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Patterns of gene flow and genetic structure in cave-dwelling crickets of the Tuscan endemic, *Dolichopoda schiavazzii* (Orthoptera, Rhaphidophoridae)

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Dolichopoda schiavazzii is a cave cricket species endemic to Tuscany, Italy. This species inhabits natural limestone caves and also man-made hypogean environments. *Dolichopoda schiavazzii* can colonize new environments both passively and actively. This species shows a metapopulational structure depending on both the cave's external bioclimatic conditions and the geographical distance. This paper reports data on 26 allozyme loci in nine populations of *D. s. schiavazzii* and in one of *D. s. caprai*, investigates their genetic structuring and provides measures of gene flow between them at different geographical scales. Some loci showed heterozygote deficiencies, probably owing to the Wahlund effect, caused by the mixing of individuals belonging to two different cohorts. Genetic subdivision is high, particularly among populations inhabiting caves located on the Tyrrhenian coast. The mean F_{ST} (θ estimator) across populations was 0.34. An analysis of the gene flow levels, carried out by comparing pairwise *Nm* values, indicates that the number of migrants drops as the geographical distance increases, suggesting the actual occurrence of gene flow only between geographically close populations in an inner area of Tuscany where the occurrence of mesophilous woods might favour migration between caves. The general picture, however, is one of a substantial lack of gene flow, even if a significant trend of isolation by distance is found, probably reflecting historical gene flow.

Keywords: allozymes, cave crickets, gene flow, population genetics.

Introduction

Over the past several years, our laboratory has carried out geographical surveys of genetic variability within a number of cave cricket species, belonging to the genus *Dolichopoda*, distributed throughout peninsular and insular Italy. These studies were carried out by combining several methodologies and approaches to elucidate patterns of adaptation, population divergence and speciation (Sbordoni *et al.*, 1985, 1987, 1991; Allegrucci *et al.*, 1992; Venanzetti *et al.*, 1993).

Most species of this genus are dependent on caves. However, several populations inhabit cave-like habitats, such as rock crevices and ravines, and individuals are sometimes observed in moist or mesic woods. *Dolichopoda schiavazzii* is one of the nine species present in Italy; it is endemic to Tuscany, being distributed throughout the western part of this region, and the two islands of Elba and Pianosa. In addition to natural limestone caves, several populations inhabit man-made hypogean environments, such as cellars, catacombs, aqueducts and Etruscan tombs. Within the species range, some populations seem to exhibit a metapopulational structure, in which gene flow could occur to some extent between geographically close demes. Unlike other *Dolichopoda* species, *D. schiavazzii* is susceptible to passive dispersal and capable of colonizing new environments. In a few artificial caves of the Argentario promontory, it coexists with *D. baccettii*, where secondary contact and hybridization pheno-

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mena have also been reported (Allegrucci *et al.*, 1982). The occurrence of *D. schiavazzii* in this area seems to result from anthropocore dispersal, probably dating back to the middle of the eighteenth century, after the foundation of the religious house 'Padri Passionisti', located near the caves. Morphologically, *D. schiavazzii* can be clearly discriminated from other *Dolichopoda* species by its spines on the femurs of the second and third pairs of legs. On this basis, a different subspecies, *D. schiavazzii caprai*, has been described with a reduced number of spines along the inferior borders of the femurs of the second pair of legs (Lanza, 1957).

A previous study (Sbordoni *et al.*, 1985) revealed the occurrence of genetic differentiation between populations of *D. schiavazzii*. On the other hand, the analysis of a species-specific satellite DNA sequence of a few populations of *D. schiavazzii* revealed a very high degree of sequence homogeneity within the species with no single fixed nucleotide substitution discriminating any populations (Bachman *et al.*, 1994). This outcome was unexpected from the early allozyme analyses that revealed substantial genetic differentiation between populations.

In order to obtain additional detailed information on the population genetic structure of *D. schiavazzii*, we increased the number of allozyme loci and populations from the island of Elba to coastal and inland Tuscany. Some populations were the same as those analysed for satellite DNA sequence. Two populations from the Argentario promontory, which were the result of human introduction (Allegrucci *et al.*, 1982), were also included, and one population of *D. s. caprai* was also studied. The population samples represented the distribution range of *D. schiavazzii*.

