock, garigue, and rubble were recorded. Each  $1 \times 1$  m square was assigned a code between 00 and 99. Adult snails were marked with India ink by writing the code of the square in which they were found on the dorsal side of the penultimate whorl. This method of marking was first tested on empty shells exposed to outdoor conditions in The Netherlands for several months. The India ink was found to adhere to the shells quite well, mainly because they lack a periostracum, and the ink is partly absorbed by the calcite matrix of the shells. Some of the test markings, however, did become more vague over time. Therefore, in the field experiments, a certain amount of mark loss could not be excluded. Juvenile snails, mostly too small for marking the way adults were marked, received a single dot on one of the whorls. Marking was carried out during dry days, when the snails were inactive, having stuck their apertures to the rocks by means of epiphragms (tests at other sites within the same area confirmed that virtually all snails found in this position were alive). During marking, they were not removed or otherwise disturbed to avoid any disturbance-induced dispersal (see Cameron and Williamson 1977, for such complications). The numbers of both adult and juvenile snails in each  $1 \times 1$  m square were recorded. To assess collecting efficiency, site I was visited several weeks after marking, during rainy weather, when the snails were actively crawling around. After searching the area with the same efficiency as during the first visit, only 4 unmarked adults and 6 unmarked juveniles were discovered (and subsequently marked), whereas 129 and 151, respectively, had been marked during the first visit.

This would indicate that sampling intensity had been nearly 100%. The numbers of marked adult snails, therefore, are treated as the actual numbers of adult snails present (this means that any mark loss during the experiment would not influence our estimate of effective population size). Because of their often very small size, the juveniles, however, may still have been undersampled. The two sites were visited again 1 yr later, on October 3 and 4, 1992. Each grid and the eight  $10 \times 10$  m squares around it were searched intensively. Again, the numbers of adult and juvenile snails were recorded for each  $1 \times 1$  m square. All positions of retrieved marked snails were taken down. From the data, density and patterns of dispersal and dispersion could be assessed. For determining the latter parameter, the index of dispersion (Southwood 1978) was used:  $I_D = s^2(n-1)/x$ , in which  $x$  is the mean density per  $m^2$ ,  $s^2$  is the variance and  $n$  equals 100, the number of square meters sampled.  $I_D$  is approximately distributed as  $\chi^2$  with  $n-1$  degrees of freedom, and departure from random dispersion can hence be tested.

As juvenile snails were marked only with single dots, their dispersal could not be estimated as accurately as the adults. Therefore, a separate experiment was set up. On October 4, 1992, all 58 juvenile snails present on a single boulder (largest diameter 2 m) close to site I, were marked with a single dot of India ink, without disturbing the animals. At the end of the rainy season, on April 2, 1993, the boulder and its surroundings were searched intensively and the positions of marked snails were recorded.

**Sampling.**—Twenty-three samples were taken for allozyme electrophoresis. To investigate the influence of spatial scale on levels of gene flow, 20 samples were taken along two transects, A and B, with intervals between sample sites increasing from 4 to 230 m. The amount of suitable habitat along transect A was clearly less than along transect B, where long stretches of bare rock were present. Additionally, three samples were taken at 1 to 5 km from each other and from the other 20 sample sites (fig. 2). All samples were grouped into four levels of spatial scale: samples 1–4 and 11–14 constitute two groups at level 1, with intervals along the transect of up to 10 m; samples 1, 4, 5, 6, 7, and 11, 14, 15, 16, and 17 constitute two groups at level 2, with intervals along the transect of 20 to 60 m; samples 1, 7, 8, 9, 10, and 15, 17, 18, 19, and 20 make up two groups at level 3, with intervals

