

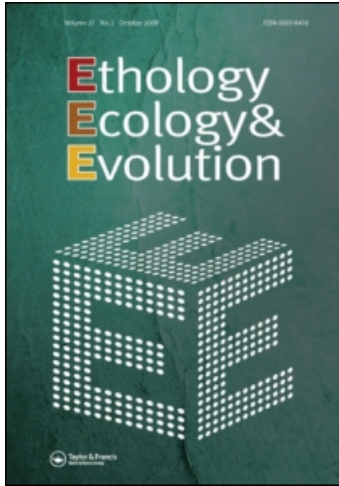
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Variation of genetic and behavioural traits in the sandhopper *Talitrus saltator* (Crustacea Amphipoda) along a dynamic sand beach

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In an attempt to unravel the possible relationships among coastal stability, genetic variability and orientation behaviour in the sandhopper *Talitrus saltator*, we have screened four subpopulations of this species. Sandhoppers along 3 km of a dynamic sand beach were studied for orientation performance (i.e. adherence to the seaward theoretical escape direction (TED)) and sequence variation for a fragment of the mitochondrial (mt) gene encoding for the cytochrome oxidase subunit I (COI). The sampling sites differed in the stability of the shoreline (from severely eroded to accreting). Analysis of molecular variance (AMOVA) revealed significant subdivisions among the four locations. The subpopulation sampled at the most eroded point showed no haplotype diversity and had the highest scatter in orientation. Genetic diversity increased with decreasing erosion. Orientation performances were best where the beach is in dynamic equilibrium, and were progressively more scattered at the accreting points. Multiple linear regression analyses demonstrated that the variables and factors that affected orientation significantly were daytime, global solar radiation, sex of individuals and haplotype diversity. Environmental factors are likely to influence sandhopper behaviour on a short and medium time scale (i.e. from single individual life span to a few generations). The data also suggest that temporal stability of the shoreline has a positive effect on the genetic variability of the resident sandhopper populations.

KEY WORDS: *Talitrus saltator*, orientation, mitochondrial DNA, cytochrome oxidase I, sandy beach.

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INTRODUCTION

Organisms exist in a state of dynamic equilibrium with their environment. Initial responses to environmental change involve changes in animal behaviour, which are immediate and overt. In contrast, changes in the genome occur over a much longer time-scale, and are only evident following molecular analysis (SCAPINI 2002). Some estimate of intra-specific variation in genotype and phenotype is required if we are to understand the way in which animal populations adjust to environmental changes. In this paper we describe genetic and behavioural variation in four sub-populations of the sandhopper *Talitrus saltator* (Amphipoda) in light of the erosion/accretion dynamics of the shoreline of a single extended sand beach located in southern Tuscany (Italy). We selected this species because it is a key species of sandy beach communities and has a suite of features that makes it a very interesting organism for evolutionary studies.

Sandhoppers, though occurring on land, respire branchially. Their gills need a film of water in order to work. However, efficient morphological and physiological adaptations to prevent dehydration are lacking. Consequently, environmental humidity is the main limiting factor, especially in summer, when sand surface temperature may reach 60 °C. Talitrid amphipods have developed behavioral adaptations to avoid these hostile conditions (SCAPINI et al. 1988; NAYLOR 1989; SCAPINI 1997). *T. saltator* is widespread around the Mediterranean Sea and up to north Atlantic European shores. During the last decade, the species has been studied extensively with regard to its ecology, population dynamics, behaviour and population genetics (SCAPINI et al. 1992; DE MATTHAEIS et al. 1994, 1995, 1998, 2000; SCAPINI 1997, 2002; ANASTÁCIO et al. 2003; FALLACI et al. 2003; KETMAIER et al. 2003, 2005; MARQUES et al. 2003; NARDI et al. 2003).

T. saltator typically lives above the high tide marks, where it is concentrated near the waterline in summer, but dispersed up to the dune zone in winter (FALLACI et al. 2003). Individuals migrate daily across the beach to feed (SCAPINI et al. 1992, 1997). Where tides have small ranges, as is the case in parts of the Mediterranean Sea, sandhoppers move landwards beyond high water to forage at night, but they navigate back to the supralittoral zone before dawn (SCAPINI et al. 1992, 1997). This activity has a circadian rhythm (NARDI et al. 2003). When the sand dries up for increasing insulation, and/or animals are removed from the substratum during the day, they regain the wet zone seawards (SCAPINI et al. 1997; BORGIOLI et al. 1999). The expected orientation seawards is called the theoretical escape direction (TED); it is assumed to be perpendicular to the coastline and changes according to the orientation of the shoreline. Thus, this trait is typical for each single population (SCAPINI & FASINELLA 1990). The better individuals adhere to the TED, the higher the chances for population maintenance in time through generations. SCAPINI et al. (1995), BORGIOLI et al. (1999) and ELGTARI et al. (2000) found that populations orient significantly seawards around the TED on beaches with dune belts stable and/or in accretion and a coastline with no signs of recession and/or accretion. On the other hand, populations living on eroded beaches or on beaches impacted by constructions and/or human frequentation show a more scattered distribution around the TED (SCAPINI et al. 2005). A likely explanation for these results is that adaptation to a given environment requires time, and only populations living on relatively stable coasts can persist long enough to reach a good match between their intrinsic features and those of the beach (SCAPINI 2006).