The object of this paper is to determine the amount of genetic divergence and levels of gene flow within this discontinuously distributed species. This is an attempt to assess the genetic structure of a species in which the process of adaptation to cave life and isolation in caves has still not been completed.

The allozyme data will also be compared with the satellite DNA sequence data.

Materials and methods

In Table 1, the populations studied are reported with details on their geographical locations, estimated population sizes and sampling dates.

Dolichopoda populations were assayed electrophoretically at 26 gene loci, coding for 21 enzymes. Details on technical procedures are the same as in Allegrucci *et al.* (1992).

Allele frequencies, Nei's (1978) genetic distance, heterozygosities and other genetic parameters were calculated using the BIOSIS-1 program of Swofford & Selander (1981). A tree was drawn using the Reynolds *et al.* (1983) index and the neighbour-joining (NJ tree; Saitou & Nei, 1987) method to highlight genetic relationships between the studied populations. Both distances and the tree were obtained using the phylogenetic package PHYLIP 3.57 (Felsenstein, 1995). Robustness of each node was evaluated by bootstrapping allele frequencies 100 times, using the program SEQBOOT in PHYLIP 3.57.

Table 1 *Dolichopoda schiavazzii* populations: basic and ecological information on the sampled caves

Population	Locality	Name	Altitude (m)	Latitude	Longitude	*N* average	Sampling date
D.s. caprai							
Fichino	Bagni di Casciana, PI	FIC	242	43.47	10.52	50	05/94
D.s. schiavazzii							
Cisternino	Cisternino aqueduct, LI	CIS	45	43.53	10.40	1877	05/94
Tomba del Belagaio	Roccastrada, GR	BEL	250	43.07	11.15	360	11/92
Necropoli di Populonia	Populonia, LI	POP	60	42.95	10.50	1500	02/92
							11/92
Grotta dei Pipistrelli	Montorsaio, GR	ORS	250	42.88	11.20	150	04/92
							11/92
Cabinovia di Marciana	Isola d'Elba, LI	MRC	350	42.78	10.17	200	04/94
Pozzetto di S. Felo	Isola d'Elba, LI	ELB	150	42.77	10.40	—	05/92
Necropoli di Vetulonia	Vetulonia, GR	VET	120	42.83	10.97	1000	11/92
Buca sopra Cimitero	M.te Argentario, GR	BSC	520	42.42	11.01	150	03/92
Convento Passionisti	M.te Argentario, GR	CPS	520	42.40	11.02	800	11/92

The coancestry index of Reynolds *et al.*, (1983) was used because it is appropriate for short-term evolution, when the divergence between populations with a common ancestral population may be regarded as being caused solely by drift. Nei's index was used to compare the present data with those from other *Dolichopoda* species.

Wright's measure of the among-population component of genetic variance, F_{ST} (Wright, 1951, 1965), was used to study the population structure of *D. schiavazzii*. Cockerham's coancestry coefficient (θ) was used as an estimator of F_{ST} (Cockerham, 1969; Weir & Cockerham, 1984).

A statistical test of the neutrality hypothesis was used to verify neutrality for the set of polymorphic loci (Slatkin, 1982). The sequential Bonferroni procedure was employed to control for the probability of incorrectly rejecting one or more true null hypotheses at a 0.05 'table-wide' level of significance (Rice, 1989).

To investigate whether isolation by distance is detectable from the data, a correlation between pairwise Nm and geographical distances was performed by using log transformations (Slatkin, 1993). The significance of the resulting regression was tested using a Mantel test between the two matrices (Mantel, 1967).

Estimates of gene flow were computed from the coancestry coefficients, assuming an infinite island model at equilibrium, as $Nm = (1-\theta)/4\theta$ (Wright, 1969).