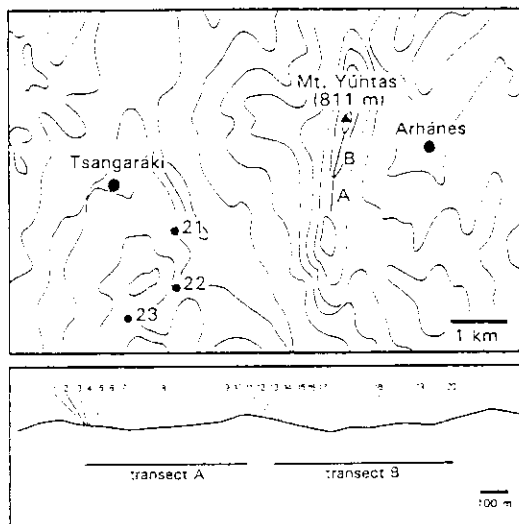


FIG. 2. Map of a part of Crete, with sample sites indicated. Sample sites 1–20 are situated along transects A and B, which are shown in profile in the bottom part of the figure. Contours are given at 50-m intervals.

along the transect of 100 to 230 m and samples 1, 20, 21, 22, and 23, finally, form the group that represents spatial level 4, with between-sample distances of 950 to 4900 m. The snails were collected from single boulders, each sampling area not exceeding  $2 m^2$ . Sample size ranged from 14 to 52 specimens. The animals were transported alive and frozen at  $-70^\circ\text{C}$  upon arrival in the laboratory.

**Electrophoresis.**—Each snail, including the shell, was ground by hand in 2 to 5 drops of homogenizing buffer. Homogenates were then centrifuged at 9617 g for 5 min, and the supernatants were immediately applied to sample wicks for loading the gels. Standard procedures for horizontal starch gel electrophoresis were employed (see Selander et al. 1971). Gels were prepared from 12% (weight per volume) Connaught hydrolyzed starch, 15 mg of NAD, and 15 mg of NADP were added to the gel buffer mixture after boiling and to the cathodic buffer compartment before electrophoresis. After 15 initial running minutes at a lower voltage, the sample wicks were removed, and the voltage was raised to the desired value. All gels were maintained at approximately  $4^\circ\text{C}$  during electrophoresis. On the basis of previous allozyme data for this population (Schilthuisen, unpubl. data), six enzyme systems were selected and used to detect six polymorphic loci (the nomenclature used follows the

TABLE 1. Data on population size, density and aggregation for both sites in both years.

	Site 1	Site 2
Total of adults marked (1991)	133	182
Marked adults recaptured (1992)	38	47
Total of adults found (1992)	155	241
Total of juveniles found (1991)	157	174
Marked juveniles recaptured (1992)	11	42
Total of juveniles found (1992)	128	230
Index of dispersion (ID)		
Adults in 1991	838*	1020*
Juveniles in 1991	588*	722*
Adults in 1992	639*	1530*
Juveniles in 1992	490*	1660*

\* Significant aggregation ( $\chi^2$  test,  $P < 0.05$ ).

recommendations by the Fish Genetics Nomenclature Committee 1989): soluble Aspartate aminotransferase (sAAT, E.C. 2.6.1.1), Esterase (EST, E.C. 3.1.1.—), soluble Isocitrate dehydrogenase (NADP<sup>+</sup>) (sIDHP, E.C. 1.1.1.42), Leucine aminopeptidase (LAP, E.C. 3.4.11.1), soluble Malate dehydrogenase (sMDH, E.C. 1.1.1.37), and Phosphogluconate dehydrogenase (PGDH, E.C. 1.1.1.44). Staining recipes were taken from Selander et al. (1971) for sAAT and from Shaw and Prasad (1970) for the other enzymes. Buffer systems used were tris-citrate, pH 8.0 (Shaw and Prasad 1970) for sAAT, LAP, and PGDH (running time 3 h at 15V/cm) and tris-citrate, pH 7.0 (Shaw and Prasad 1970) for EST, sIDHP, and sMDH (running time 3 h at 15V/cm). Alleles were assigned numbers, reflecting the electrophoretic mobility of the corresponding allozyme relative to the one with the lowest mobility.