Extensive allozyme data exist for *T. saltator*. They reveal a deep phylogeographic break in the genetic structuring of the species at the scale of the Mediterranean Sea coasts. Tyrrhenian, Adriatic, and Aegean populations are highly differentiated from each other (DE MATTHAEIS et al. 1994, 1995, 1998, 2000; KETMAIER et al. 2003). On a

macro- and meso-geographical scale the species is likely to have reached migration-drift equilibrium under a pattern of isolation by distance with low levels of gene flow (DE MATTHAEIS et al. 2000; KETMAIER et al. 2003). A preliminary study based on mitochondrial DNA (mtDNA) confirmed the allozymic results (KETMAIER et al. 2005).

In an attempt to examine the possible interplay between genetic and behavioural traits, SCAPINI et al. (1995) analysed simultaneously 19 allozyme loci and the adherence to TEDs in 13 sandhopper populations, sampled from different beaches on the Tyrrhenian and Adriatic coasts along the Italian peninsula. The authors found significant positive correlations between shoreline stability mean seaward orientation and mean heterozygosity. These results imply that the temporal stability of beaches may affect both phenotypic and genotypic traits. Allozymes proved to be useful to assess differences among geographically distinct populations, but the relatively low variability of these markers prevented an in-depth analysis (i.e. at the intra-population level).

To pursue such an intra-population analysis and to investigate further the possible relationships among shoreline stability, orientation and genetic variation, we screened for orientation performance and mtDNA polymorphism a single population of *T. saltator* along an extended shore. The chosen beach shows a clear erosion/accretion gradient (see the following section) and therefore offers a unique opportunity to estimate variation in behaviour and genetic traits within a single population along such a gradient. As a genetic marker we used the mtDNA gene encoding for the cytochrome oxidase subunit I (COI). The fragment we sequenced encompasses most of the highly variable domains of the gene (LUNT et al. 1996). Recently, we found this fragment to be highly variable in *T. saltator* (KETMAIER et al. 2005), thus enhancing our confidence in the usefulness of this marker on a micro geographical scale. It is important to emphasise that the samplings for the genetic analyses were contemporary with the samplings for the acquisition of behavioural and ecological data to avoid the introduction in the study of possible biases due to seasonality and temporal effects of morphodynamic changes.

We designed this study to test whether: (a) subpopulations at distinct locations along the beach show diverse orientation performances as an adaptive response to differences in the dynamics of the shoreline; (b) the subpopulations sampled along the beach are genetically distinct from each other; and (c) variations of behavioural and genetic traits parallel each other or not. According to SCAPINI et al. (1995), we would expect subpopulations from stable or gradually accreting sections of the beach to show a better adherence to TED and a higher level of genetic polymorphism than subpopulations sampled where the shoreline shows signs of erosion. Our ultimate goal is to combine geomorphological (shoreline dynamics), genetic (mtDNA polymorphism) and behavioural (orientation) data to obtain a multidisciplinary picture of the relationships of the species with the characteristics of the environment, namely the morphodynamic changeability.

MATERIALS AND METHODS

Study sites

The animals tested were sampled at four points along 3 km on the dynamic sandy beach of Collelungo (Maremma Regional Park, Grosseto, Tuscany, Italy). For simplicity, we will designate individuals sampled at each point as belonging to different subpopulations. The Collelungo beach extends over about seven kilometers; we selected this beach because it is a relatively closed system, located between two geographical barriers (the Ombrone river mouth and the Collelungo rocky

promontory; Fig. 1). The points are located at about 3, 4, 5 and 6 km from the Ombrone river mouth (Fig. 1; nearer to the mouth at 0, 1, and 2 km, no talitrids were found) and they differ considerably in the erosion/accretion dynamics. The beach-dune is severely eroded at point C3 (3 km), well developed at point C4 (4 km), has an accreting foredune at point C5 (5 km) and it is of new formation and backed by marshes at point C6 (6 km) (INNOCENTI & PRANZINI 1993). Detailed descriptions of beach mean slope, width, substrate characteristics and TED for each point are presented elsewhere (COLOMBINI et al. 2005; SCAPINI et al. 2005) and are also summarised in Table 1.

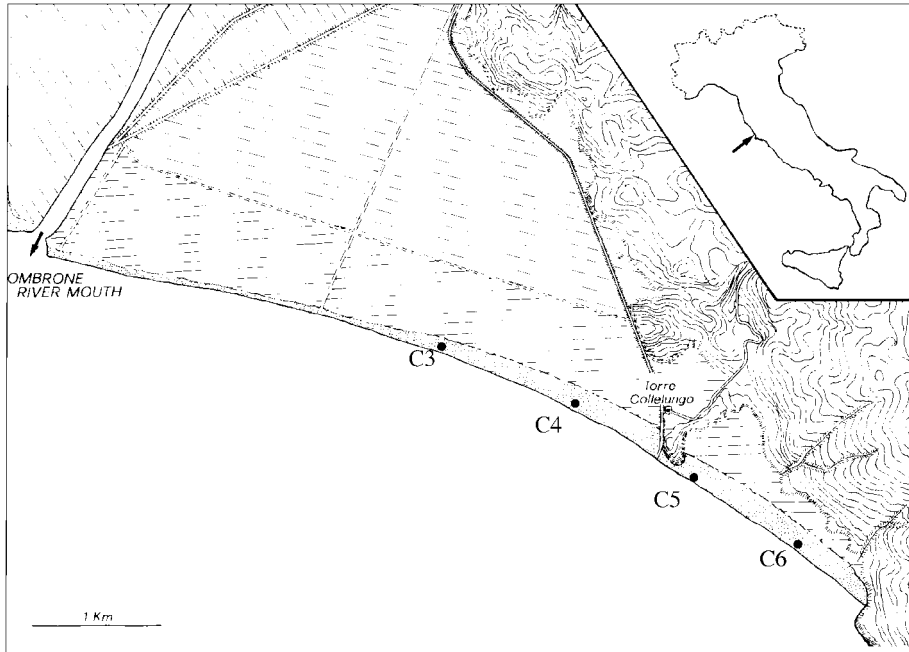


Fig. 1. — Schematic map of the Collelungo beach with an inset showing its location with respect to Italy. Circles with different shadings identify the four collecting sites; see Table 1 for details on environmental parameters for each site.