Results

Table 2 reports allele frequencies for each population for all loci. Sixteen loci were polymorphic

Table 2 Allele frequencies at 26 allozyme loci in one population of *Dolichopoda s. caprai* (FIC) and nine of *D. s. schiavazzii*

Locus	FIC	CIS	BEL	POP	ORS	MRC	ELB	VET	BSC	CPS
*N**	10	20	10	30	25	40	18	25	10	30
Aat										
A	0.000	0.000	0.000	0.081	0.000	0.243	0.028	0.000	0.000	0.000
B	1.000	1.000	1.000	0.919	1.000	0.757	0.972	1.000	1.000	1.000
Acp										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ada										
B	0.850	0.912	0.643	0.375	0.889	0.000	0.375	0.167	0.500	0.750
C	0.150	0.088	0.357	0.281	0.111	0.861	0.625	0.750	0.500	0.179
D	0.000	0.000	0.000	0.344	0.000	0.139	0.000	0.083	0.000	0.071
Aldo										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ao-3										
A	0.286	0.375	0.167	0.341	0.559	0.440	0.143	0.342	0.187	0.444
B	0.714	0.625	0.833	0.659	0.441	0.560	0.857	0.658	0.812	0.556
Aph-1										
B	0.125	0.269	0.500	0.591	0.667	0.630	1.000	0.808	1.000	0.850
C	0.875	0.731	0.500	0.409	0.333	0.370	0.000	0.192	0.000	0.150
Enol										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Est-1										
A	0.200	0.175	0.167	0.125	0.062	0.306	0.000	0.023	0.000	0.014
C	0.800	0.825	0.833	0.875	0.937	0.694	1.000	0.977	1.000	0.986
Est-2										
A	0.550	0.700	0.167	0.062	0.038	0.733	0.900	0.000	0.000	0.028
B	0.450	0.025	0.833	0.937	0.962	0.267	0.100	1.000	0.812	0.750
C	0.000	0.275	0.000	0.000	0.000	0.000	0.000	0.000	0.187	0.222
Gal-1										
A	0.400	0.550	1.000	0.790	0.935	0.613	0.500	1.000	0.750	0.985
B	0.600	0.450	0.000	0.210	0.065	0.387	0.500	0.000	0.250	0.015

Table 2 *Continued*

Locus	FIC	CIS	BEL	POP	ORS	MRC	ELB	VET	BSC	CPS
Gal-2										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Gpi										
D	0.650	0.850	1.000	1.000	1.000	0.898	1.000	1.000	1.000	1.000
F	0.000	0.150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G	0.350	0.000	0.000	0.000	0.000	0.102	0.000	0.000	0.000	0.000
Hk										
C	0.625	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
D	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Idh-1										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Idh-2										
B	1.000	1.000	0.944	1.000	1.000	1.000	1.000	1.000	1.000	1.000
C	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lap-2										
A	0.950	0.833	0.937	0.750	0.850	0.864	1.000	0.900	1.000	0.818
B	0.050	0.167	0.062	0.250	0.150	0.136	0.000	0.100	0.000	0.182
Mdh-1										
A	0.556	0.667	0.611	0.655	0.407	0.608	0.417	0.458	0.812	0.609
C	0.444	0.333	0.389	0.345	0.593	0.392	0.583	0.542	0.187	0.391
Mdh-2										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me-3										
A	0.000	0.000	1.000	0.444	1.000	0.042	0.000	0.944	0.250	0.319
C	1.000	1.000	0.000	0.556	0.000	0.903	1.000	0.056	0.750	0.681
D	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000
Mpi										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Np										
C	0.125	0.525	0.833	0.357	0.900	0.767	1.000	0.342	0.500	0.500
D	0.875	0.475	0.167	0.643	0.100	0.233	0.000	0.658	0.500	0.500
Pep-1										
A	0.000	0.056	0.000	0.014	0.000	0.012	0.000	0.000	0.000	0.000
B	1.000	0.944	1.000	0.986	1.000	0.988	1.000	1.000	1.000	1.000
Pgm										
B	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000	0.000
C	0.944	1.000	1.000	1.000	1.000	0.987	1.000	0.963	1.000	1.000
D	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000
Pk-2										
A	0.000	0.000	0.000	0.000	0.000	0.026	0.000	0.000	0.000	0.000
B	1.000	1.000	1.000	1.000	1.000	0.974	1.000	1.000	1.000	1.000
Pt-4										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pt-5										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

*Average number of assayed individuals per locus.

within and/or among populations. Several populations had genotype frequencies at eight loci not in agreement with Hardy–Weinberg expectations, usually showing a heterozygote deficit (Table 4).