*Genetic Data Analysis.*—The genotypic frequencies were analyzed with the BIOSYS-1 package of Swofford and Selander (1981). Every sample was tested for conformance to the Hardy-Weinberg equilibrium at each polymorphic locus, using the exact probability test provided by BIOSYS-1. As recommended by Rice (1989) and Lessios (1992), the sequential Bonferroni test (Holm 1979) was used on all 110 tests to determine if any cases showed significant departure from Hardy-Weinberg expectations, with  $\alpha = 5\%$ .

Rogers' (1972) genetic distances ( $D_R$ ) were calculated for all pairs of samples and used for the construction of a UPGMA dendrogram. The values for  $D_R$  were also plotted against geographic distances as suggested by Sokal (1979).

Values for  $F_{ST}$ , the standardized variance of allelic frequencies (Wright 1943, 1969), were calculated per locus for each group of samples with-

in the four spatial levels described above. Where  $F_{ST}$  values proved to be in the same order of magnitude for each locus (see Results), they were averaged across the polymorphic loci to produce a single  $F_{ST}$  value for each group (as recommended by Slatkin 1987). Gene flow was estimated from the  $F_{ST}$  values using the relationship  $F_{ST} \approx 1/(4Nm + 1)$ , in which  $N$  is the effective size of an isolated, panmictic population ("deme"), and  $m$  is the proportion of the deme that are migrants (Wright 1943). This equation was developed for the infinite-island model of population structure, but simulations by Crow and Aoki (1984) showed it is also applicable to the stepping-stone model. The private-allele method of Slatkin (1985a) for estimating  $Nm$  was not used because of the presence of private alleles in only one sample.

## RESULTS

*Density and Dispersion.*—The numbers of snails found in both years at sites I and II are presented in table 1. The high indices of dispersion indicate significant aggregation on the limestone boulders within each site for both age classes.

*Dispersal.*—At site I, 38 out of the 133 marked adults were retrieved after 1 yr. At site II, this figure was 53 out of 182. The mean rate of adult recapture, therefore, amounts to 29%. The fate of the remaining 71% is unclear. These may have gone undetected because of loss of marks, or they may have been taken by predators. If the latter possibility were true, then all unmarked adults in 1992 must have grown up from juveniles that were observed the previous year. However, only 8 adults at site I and 27 at site II carried the ink dot that was applied to the juveniles in the pre-

vious year (see below). Probably, a combination of adult mark-loss, predation, and insufficient sampling of juveniles in 1991 explains the high proportion of unmarked adults in 1992. It should be noted that both mark loss and predation may be expected to have influenced the marked individuals randomly. Therefore, the recaptured 29% may be regarded as an unbiased sample for estimating dispersal.

In figure 3, patterns of minimal dispersal within each site are shown. At both sites, about 90% of the adult individuals had dispersed less than 2 m. The longest distance traveled was between 4 and 5 m. Most individuals remained within the rocky patch where they had been marked. In all, only six of them probably crossed inhospitable habitat as they migrated from one patch to another (marked with asterisks in fig. 3).

**Juvenile Dispersal.**—Eleven (site I) and 42 (site II) individuals were recaptured that had been marked as juveniles. Of these, 8 and 27 had grown mature, respectively. As juveniles had been marked only with a single dot, their exact pattern of dispersal could not be established. However, none of the retrieved individuals were found outside their original 10 × 10 m squares, indicating that juvenile dispersal is similar to adult dispersal. This was further examined in the separate experiment described in the previous section, in which all juveniles on a single boulder were marked. Of the 58 juveniles that were marked in 1992, 10 were recaptured the next year. Nine of these were retrieved from the same boulder they had been found on, whereas one individual had crossed to a neighboring boulder. This pattern of dispersal strongly resembles the pattern of adult dispersal. There seems to be no reason, therefore, to assume different rates of dispersal for juveniles.