Table 1.

Environmental parameters at the four study sites along the Collelungo beach. We considered the mean slope (MS), width (W) and theoretical escape direction (TED). The sand parameters were calculated following standard techniques (FOLK & WARD 1957). Data from COLOMBINI et al. (2005).

Distance from the Ombrone river mouth (km)	Code	MS (%)	W (m)	TED (°)	Sand moisture (%)	Sand size (φ)
3	C3	9.8	13	205	1.211 ± 0.792	1.755 ± 0.011
4	C4	3.8	22	205	1.272 ± 0.778	1.900 ± 0.026
5	C5	1.8	34	210	1.273 ± 0.705	2.234 ± 0.033
6	C6	1.6	42	215	3.585 ± 1.279	2.538 ± 0.015

Behavioural experiments

At each point (C3, C4, C5, and C6) sandhoppers were collected using pitfall traps placed above the water's edge the night before each test. Animals were kept in containers filled with moist sand prior to testing. Four teams carried out the orientation tests at the four sites simultaneously to obtain quantitative non-biased estimates of orientation. For the experiments we used circular orientation chambers, which permitted the vision of the sun and sky but not that of landscape (SCAPINI 2002). The theoretical sampling effort was designed to have 40 adult individuals tested in each experiment; these were subdivided in 4 groups of 10 released in the arena every 15 min. It is important to point out here that because of the methodological approach adopted, the theoretical (as expected on the basis of the sampling effort) and the actual number of individuals tested may vary. These eventual differences result from the experimental protocol itself, which is based on releasing the collected animals in the centre of the orientation chamber. This discourages us to make samplings perfectly even as any additional manipulation would stress the animals (and thus possibly affect their behaviour), plus it would introduce an undesirable a priori selection of individuals (SCAPINI 2006). For these reasons, and also to avoid any a posteriori selection of data, we present in Table 2 the total number of animals tested at each site. We repeated this 1 hr session twice a day (once in the morning and once in the afternoon) for 3 consecutive days (19–21 May 2003). The angles of orientation with respect to North were registered from the traps placed at the circumference of the orientation chamber, where the individuals were caught after each release. During the tests we measured the following weather variables: air temperature and humidity, global radiation, solar time of the releases from which sun azimuth was calculated. After the test we checked the sex of each animal tested and measured its cephalic length as an estimate of size (MARQUES et al. 2003) and the number of tagma of the second antennae as an estimate of age (WILLIAMS 1987).

Angular distributions relative to TED were analysed for each point using the statistics of circular distributions (SCAPINI et al. 2005). We calculated mean angles, mean resultant lengths, sample circular dispersions and confidence intervals (95%) of the mean angle for each angular distribution (FISHER 1993).

Genetic analyses

We sampled a total of 70 individuals from the Collelungo beach (10 at point C3 and 20 each at points C4, C5, and C6). We applied the same sampling effort at each site, with animals collected using an aspirator or by hand over a 2 hr time span. The sampling for the genetic analyses was conducted independently (but on the same days) from that for the behavioural experiments,

Table 2.

Summary statistics of orientation experiments at increasing distances from the Ombrone river mouth. TED is the theoretical escape direction, MAO is the mean angle of orientation, MRL is the mean resultant length, CD is the circular dispersion of samples, CI is the confidence interval of the mean direction and N is the number of individuals tested at each study point. MRL and CD give information on the concentration of individuals; MRL may vary between 0 (all individuals scattered in all directions) and 1 (all individuals oriented in the same direction).

Subpopulation	TED (°)	MAO (°)	MRL	CD	CI (°)	N
C3	205	179.6	0.3409	3.984	163.1–196.0	191
C4	205	189.5	0.6493	0.738	183.3–195.6	250
C5	210	214.5	0.6464	0.743	208.1–220.9	231
C6	215	192.8	0.4784	2.100	182.4–203.3	245

with no replicates. This prevented the introduction of biases in the study in that: (i) individuals analysed genetically were not a priori selected; and (ii) behavioural and genetic variables were truly independent from one another. The latter point is particularly important for the correctness of the correlation analyses of environmental, behavioural and genetic data (see below). To make sure that we are sampling the genetic data appropriately and to confirm our previous allozymic and mtDNA results (see references in the Introduction), we included in the study one Tunisian (Rtiba beach, Mediterranean Sea; RT), one Moroccan (Kabyla beach, Mediterranean Sea; KB) and one Polish (Hel Peninsula, Baltic Sea; PL) population of *Talitrus saltator* (3 individuals each). As an outgroup in the phylogenetic analyses we used 3 individuals of *Deshayesorchestia deshayesii*, a talitrid species closely related to *T. saltator* (DE MATTHAEIS et al. 2000; TAFANI et al. 2004). Total DNA was extracted from ethanol-preserved specimens, using the Easy-DNA kit from Invitrogen (Invitrogen, CA). PCR amplifications and sequencing of about 400 base pairs (bp) of the cytochrome oxidase I (COI) gene were carried out following KETMAIER et al. (2005). Strands were sequenced in both directions for each individual.