In Table 3, genetic variability estimates for all populations at 26 gene loci are reported. *Dolichopoda schiavazzii* populations showed 19–46 per cent polymorphic loci with mean observed heterozygosities ranging from 0.074 to 0.163.

Results from the Slatkin (1982) neutrality test indicated that six loci (*Ada*, *Ao-3*, *Aph-1*, *Gal-1*, *Mdh-1* and *Np*) deviated significantly from neutrality

Table 3 Genetic variability estimates of 26 loci in the studied populations of *Dolichopoda schiavazzii*

Population	$A \pm$SE	P	$H_o \pm$SE	$H_e \pm$SE
Fichino (FIC)	1.5±0.1	46.2	0.163±0.041	0.163±0.041
Cisternino (CIS)	1.5±0.1	42.3	0.150±0.039	0.151±0.039
Belagaio (BEL)	1.3±0.1	34.6	0.093±0.034	0.114±0.036
Populonia (POP)	1.5±0.1	42.3	0.124±0.035	0.166±0.044
Orsaio (ORS)	1.3±0.1	30.8	0.077±0.030	0.094±0.032
Marciana (MRC)	1.6±0.1	46.2	0.146±0.034	0.172±0.039
Elba (ELB)	1.2±0.1	19.2	0.074±0.033	0.080±0.034
Vetulonia (VET)	1.4±0.1	26.9	0.091±0.032	0.099±0.034
Buca s. Cim. (BSC)	1.3±0.1	26.9	0.130±0.049	0.115±0.040
Passionisti (CPS)	1.5±0.1	30.8	0.111±0.040	0.130±0.039

A, average number of alleles per locus; P, percentage of polymorphic loci (95% criterion); H_o and H_e, observed and expected heterozygosity.

($P \leq 0.05$). The sequential Bonferroni procedure indicated that only three of the above loci, *Ao-3*, *Mdh-1* and *Np*, actually depart from neutrality. Therefore, these three loci were removed from the genetic structure analysis (θ and *Nm* calculations) and from phylogenetic intraspecific analysis (Reynolds genetic distance).

Figure 1 shows the genetic relationships between the studied populations. The dendrogram is a consensus tree from 100 NJ trees obtained using the Reynolds *et al.* (1983) index. The two innermost populations (BEL and ORS) are the closest relatives (bootstrap value 95 per cent) and cluster with another inland population (VET, bootstrap value 65 per cent). The relationship of this cluster to the coastal populations (POP, BSC and CPS) is not well defined. The two northernmost populations (FIC and CIS) link together (bootstrap value 70 per cent) and they are the closest relatives of the island populations (ELB and MRC).

Table 5 reports Nei's (1978) genetic distance values between populations. These values range from 0.005 to 0.125.

Results from the Mantel test indicated a significant correlation ($Z = -0.643$, $P < 0.001$) between *Nm* and geographical distances, suggesting that isolation by distance cannot be rejected as a model for genetic structure in *D. schiavazzii*. The allochtonous populations in the Monte Argentario promontory were removed from this analysis. The pairwise *Nm* estimates are reported in Table 5.

The average multilocus θ value was 0.340. Removing the island populations from the analysis did not reduce this value (0.330), and it was lowered to 0.200 by excluding the northernmost populations.

Discussion

Some loci showed genotype frequencies deviating from Hardy–Weinberg proportions, generally attributable to heterozygote deficiencies (Table 4). Heterozygosity deficit can be caused by several factors, such as Wahlund effect, inbreeding, selection against heterozygotes, or some combination of these. It is difficult to determine which of these factors is responsible for the observed phenomenon. The Wahlund effect is caused by the occurrence of subunits that are present inside the samples but not detected. Like other organisms with a semivoltine cycle, *Dolichopoda* populations are known to be composed of two-yearly cohorts, characterized by individuals of different age and size (Di Russo *et al.*, 1987, 1994). A possible explanation of our finding is that the sampled individuals could belong to two separate cohorts. Therefore, the observed heterozygote deficiencies could be the result of a sample raised by nonrandom union of gametes. This hypothesis has been tested in two populations, POP and ORS, in which at least one cohort for each population (POP1 and ORS1) could be identified on the basis of sampling date and individual age. The results indicated that the loci not in H–W equilibrium in the total population were either in H–W equilibrium or, generally, had lower F-values in the subsamples (Table 4).