**Population Structure.**—The high degree of aggregation and the patterns of dispersal strongly suggest that the population is concentrated in demes, corresponding with single boulders, among which migration is extremely limited. Such a pattern of population structure would agree with the two-dimensional stepping-stone model of Kimura (1953), in which the demes are arranged in a rectangular lattice, with gene flow occurring only between adjacent demes. If this model is applied to the present data, the gene flow parameter,  $Nm$ , may be calculated as follows:

At site I, six inhabited rocky patches are found that are separated from each other by stretches

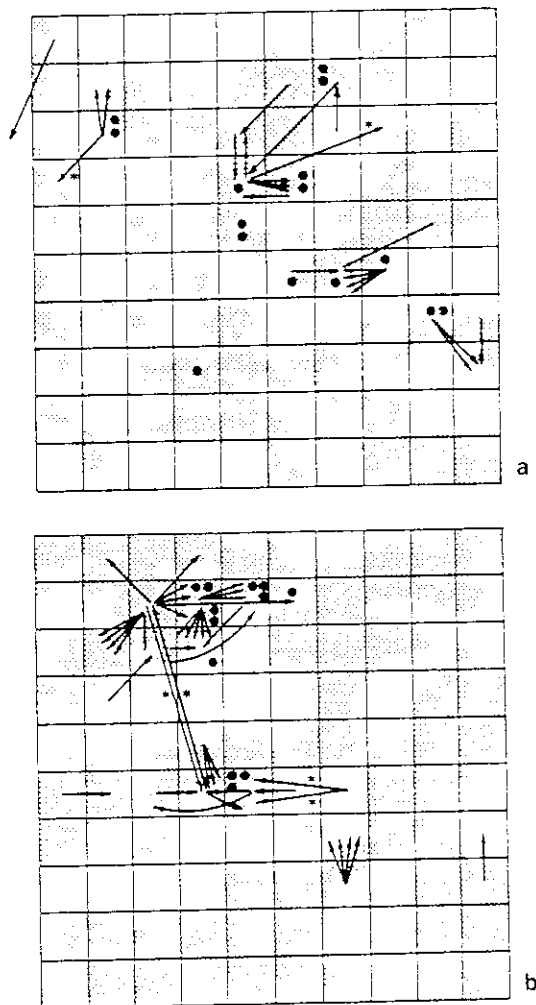


FIG. 3. Patterns of minimal dispersal of adult snails in site I (a) and site II (b) during the season 1991/1992. The distributions of limestone rock and inhospitable habitat ("garigue" and rubble) are indicated with stippled and white areas, respectively. Dots indicate snails that were recaptured in the same 1 × 1 m square in which they were marked. Arrows indicate individuals that had migrated from their original 1 × 1 m square to another. Asterisks (\*) show which migrating individuals probably crossed inhospitable habitat.

of inhospitable habitat. At site II, five such patches are found. Dividing the total number of adult snails at each site (in 1991) by the number of patches there, yields minimum values for  $N$  of 22 at site I and 36 at site II. Hence, the mean value for  $N$  is 29. The value for  $m$  may be obtained as follows. The average number of migrants encountered per deme in 1991 is  $6/11 =$

0.55; the rate of recapture was the number of recaptured snails/the number of marked snails = 0.29. The estimated fraction of migrants per deme per year would then be  $[(0.55/0.29)/29] = 0.065$  if there were one generation per year. However, several field experiments with marked snails and the presence on adult shells of juvenile aestivation-apertures (Schilthuisen, unpubl. data) indicate that most *Albinaria* individuals will need at least 2 yr to reach maturity. Adult life was found to last for more than 3 yr under laboratory conditions, but field data (Schilthuisen unpubl. data) indicate that under natural conditions, adult mortality is so high that the majority of breeding individuals are replaced by a new generation each year. In fact, the present study also supports this idea, as most adults found in 1992 seem to have been recruited from the juveniles of the previous year (see above). Hence, it seems legitimate to assume a generation time of 3 yr for *Albinaria*, which makes (assuming equal annual dispersal for juveniles and adults)  $m = 3 \times 0.065 = 0.195$ . The gene flow parameter,  $Nm$ , therefore, equals 5.7.