Sequences were edited using Sequencher 4.6 (Gene Code Corporation, Ann Arbor, MI) and aligned by eye following the guide provided by the reading frame. We constructed a haplotype network based on the statistical parsimony method of TEMPLETON et al. (1992) as implemented in the program TCS 1.13 (CLEMENT et al. 2000). We also analysed data phylogenetically by neighbor-joining (NJ); (SAITOU & NEI 1987) and Bayesian methods (RANNALA & YANG 1996; MAU & NEWTON 1997; LARGET & SIMON 1999; MAU et al. 1999; HUELSENBECK 2000) to derive a haplotype tree. We used MODELTEST (POSADA & CRANDALL 1998) to determine the best model of sequence evolution, and in the subsequent calculation of maximum likelihood (ML) distances for NJ analyses. For the Bayesian approach, we employed the same models of sequence evolution, allowing site-specific rate variation partitioned by codon positions. MRBAYES was run for 2 million generations with a sampling frequency of 100 generations. We ran one cold and three heated Markov chains. To establish if the Markov chains had reached stationarity, we plotted the likelihood scores of sampled trees against generation time. Trees generated before the stationarity phase were discarded as "burn-in" (first 10% of the sampled trees), and posterior probability values for each node were calculated based on the remaining 90% of sampled trees. These trees were used to construct a 50% majority rule consensus tree using PAUP* 4.0 β 10 (SWOFFORD 2003). The robustness of the NJ phylogenetic hypotheses was tested by 1000 bootstrap replicates (FELSENSTEIN 1985).

We used ARLEQUIN 3.0 (EXCOFFIER et al. 2005) to calculate the following parameters of genetic diversity: haplotype diversity (h), mean number of pairwise differences between all pairs of haplotype (π), and the nucleotide diversity (π_n). Levels of genetic diversity were tested by a hierarchical analysis of molecular variance (AMOVA) (EXCOFFIER et al. 1992) using ARLEQUIN 3.0 (EXCOFFIER et al. 2005) with 1000 permutations. We carried out multiple AMOVA analyses on sequences arranged as follows: (1) subpopulations from points C3, C4, C5, C6 (a single group); and (2) all subpopulations pooled together + RT, KB, PL. In the latter case we grouped populations into three groups: Collelungo beach, North African populations (RT + KB) and the Polish population (PL). To test for past changes in the demographic history of the Collelungo population, we carried out a mismatch analysis (SCHNEIDER & EXCOFFIER 1999) as implemented in ARLEQUIN 3.0 (EXCOFFIER et al. 2005). Also for this analysis, we either considered each subpopulation separately or we pooled them together.

Correlation analysis of environmental, behavioural and genetic data

Multiple linear regression models (SPLM, spherical projected linear models, adapted for angular data; MARCHETTI & SCAPINI 2003) were used to estimate the effects of individual variation (sex, size and age), population genetics (number of haplotype, H), weather (air temperature and humidity and sun global radiation) and astronomic (azimuth) variables to orientation (angular response variable).

The beach and sand characteristics are related to the beach morphodynamics and may influence population abundance, but obviously did not vary throughout the orientation experiments.

The factor number of haplotypes (H) includes site differences, which in turn may have affected genetic variability. The variables were checked for linear correlations and, when these were found, we selected the more informative variable for the model. We started with a model containing all variables that could affect orientation and compared models according to the Akaike information criterion (AIC) (AKAIKE 1974), ending to a best model with a minimum number of variables and best likelihood. The effects of single factors and variables were then estimated by comparing the best model with a simpler one not containing the factor or variable. The difference between the likelihood of the two nested models gave the probability levels for the factor or variable under test (MARCHETTI & SCAPINI 2003).

RESULTS

Behavioural descriptors

Results of the orientation tests are summarised in Table 2 (see Materials and methods for eventual differences between theoretical and actual sample sizes). All distributions were significantly different from the uniform distribution according to the Rayleigh test ($P < 0.05$). The mean angles of orientation (MAO) changed slightly along the beach. These changes did not completely match those of the TED. Indeed, at point C5 we detected the most westward oriented angle for MAO (214.5°) but not for TED (210° ; TED was 215° at C6). The confidence intervals of the mean direction did contain the TED at point C5 only, showing significant deflections southwards at the other points along the shoreline (Table 2). The mean resultant lengths (MRL), which indicate the degree of concentration of the angular distribution in the tested samples, were low at point C3, intermediate at point C6 and high at points C4 and C5; the circular dispersion (CD) values varied accordingly (Table 2).

Genetics

We sequenced 407 bp of the COI gene (both strands) for each of the 80 individuals included in the study; all sequences were deposited in GenBank (Accession numbers EF535006-EF535026). No indels were found in the alignment and no stop codons were observed in the sequences. These results, together with the excess of As (28.8%) and the anti-Gs bias in 3rd codon positions (6.1%), are typical of the mitochondrial genome. A χ^2 test for base homogeneity across taxa suggests that base frequencies do not deviate from the expected values ($P = 1$ for all codon positions combined and for each codon position separately). These sequences defined a total of 20 unique haplotypes for *Talitrus saltator*, 17 of which were found at Collelungo. Haplotype frequencies are given in Table 3. We detected no haplotype variation within the Tunisian, Moroccan and Polish populations (but only three individuals each were sequenced in these cases). *T. saltator* haplotypes differed from each other from 1 to 88 substitutions; the mean number of pairwise substitutions among the haplotypes found at the Collelungo beach was 10.946 ± 5.036 , but this value drops to 3.901 ± 1.982 when haplotype 17 is removed from the analysis (Table 4; this table also shows for each subpopulation comparisons of various measures of molecular diversity calculated alternatively including or excluding haplotype 17). Indeed, haplotype 17 (found at both C5 and C6) shows a remarkably high number of substitutions when compared to the average number of differences we found among haplotypes 1–16. These differ by 1 to 8

Table 3.