Genetic variability estimates (Table 3) are of the same order of magnitude as those determined for other cave organisms, including species of *Dolichopoda*. Heterozygosity is not correlated to population size, N ($r = 0.227$; $P = 0.550$), as would be expected by the neutral theory of molecular evolution. This

Table 4 *F*-values and chi-square values for each of eight of the most polymorphic loci in 10 populations of *Dolichopoda schiavazzii*

	Ada		*Aph-1*		*Est-1*		*Est-2*		*Gal-1*		*Gpi*		*Mdh-1*		*Me-3*	
	F	χ^2	*F*	χ^2	*F*	χ^2	*F*	χ^2	*F*	χ^2	*F*	χ^2	*F*	χ^2	*F*	χ^2
FIC	−0.118	0.199	−0.071	0.077	−0.188	0.450	0.040	0.018	−0.187	0.394	−0.044	0.022	0.150	0.229	0.000	0.000
CIS	−0.064	0.103	0.060	0.053	0.156	0.555	0.101	0.751	0.015	0.005	−0.147	0.508	0.215	0.897	0.000	0.000
BEL	0.711	4.27*	−0.080	0.040	0.622	4.98*	0.622	4.978*	0.000	0.000	0.000	0.000	−0.104	0.111	0.000	0.000
POP	0.177	9.01*	0.820	8.21*	−0.125	0.564	−0.033	0.034	0.138	0.633	0.000	0.000	−0.200	1.211	0.667	16.495*
POP1	0.000	0.000	0.686	3.429	−0.107	0.238	0.000	0.000	0.000	0.000	0.000	0.000	−0.222	0.957	0.606	7.741*
ORS	−0.063	0.067	0.641	5.503	0.652	14.93*	0.000	0.000	0.651	14.33*	0.000	0.000	−0.355	3.546	0.000	0.000
ORS1	−0.100	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	−0.214	0.527	0.000	0.000
MRC	−0.129	0.366	0.361	3.156	−0.162	0.980	0.472	9.942*	0.012	0.006	0.383	7.383*	0.049	0.092	−0.064	0.353
ELB	0.533	1.600	0.000	0.000	0.000	0.000	0.000	0.000	0.240	0.640	0.000	0.000	−0.444	3.778	0.000	0.000
VET	0.061	11.85*	0.286	1.286	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	−0.150	0.566	−0.039	0.061
BSC	−0.500	1.000	0.000	0.000	0.000	0.000	−0.154	0.269	−0.222	0.417	0.000	0.000	−0.154	0.269	0.375	1.432
CPS	−0.032	1.686	1.000	23.49*	0.000	0.000	0.303	2.954	0.000	0.000	0.000	0.000	−0.357	4.222*	0.559	11.672*

*$P < 0.05$.

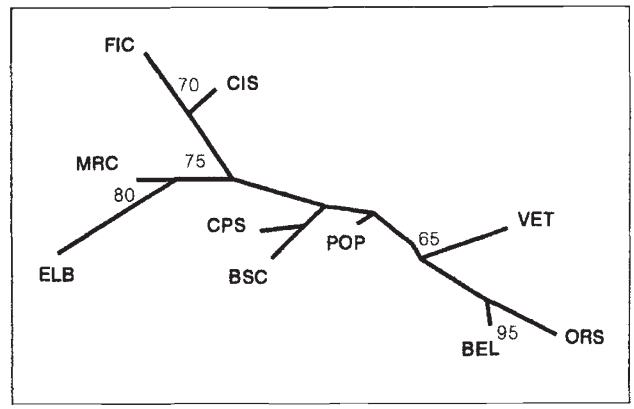

Fig. 1 NJ tree illustrating the genetic relationships between populations of *Dolichopoda schiavazzii*. Bootstrap values are from a consensus tree. Only values above 65 per cent are indicated.

confirms previous studies on other *Dolichopoda* populations and species and again raises the issue of the maintenance of high polymorphism levels in small isolated cave populations (Sbordoni *et al.*, 1987).