**Genetic Analysis.**—Table 2 presents the frequencies of the various alleles in all samples. The patterns of frequencies of alleles along transects A and B have a fairly random appearance, with the exception of *Lap*, of which the 100-allele increases in frequency in a northerly direction, at the expense of *Lap-125* (fig. 4). The statistical tests proved deviation from Hardy-Weinberg equilibrium to be insignificant in all cases, indicating that every sample may be considered representative of a single deme.

Rogers' genetic distance ( $D_R$ ) between samples ranged from 0.028 to 0.605. Figure 5 shows the result of a UPGMA cluster analysis. The high cophenetic correlation (0.98) indicates that the clustering pattern accurately reflects the pattern of genetic variation in the matrix of genetic distances (Sneath and Sokal 1973). The dendrogram shows two major groups: one containing all 20 samples along transects A and B, the other containing the three samples several kilometers to the west (see fig. 2). Within the cluster containing samples 1–20, however, concordance with geographic positions of the samples sites is completely absent: nowhere in the dendrogram do two adjacent sample sites cluster together. For example, sample sites 11, 12, 13, and 14, each separated from the next by a distance of about 7 m, fail to show any genetic resemblance that makes them cluster together. Conversely, sam-

ples 1 and 20 are genetically so similar that they are clustered together, a similarity that does not seem to agree with the fact that they are separated by a distance of more than 1300 m. However, a plot of all pairs of genetic and geographic distances for samples 1–20 (fig. 6) does show a significant correlation between the two variables ( $r = 0.16$ ,  $P = 0.03$ ), although the scatter of the coordinates is considerable. However, this correlation disappears when *Lap* is excluded from the calculations of  $D_R$  ( $r = 0.09$ ,  $P = 0.23$ ). Apparently, the correlation between genetic distance and geographic distance is caused by the alleles of this locus, which (as mentioned previously) show a cline along the transects.

$F_{ST}$  statistics and gene flow estimates are presented in table 3. With the exception of the  $F_{ST}$  value for the *sAat* locus at the 950–4900 m level, a close correspondence in  $F_{ST}$  values is seen for all loci in each group. This justifies the calculation of a single, average  $F_{ST}$  figure for each group at the three lower spatial levels (Slatkin 1987). For the 950–4900 m level, the mean  $F_{ST}$  was calculated without the value for the *sAat* locus. The  $F$ -statistics show that among all three lower spatial levels, genetic differentiation is roughly equal, although  $F_{ST}$  values for samples along transect B (where long stretches of suitable habitat were present) are about twice as high as for those along transect A. As the UPGMA dendrogram already showed, genetic differentiation at the highest spatial level is distinctly increased in relation to the three lower levels.

Gene-flow estimates as calculated from the  $F_{ST}$  values are relatively high among sample sites at the three lower spatial levels. Only among the five sample sites constituting the 950–4900 m spatial level is the gene flow parameter less than 1, and may be considered sufficiently reduced for fixation of alleles caused by drift to occur (Wright 1969; Slatkin and Barton 1989).

#### DISCUSSION

Our observations on dispersal and aggregation of *Albinaria corrugata* closely correspond with similar studies in other rock-dwelling pulmonates: Baur (1988) reports an average annual dispersal by adult *Chondrina clienta* of 0.83 to 2.97 m (dependent on the microhabitat); in this species, too, habitat fidelity was strong, and no snails were found crossing inhospitable habitat between rocky patches. Giokas (1990, pers. comm.) reported that in other *Albinaria* species the mean lifetime dispersal amounted to only 2 m.