Absolute haplotype frequencies for all sequenced individuals of *T. saltator*. C3–6 are the different localities sampled at the Collelungo beach (Fig. 1); RT, KB and PL identify Tunisian, Moroccan and Polish collecting sites, respectively.

Hapl./Subpop.	C3	C4	C5	C6	RT	KB	PL
1	10	5	5	2	0	0	0
2	0	4	0	0	0	0	0
3	0	6	0	0	0	0	0
4	0	3	0	0	0	0	0
5	0	2	0	0	0	0	0
6	0	0	2	0	0	0	0
7	0	0	3	0	0	0	0
8	0	0	4	0	0	0	0
9	0	0	4	0	0	0	0
10	0	0	0	3	0	0	0
11	0	0	0	2	0	0	0
12	0	0	0	2	0	0	0
13	0	0	0	3	0	0	0
14	0	0	0	3	0	0	0
15	0	0	0	2	0	0	0
16	0	0	0	1	0	0	0
17	0	0	2	2	0	0	0
18	0	0	0	0	3	0	0
19	0	0	0	0	0	3	0
20	0	0	0	0	0	0	3

substitutions, while haplotype 17 differs from them by 59 to 63 substitutions. It is worth noting that these numbers are in the range of variation found among geographically distant populations (i.e. haplotypes 1–17 differ by 77 to 88 substitutions from Tunisian, Moroccan and Polish populations, while differences among North African and Polish haplotypes range from 39 to 47 substitutions). Measures of molecular diversity reveal a progressive increase in genetic variability from C3 to C6 (Table 4). We found no haplotype diversity at point C3; levels of genetic variability are comparable between C5 and C6, while they are substantially lower at point C4.

Fig. 2 shows the Bayesian tree based on the TIM + Γ model of sequence evolution ($\alpha = 0.426$ model chosen with MODELTEST) and summarises the results of the NJ search based on ML distances (the same model of sequence evolution as in the Bayesian analyses). Bayesian and NJ methods gave fully compatible trees with similar statistical supports. Haplotypes from the Collelungo beach cluster together in a strongly supported clade and there is a remarkable support for the placement of Morocco and Tunisian populations basal to it; these two populations are placed as each other's closest

Table 4.

Estimates of molecular diversity for the *T. saltator* population from the Collelungo beach calculated both for each subpopulation separately and for all subpopulations combined. For points C5, C6, and for all subpopulations combined, estimates of molecular diversity have been alternatively calculated on all haplotypes (all hapl.) and also excluding the highly divergent haplotype 17 (hapl. 17 excl.). The sample size (n), the number of haplotypes (H), the haplotype diversity (h), the mean number of pairwise differences between all pairs of haplotypes (π) and the nucleotide diversity (π_n) are given.

Subpopulation	n	H	h	π^a	π_n^a
C3	10	1	NA	NA	NA
C4	20	5	0.816 ± 0.041	2.662 ± 1.480	0.007 ± 0.004
C5 (all hapl.)	20	6	0.858 ± 0.036	16.984 ± 7.888	0.041 ± 0.021
C5 (hapl. 17 excl.)	18	5	0.830 ± 0.049	4.941 ± 2.522	0.012 ± 0.006
C6 (all hapl.)	20	9	0.926 ± 0.026	17.164 ± 7.968	0.042 ± 0.021
C6 (hapl. 17 excl.)	18	8	0.815 ± 0.031	5.084 ± 2.587	0.012 ± 0.007
All subpopulations (all hapl.)	70	17	0.879 ± 0.030	10.946 ± 5.036	0.026 ± 0.013
All subpopulations (hapl. 17 excl.)	66	16	0.867 ± 0.033	3.901 ± 1.982	0.010 ± 0.005

^aWe used the TAMURA & NEI (1993) distance correction with equal rates among sites to calculate π and π_n ; model selected by MODELTEST (POSADA & CRANDALL 1998) for the data set including only the 17 haplotypes from the Collelungo beach.

relatives. Within the Collelungo population the highly divergent haplotype 17 is placed basal; all the remaining haplotypes cluster together with maximum supports for both Bayesian and NJ analyses. Very little structuring by the geographic origin of haplotypes 1–16 is revealed by these analyses. The network analysis based on haplotypes 1–16 (Fig. 3) yielded similar results. This genealogy has a typical star-like pattern; the most common haplotype 1 lies at the centre and is connected by independent mutational steps to other haplotypes with much lower frequencies. Missing haplotypes are always necessary to connect haplotype 1 to the remaining ones but for the link between haplotypes 1 and 5. Fig. 3 also shows that haplotype 1 is the only one shared among different subpopulations; all other haplotypes are unique to single subpopulations.

Table 5 shows the results of the hierarchical analysis of molecular variance (AMOVA) conducted on different groupings of populations. This analysis reveals a high degree of genetic structuring either when all populations are pooled together ($\Phi_{ST} = 0.203$; $P < 0.001$) or when haplotypes from Collelungo beach are analysed separately ($\Phi_{ST} = 0.190$; $P < 0.001$). In both cases the vast majority of detected variation is due to differences within (sub-) populations (80.93% and 75.74%) rather than among them (17.99% and 19.07%, respectively).