The analysis of genetic distance values (Table 5) suggests that, within *D. s. schiavazzii* species, the two island populations, ELB and MRC, are quite differentiated from the continental ones. The mean value of Nei's genetic distance between these groups was 0.074. This provides a rough estimation of divergence time of 368 000 years, suggesting that isolation of Elba's populations could have started during a marine transgression in the Mindel–Riss interglacial.

The population from the Fichino cave (FIC, *D. s. caprai*) is rather divergent from the others (average Nei's $D = 0.082$); this finding would corroborate its taxonomic status of subspecies (Lanza, 1957). However, the FIC population is genetically very similar to its geographically closest population, CIS (Nei's $D = 0.021$). Because the latter population is taxonomically assigned to *D. s. schiavazzii* and is located only 2 km from the type locality of *D. schiavazzii*, the status of *D. s. caprai* needs to be reconsidered.

Based on the NJ tree, topological relationships among *D. schiavazzii* populations basically resemble geographical proximities (Fig. 1). Although BSC and CPS are only 100 m apart, the bootstrap value associated with their node is quite low, probably owing to the sample sizes. However, whereas CPS is a pure *D. schiavazzii* population, the BSC sample comes from a cave where *D. schiavazzii* is sympatric with *D. baccettii*. In this cave, the two species are known to

Table 5 *Nm* values (above diagonal) and genetic distance (below diagonal) estimates (Nei, 1978) between the studied populations of *Dolichopoda schiavazzii*

	FIC	CIS	BEL	POP	ORS	MRC	ELB	VET	BSC	CPS
FIC	—	2.967	0.310	0.668	0.211	0.727	0.427	0.163	0.401	0.388
CIS	0.021	—	0.311	0.570	0.230	0.730	0.651	0.163	0.424	0.455
BEL	0.106	0.086	—	2.031	53.89	0.426	0.237	1.260	0.679	0.832
POP	0.057	0.056	0.028	—	1.219	0.861	0.588	1.087	7.517	2.826
ORS	0.125	0.100	0.008	0.038	—	0.271	0.160	0.671	0.464	0.825
MRC	0.073	0.047	0.081	0.050	0.105	—	2.546	0.367	0.885	0.494
ELB	0.093	0.052	0.091	0.078	0.107	0.022	—	0.155	0.607	0.430
VET	0.124	0.125	0.022	0.022	0.034	0.083	0.107	—	0.535	0.517
BSC	0.072	0.059	0.038	0.011	0.052	0.045	0.043	0.031	—	27.67
CPS	0.070	0.051	0.033	0.011	0.030	0.061	0.064	0.034	0.005	—

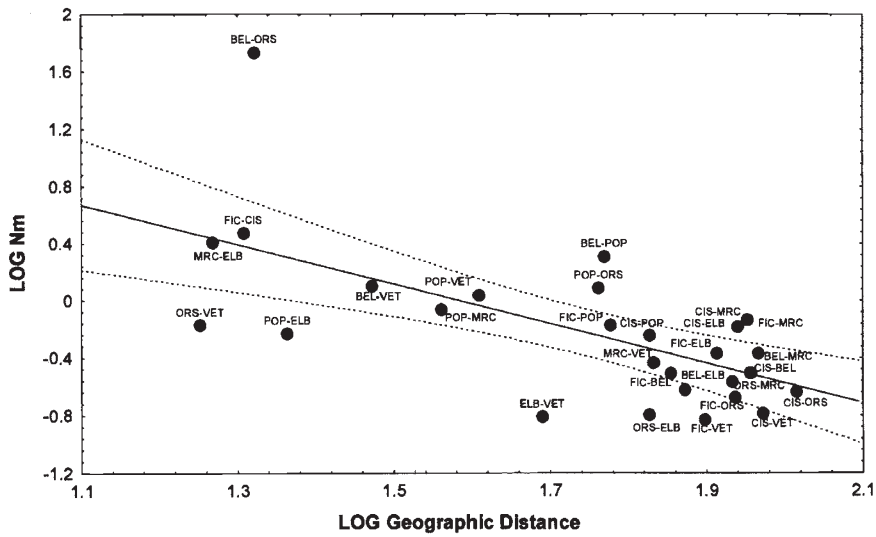

Fig. 2 Regression line based on $\log_{10}$ *Nm* values and $\log_{10}$ geographical distance (in km) for the studied populations of *Dolichopoda schiavazzii*. There is a significant correlation between the two matrices (Mantel test: $Z = -0.643$, $P \leq 0.001$). Broken lines represent 95% confidence limits.

hybridize, and some introgression of *D. baccettii* genes into *D. schiavazzii* has been documented (Allegrucci *et al.*, 1982).