TABLE 2. Allele frequencies for all twenty-three samples.

Locus	Sample																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
<i>s4ur</i>	(n)	38	24	27	15	31	17	14	24	26	22	43	32	29	30	30	35	28	43	40	52	20	20	
	125	0.316	0.396	0.537	0.333	0.339	0.588	0.214	0.542	0.346	0.432	0.233	0.281	0.328	0.133	0.283	0.314	0.375	0.128	0.175	0.269	1.000	0.975	0.900
	118	0.645	0.500	0.444	0.667	0.661	0.353	0.786	0.438	0.577	0.300	0.628	0.578	0.586	0.667	0.467	0.586	0.482	0.651	0.700	0.587	0.000	0.025	0.100
	108	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	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.039	0.104	0.019	0.000	0.000	0.059	0.000	0.021	0.077	0.068	0.140	0.141	0.086	0.167	0.250	0.100	0.143	0.221	0.125	0.144	0.000	0.000	0.000
<i>s4tdh</i>	(n)	38	24	27	15	31	17	14	24	26	22	43	32	29	30	30	35	28	43	40	52	20	20	
	300	0.092	0.042	0.019	0.067	0.113	0.206	0.000	0.021	0.019	0.045	0.035	0.016	0.086	0.067	0.100	0.029	0.196	0.093	0.075	0.038	0.000	0.000	
	230	0.908	0.958	0.981	0.933	0.887	0.794	1.000	0.979	0.981	0.955	0.965	0.984	0.914	0.917	0.900	0.971	0.804	0.907	0.925	0.962	1.000	1.000	1.000
	100	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	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	(n)	38	24	27	15	31	17	14	24	26	22	43	32	29	30	30	35	28	43	40	52	20	20	20
<i>s4tdp</i>	(n)	36	23	27	15	31	17	14	24	26	22	42	32	29	29	30	35	28	43	40	52	20	20	
	100	0.042	0.087	0.037	0.000	0.113	0.059	0.107	0.021	0.154	0.000	0.095	0.047	0.069	0.052	0.150	0.086	0.036	0.058	0.000	0.038	0.225	0.150	0.525
	118	0.000	0.304	0.093	0.133	0.048	0.118	0.000	0.021	0.038	0.045	0.000	0.000	0.000	0.000	0.000	0.014	0.089	0.070	0.013	0.000	0.775	0.450	0.075
	136	0.958	0.609	0.870	0.867	0.839	0.824	0.893	0.958	0.808	0.955	0.905	0.953	0.931	0.948	0.850	0.900	0.875	0.872	0.988	0.962	0.000	0.400	0.400
	(n)	38	24	27	15	31	17	14	24	26	22	43	32	29	30	30	35	28	43	40	52	20	20	20
<i>s4tdp</i>	(n)	38	24	27	15	31	17	14	24	26	22	43	32	29	30	30	35	28	43	40	52	20	20	
	142	0.000	0.125	0.093	0.000	0.000	0.059	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	0.975	0.800	0.975
	100	1.000	0.875	0.907	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.025	0.200	0.025
	(n)	38	23	27	15	31	17	14	24	26	22	43	32	29	30	30	35	28	43	40	52	20	20	20
	161	0.408	0.609	0.481	0.433	0.290	0.471	0.214	0.333	0.308	0.341	0.349	0.203	0.310	0.250	0.317	0.429	0.286	0.267	0.338	0.413	1.000	0.975	1.000
100	0.592	0.391	0.519	0.567	0.710	0.529	0.786	0.667	0.667	0.659	0.651	0.797	0.690	0.750	0.683	0.571	0.714	0.733	0.663	0.587	0.000	0.025	0.000	