Mismatch analyses support a model of sudden population expansion for point C4 (graphs of mismatch distribution not shown). For this point we obtained a moment estimator of time to the expansion $\tau = 3.914$ and a mismatch observed mean = 2.621; the probability of observing a less good fit between the model and the observed distribution by chance P_{SSD} (SSD = sum of squared deviations) is $P_{SSD} = 0.13$. The hypothesis of a sudden population expansion is rejected at points C5 and C6 ($P_{SSD} < 0.001$) and when all subpopulations are analysed simultaneously ($P_{SSD} = 0.02$). The results of the

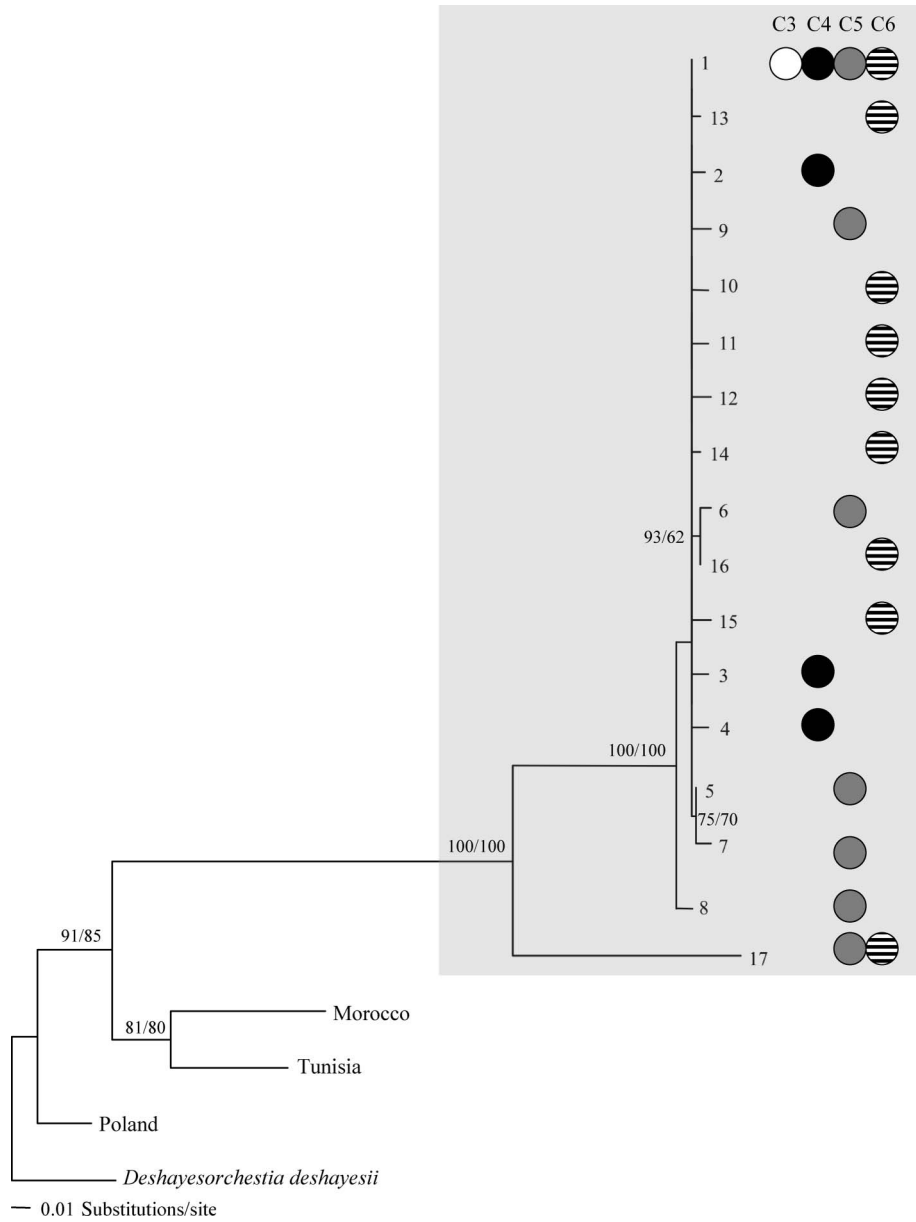


Fig. 2. — COI haplotype tree obtained by the NJ method under the TIM + Γ model of sequence evolution selected with MODELTEST (POSADA & CRANDALL 1998). Only statistical supports $\geq 75\%$ for both the NJ (1000 bootstrap replicates) and Bayesian searches (2.000.000 generations; same model of sequence evolution as in the NJ search) are reported on the branches. The shaded area encompasses haplotypes from the Collelungo beach. The numbers for haplotypes are as in Table 3; the shading of circles next to each haplotype corresponds to that in Fig. 1 and identifies the provenance of samples along the Collelungo beach.