Population structure analysis suggests a considerable degree of genetic structuring among populations, because about 34 per cent of the overall genetic variability is attributable to interpopulation variation. Removing the island and the northernmost populations does not lower this estimate.

At a first glance, isolation by distance seems to explain the geographical structure of the data. A statistically significant correlation is found between the log *Nm* and log geographical distance matrices, indicating a significant trend using the Slatkin method (Mantel test: $Z = -0.643$; $P \leq 0.001$; Fig. 2). To establish whether this apparent migration traces historical and/or actual gene flow, we extend the analysis on the *Nm* values. We used *Nm* pairwise estimates between insular and continental populations to calibrate *Nm* values. This permits quantification of lower threshold values, ranging from 0.155 to 0.730 (Table 5), in which the existence of any present gene flow can be excluded. On the other hand, the estimated *Nm* value between the two populations (BSC and CPS) in the Monte Argentario promontory, which are only 100 m apart, clearly reflects the existence of substantial gene flow presently occurring between them ($Nm = 28$, Table 5). As already outlined, the occurrence of *D. schiavazzii* in this area, where the autochtonous *D. baccettii* occurs, most probably results from anthropocore dispersal (Allegrucci *et al.*, 1982). The presence of *D. schiavazzii* in the Monte Argentario promontory seems to be limited to these two caves, and the two collection samples probably represent the same population with the limitation owing to introgression

outlined before. Moreover, a rather extensive amount of biological information on *Dolichopoda* cave crickets has been gathered over the last 20 years for microevolutionary studies. Such studies, carried out on the *D. laetitiae–geniculata* complex, suggest that the overall geographical variation of allele frequencies is mainly influenced by the degree of isolation of populations, depending more on the different bioclimatic and vegetational conditions occurring outside the caves than on the geographical distance between them (Sbordoni *et al.*, 1985, 1987, 1991). From this view, actual gene flow cannot be accepted among populations inhabiting coastal caves, even if *Nm* values are >1. The occurrence outside the caves of the dry Mediterranean 'macchia' prevents the occurrence of *Dolichopoda* outside caves, eliminating gene flow among cave populations. This is the case with the POP and CIS populations analysed in this study. Moreover, data obtained from populations of other *Dolichopoda* species suggest that ongoing exchange among populations can be safely accepted only when *Nm* values are $>>1$ (unpublished data). The highest *Nm* value has been found between the BEL and ORS populations ($Nm = 54$, Table 5). These are geographically rather close and inhabit caves surrounded by mesophilous woods that might favour migration between them. Based on this *Nm* value and records of *Dolichopoda* from similar surface environments (extensive data from pitfall traps in mesophilous woods, unpublished data), we argue that gene flow is presently occurring between these two populations.

In conclusion, isolation by distance seems to represent a powerful tool for investigating population structures, but it needs to be carefully evaluated. *Nm* values >1 obtained in this study, except for the case discussed above, may be more indicative of past gene flow rather than reflecting actual gene exchange among populations.

Interestingly, a number of the *D. schiavazzii* populations analysed in this study have also been investigated for a satellite DNA family specific to this cave cricket species. A sequence comparison of 31 clones from three isolated populations revealed a very high degree of sequence homogeneity within the species, with no evidence of any specific population feature (Bachmann *et al.*, 1994). This result appeared to contrast with the allozyme data, which revealed genetic differentiation among populations. This discrepancy could be reconciled by considering the different genetic nature of the two markers. Satellite DNA is a class of noncoding DNA typically organized in large tandemly arranged repeat units. The evolutionary forces shaping its variation may be substantially different from those controlling allozymes (usually located in a single-copy region). The high mutation rate of satellite DNA, both intraindividually and interpopulationally, and the high homogenization mechanisms controlling the distribution of this variation (i.e. concerted evolution) may be responsible for the lack of genetic differentiation between populations revealed by this marker.