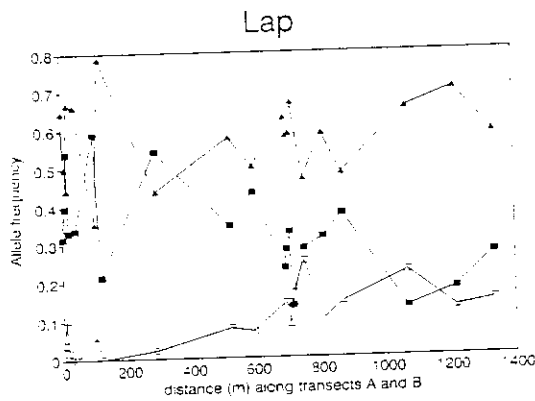


FIG. 4. Pattern of frequencies of four alleles of *Lap* along transects A and B (open squares, *Lap-100*; triangles, *Lap-118*; filled squares, *Lap-125*). *Lap-108*, which was detected in only one individual, is excluded.

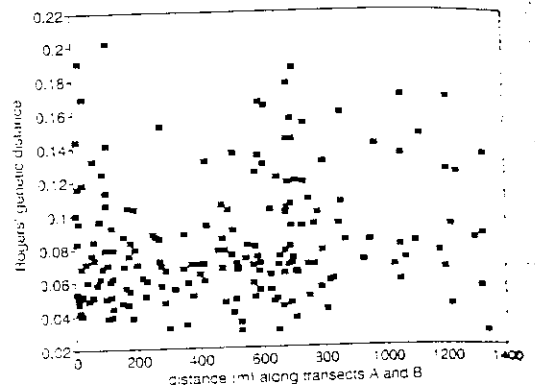


FIG. 6. Plot of Rogers' (1972) genetic distance against geographic distance for sample sites 1 to 20. The correlation is significant ( $r = 0.16$ ,  $P = 0.03$ ) but disappears when *Lap* is excluded from the data.

Many empirical studies in which direct and indirect estimates of  $Nm$  were compared show results that are incompatible: often short-term, direct studies of dispersal give an estimate of  $Nm$  that is much lower than the estimate obtained by an indirect approach (Slatkin 1985b, 1987).

A similar discrepancy is found in the present study: the value  $Nm = 5.7$  for adjacent demes would lead to the prediction of considerable genetic differentiation directly above the deme level. The indirect estimate for  $Nm$  at the lowest spatial level ( $< 10$  m, see table 3) along transect A is indeed of the same order of magnitude as the directly obtained estimate, whereas the one along transect B is somewhat higher. However, the  $Nm$  values for the spatial levels of 20–60 m and 100–230 m are similar to those for the lowest spatial level, whereas they would be expected to decrease. Only at a spatial scale in the order of kilometers is substantial genetic differentiation observed.



FIG. 5. UPGMA dendrogram of Rogers' genetic distance among samples (cophenetic correlation = 0.98).

Apparently, at the larger spatial scales, our direct estimate of  $Nm$  is too low, or our indirect estimate is too high, or both. First we discuss some factors that may make large-scale gene flow more important than our direct studies suggest.

1. *Extinction and Recolonization.* — Murphy et al. (1983 as cited in Slatkin 1985b), in explaining discrepancies in directly and indirectly estimated values for gene flow in the butterfly *Euphydryas editha*, argued that occasional extinction of demes and the subsequent recolonization may constitute an important and powerful form of gene flow. However, in view of the extremely limited powers of dispersal in *Albinaria*, extinction and recolonization may occur among nearby demes but it is difficult to envision it being of importance for long-distance gene flow.

2. *Passive Dispersal.* — Gittenberger (1991) emphasizes the possibility of passive dispersal in *Albinaria* caused by heavy winter rains that wash the snails off the rocks and down along the

TABLE 3.  $F_{ST}$  statistics and gene flow for all sampling levels.