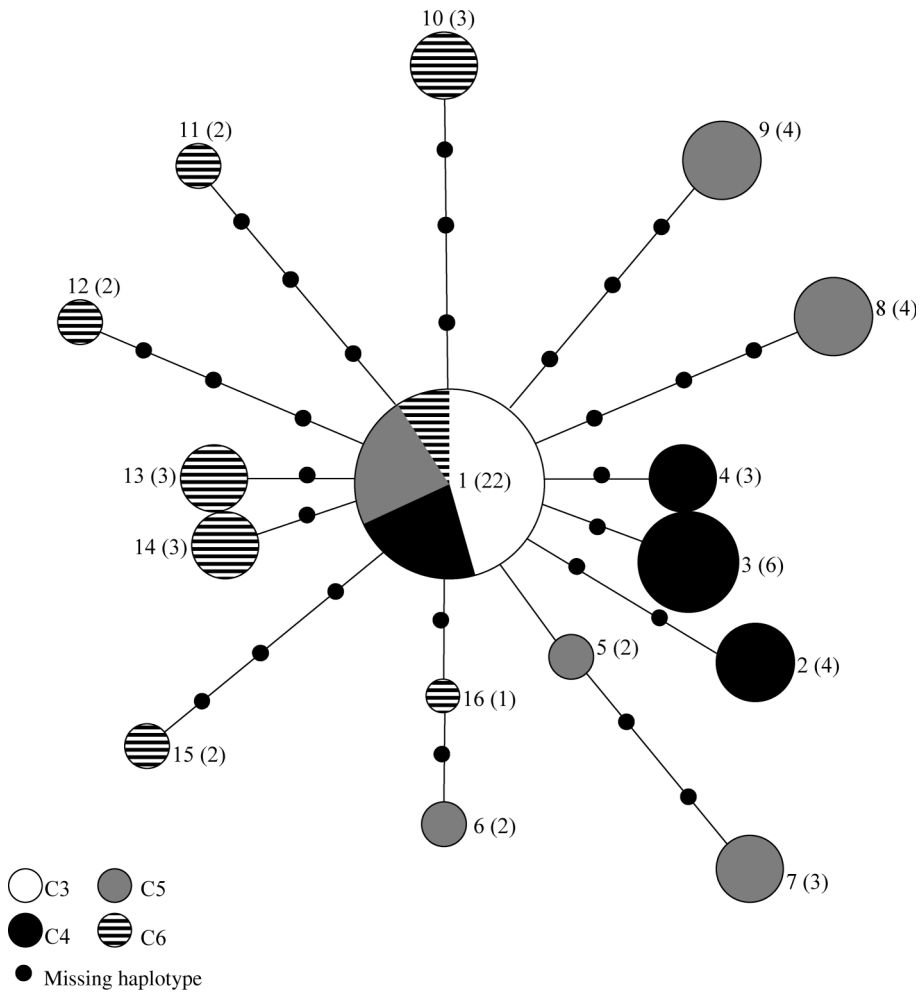


Fig. 3. — Haplotype network derived from the COI data set for the *Talitrus saltator* population from the Collelungo beach (haplotypes 1–16 only; the highly divergent haplotype 17 has been excluded from the analysis). The relative size of the circles is proportional to the number of individuals carrying that particular haplotype; pie slices indicate the fraction of each subpopulation contained within each haplotype. Different shading identifies the geographic origin of samples (see Fig. 1). The numbers close to each circle identify the haplotypes in Table 3 (first number) and how many individuals carried that particular haplotype (second number). Black dots are missing haplotypes.

mismatch analyses must, however, be considered cautiously since they are based on a relatively low number of haplotypes.

Environment, behaviour and genetics: combined analyses

To combine environmental data with behavioural and genetic results we used a multiple regression analysis, where the angles of orientation were the response variables.

Table 5.

Results from the hierarchical analysis of molecular variance (AMOVA). The analysis was conducted on haplotypes from the Collelungo beach as one hierarchical group (either including or excluding the highly divergent haplotype 17) and including all sampled populations. In the latter case we considered three different groups in the analysis (Collelungo beach, North African populations and the Polish population). The amount of variation among categories and the estimates of genetic differentiation are shown.

Hierarchy	Categories	% Variation	Φ Estimate
One group: C3-C6 (all haplotypes)	Among subpop.	19.07	$\Phi_{ST} = 0.190^*$
	Within subpop.	80.93	
One group: C3-C6 (excluding haplotype 17)	Among subpop.	20.34	$\Phi_{ST} = 0.203^*$
	Within subpop.	79.66	
Three groups: C3-C6 (all haplotypes); RT-KB (North Africa); PL (Poland)	Among groups	6.27	$\Phi_{CT} = 0.062^*$
	Among pop. within groups	17.99	$\Phi_{ST} = 0.242^*$
	Within pops.	75.74	

* $P < 0.05$.

Firstly we checked for simple correlations and found a linear correlation (though based only on four points) between the distance along the beach and number of haplotypes (H) ($R^2 = 0.954$; $P < 0.001$). The latter increased with increasing distance from the river mouth and decreasing erosion. We chose H as comprehensive descriptor of the subpopulations. The sun azimuth was included in the factor daytime (morning/afternoon). The other variables could be entered independently in the multiple regression analysis. We started with the following linear model (angle of orientation ~ daytime + air temperature + air humidity + atmospheric pressure + solar global radiation + sex + size + age + H). We compared models backwards and discarded the variables not significant to the model, according to the AIC. The best model was (angle of orientation ~ daytime + sex + global radiation + H); this model had a $-2 \log \text{Likelihood} = 2468.3128$, an AIC = 2488.3128 with 10 parameters and 844 degrees of freedom. We found no significant interaction between H (which includes differences among sites) and any other variable.

We then estimated the significance of the effect of each variable on orientation, by comparing likelihoods and degrees of freedom of the nested models. Daytime (morning vs. afternoon) and global radiation were found to be significant at the < 0.001 level; sex and H were significant at the < 0.05 level. Therefore, these variables had influence on the variation of orientation of samples.