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References

ALLEGRUCCI, G., CACCONE, A., CESARONI, D., COBOLLI SBORDONI, M., DE MATTHAEIS, E. AND SBORDONI, V. 1982. Natural and experimental interspecific hybridization between populations of *Dolichopoda* cave crickets. *Experientia*, **38**, 96–98.

ALLEGRUCCI, G., CACCONE, A., CESARONI, D. AND SBORDONI, V. 1992. Evolutionary divergence in *Dolichopoda* cave crickets: a comparison of single copy DNA hybridization data with allozymes and morphometric distances. *J. Evol. Biol.*, **5**, 121–148.

BACHMANN, L., VENANZETTI, F. AND SBORDONI, V. 1994. Characterization of a species-specific satellite DNA family of *Dolichopoda schiavazzii* (Orthoptera, Rhaphidophoridae) cave crickets. *J. Mol. Evol.*, **39**, 274–281.

COCKERHAM, C. C. 1969. Variance of gene frequencies. *Evolution*, **23**, 72–84.

DI RUSSO, C., VELLEI, A., CARCHINI, G. AND SBORDONI, V. 1987. Life cycle age structure of *Dolichopoda* populations (Orthoptera, Raphidophoridae) from natural and artificial cave habitats. *Boll. Zool.*, **54**, 337–340.

DI RUSSO, C., CARCHINI, G. AND SBORDONI, V. 1994. Life-history variation in *Dolichopoda* cave crickets. In: Danks, H. V. (ed.) *Insect Life-cycle Polymorphism*, pp. 205–226. Kluwer Academic, Netherlands.

FELSENSTEIN, J. 1995. *PHYLIP (Phylogeny Inference Package)*, Version 3.57c. Distributed by the author. Department of Genetics, University of Washington, Seattle.

LANZA, B. 1957. Speleofauna toscana. IV. Nuove notizie sulla *Dolichopoda schiavazzii* Capra. *Monit. Zool. Ital.*, **64**, 159–167.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res.*, **27**, 209–220.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

REYNOLDS, J. WEIR, B. S. AND COCKERHAM, C. C. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, **105**, 767–779.

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

SAITOU, N. AND NEI, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.

SBORDONI, V., ALLEGRUCCI, G., CESARONI, D., COBOLLI SBORDONI, M. AND DE MATTHAEIS, E. 1985. Genetic structure of populations and species of *Dolichopoda* cave crickets: evidence of peripatric divergence. In: Sbordoni, V. (ed.) *Contact Zones and Speciation. Boll. Zool.*, **52**, 139–156.

SBORDONI, V., ALLEGRUCCI, G., CACCONE, A., CARCHINI, G. AND CESARONI, D. 1987. Microevolutionary studies in Dolichopodinae cave crickets. In: Baccetti, B. (ed.) *Evolutionary Biology of Orthopteroid Insects*, pp. 514–540. Ellis Horwood, Chichester.

SBORDONI, V., ALLEGRUCCI, G. AND CESARONI, D. 1991. A multidimensional approach to the evolution and systematics of *Dolichopoda* cave crickets. In: Hewitt, G. (ed.) *Molecular Techniques in Taxonomy*. NATO Asi Series, vol. H57, pp. 171–179. Springer Verlag, Berlin.

SLATKIN, M. 1982. Testing neutrality in subdivided populations. *Genetics*, **100**, 533–545.

SLATKIN, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

SWOFFORD, D. AND SELANDER, R. B. 1981. *BIOSYS-1. A Computer Program for the Analysis of Allelic Variation in Population Genetics and Biochemical Systematics.* Release 1.7 University of Illinois, Urbana, IL.

VENANZETTI, F., CESARONI, D., MARIOTTINI, P. AND SBORDONI, V. 1993. Molecular phylogenies in *Dolichopoda* cave crickets and mtDNA rate calibration. *Mol. Phyl. Evol.*, **2**, 275–280.

WEIR, B. S. AND COCKERHAM, C. C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

WRIGHT, S. 1951. The genetical structure of populations. *Ann. Hum. Genet.*, **15**, 323–354.

WRIGHT, S. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution*, **19**, 395–420.

WRIGHT, S. 1969. *Evolution and the Genetics of Populations*, vol. 2, *The Theory of Gene Frequencies.* University of Chicago Press, Chicago.