Spatial level	Samples	$F_{ST}$ values						Mean	$Nm$
		<i>Lap</i>	<i>sAat</i>	<i>sMdh</i>	<i>Est</i>	<i>Pgdh</i>	<i>sidhp</i>		
< 10 m	1-4	0.034	0.015	0.025	0.101	0.060	0.024	0.044	5.4
	11-14	0.014	0.017	0.017	0.006	**	0.016	0.014	17.6
20-60 m	1, 4-7	0.075	0.052	0.051	0.032	0.048	0.040	0.053	4.5
	11, 14-17	0.027	0.046	0.018	0.020	**	0.017	0.024	10.2
100-230 m	1, 7-10	0.061	0.038	0.015	0.039	**	0.022	0.040	6.0
	15, 17-20	0.029	0.032	0.016	0.029	**	0.012	0.023	10.6
950-4900 m	1, 20-23	0.429	0.052	0.500	0.439	0.831	0.444	0.535*	0.2

\* Value calculated excluding the  $F_{ST}$  value for the *sAat* locus (see text for further explanation).

\*\* Locus is not polymorphic.

bare slopes. This type of dispersal may be important in *Albinaria*, but it will tend to genetically homogenize populations on steep slopes, whereas the two transects, A and B, are situated along a mountain ridge. Therefore, this form of passive dispersal is not likely to be crucial here. Other forms of passive dispersal may be postulated, such as transportation by birds or mammals, but no data exist to suggest that this type of dispersal actually takes place.

3. *Long Stretches of Suitable Habitat.*—We have shown that dispersal takes place mainly on the surface of limestone boulders. At our study sites, such boulders are relatively small and isolated. However, in other parts of the area (especially along transect B), rocky patches are more continuous and stretch for longer distances, thus creating "corridors" along which dispersal can much more easily take place (see Baur 1988 for a similar possibility in *C. clienta*). Moreover, such extended patches of suitable habitat may harbor larger demes, thus increasing the number of migrants to surrounding demes. In fact, the indirect estimates of  $Nm$  along transect B, which are about two to three times as high as those along transect A, support this idea.

Second, an important factor should be mentioned that could lead to exaggerated values for  $Nm$  as estimated from  $F_{ST}$  values: if migration among adjacent demes is indeed in the order of  $m = 0.195$ , then gene flow among demes several steps apart, will approach zero. For such low values of  $Nm$ , equilibrium values for  $F_{ST}$  are not reached for a long time. Moreover, with strongly decreasing gene flow, slight amounts of selection will have an increasing effect on allele frequencies. Therefore, on the larger spatial scales, the values for  $F_{ST}$  would not be suitable for estimating  $Nm$ , as they are no longer dependent on gene flow (Slatkin 1985b). The large-scale patterns of allele frequencies are thus expected to

depend mainly on selection and drift. The observed nonrandom pattern of the *Lap* alleles may be an example of a cline maintained by selection.

For the reasons discussed above, we expect our direct estimate of  $Nm$  ( $Nm = 5.7$  for adjacent demes) to be a slight underestimate, probably because of the existence of "corridors" of suitable habitat. This might explain the discrepancy with the indirectly obtained estimates for  $Nm$  at the lower spatial level along transect B. However, because of these low levels of gene flow, the indirect estimation of  $Nm$  at any of the higher spatial levels is not likely to produce reliable results anymore, and this may well explain the observed incompatibilities.

In summary, then, it can be stated that gene flow among demes of this (and probably other) *Albinaria* species is extremely limited, making it unlikely to act as a homogenizing force at spatial scales larger than a few tens of meters. Beyond this level, therefore, both genetic drift and natural selection may play important roles in the genetic differentiation of demes. However, the invasion of new areas by *Albinaria* may not be seriously impeded by their restricted ability for migration: in the case of a more or less regular "stepping-stone habitat," existing demes would frequently found new colonies one step ahead, thus maintaining a slow but sure invasion of the area. However the colonization of more isolated patches of suitable habitat may be a much rarer event. Such demes would be isolated for many generations and differentiating mechanisms could, in certain cases, lead to allopatric speciation.

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