DISCUSSION

Previous studies on the sandhopper *T. saltator* claimed significant relationships among orientation performance of individuals, genetic variability of populations and the dynamics of the shoreline (SCAPINI et al. 1995; KETMAIER et al. 2003). In those studies

populations from different beaches have been screened for orientation and allozyme variation. Differences among populations at those traits have been discussed in light of the stability of beaches. In this report, we have analysed for the first time mtDNA sequence variation and orientation performance simultaneously in a single population of the species at qualitatively different points along a dynamic beach to characterise this relationship further.

Genetic structure

The COI fragment sequenced in this study proved to be highly polymorphic and useful to describe in fine detail the genetic structure of the selected sandhopper population. The AMOVA analyses revealed a deep subdivision at such a small geographical scale as attested by the percentage of variation explained by differences among subpopulations and associated significant Φ_{ST} values (Table 5). On the other hand, there is no clear structuring by geographical origin of samples based on both the NJ tree and the haplotype network. In particular, the latter has a characteristic star-like pattern. Traditional wisdom holds that a haplotype star-like network is indicative of a size expansion event in the demographic story of a population. Our data, however, support a sudden population expansion only at point C4, while this hypothesis is rejected for all other points. Similarly, when all individuals from the four subpopulations are pooled together, the mismatch analysis indicates a substantial population stasis. We can take advantage of the considerable amount of genetic data we have been amassing during the last decade on *T. saltator* to discuss such a complex pattern. In an attempt to describe the genetic structuring of the species at the scale of the whole Mediterranean basin, DE MATTHAEIS et al. (2000) and KETMAIER et al. (2003) surveyed 38 populations for genetic variation at 23 allozymic loci. These studies revealed that geographic distances among populations explain most of the detected genetic heterogeneity. On this geographical scale a metapopulation model (HANSKI 1998), in which local demes are connected by dispersal events and undergo cyclical extinction and re-colonisation processes, is appropriate to describe the population dynamics of the species. According to SLATKIN (1993), such a metapopulation system should be at equilibrium between migration and drift under very low levels of gene flow. Whether and to what extent the generalisations valid at a macro-geographical scale apply to the geographically restricted case under study is unclear. Though it is granted that direct comparisons between allozymic (nuclear) loci and mtDNA are not possible because of their different evolutionary properties, we believe that the data presented in this paper only partially fit the wide geographical scale scenario derived from previous studies. The high degree of genetic subdivision detected by AMOVA does indeed match previous results based on allozymes, whereas relationships among haplotypes do not make clear geographical sense in terms of the isolation by distance hypothesis. The presence of a single haplotype (haplotype 1) shared among the four subpopulations is indicative of a certain degree of exchange of individuals. At the same time, the relatively high number of haplotypes unique to single subpopulations points to the fact that such exchanges are too infrequent to homogenise local differences and/or to originate an isolation by distance pattern. On one hand, since the four sites sampled for the present study are located along the same beach, migration of individuals among them could in theory be possible. On the other hand, it is well documented that sandhoppers preferentially migrate across the beach but only rarely along it (SCAPINI et al. 1992, 2002).

An additional point to look upon is that the mitochondrial marker we have used depicts only the female perspective. Tyrrhenian populations of *T. saltator* usually show a female-biased sex ratio (FALLACI et al. 2003), but this was not the case at Collelungo in May 2003, when the experiments took place (SCAPINI et al. 2005). On the contrary, the subpopulation at C3 was extremely male biased (male/female sex ratio = 3.35; SCAPINI et al. 2005). In addition to this, and probably more important to interpret our findings, the timing and extent of surface activity differ consistently between sexes (FALLACI et al. 2003). Females tend to stay closer to their burrowing zone and most of their movements are perpendicular to the shoreline. Males tend to cover longer distances during nocturnal foraging activity as they actively migrate along the shoreline. This implies that the male contribution to the overall genetic make up of the population could not be negligible in terms of homogenising effect, especially in those populations that are male biased. The characterisation and typing of nuclear loci (microsatellites) more variable than allozymes is necessary to properly address this point.

Overall, our data are consistent with a structure in which the population turnover is likely to be at equilibrium between migration and genetic drift under low levels of gene flow. This hypothesis is in agreement with the only slight signs of population expansion detected by our mismatch analyses. A stable metapopulation structure in which each single deme is subject to a constant rate of extinction/re-colonisation events may well produce a star-like pattern of relationships among haplotypes (MARDULYN & MILINKOVITCH 2005). Our results therefore advocate caution in deriving hypotheses based on past demographic expansions of given populations, on the basis of patterns of relationships among mtDNA sequences alone.

The multidisciplinary perspective:

inter-independence of genetic, behavioural and geomorphological patterns

Genetic diversity is not evenly distributed among the four subpopulations but increases from point C3 to point C6. Conversely, the orientation performance notably improved from point C3 to points C4 and C5, but samples were much less concentrated around the TED at point C6. The present study was specifically planned to analyse the possible interplay between genetic diversity and orientation in subpopulations from qualitatively different points along the same beach (i.e. along an erosion/accretion gradient). The use of multiple regression analysis permitted the discrimination of the searched effect from other environmental and intrinsic effects, which could also influence orientation performance. The multiple regression analysis demonstrates a significant, non-linear influence of genetic polymorphism to orientation. It is important to point out here that this result does not necessarily imply that a given haplotype (or a given array of haplotypes) would confer any adaptive advantages in terms of adherence to TED. Such a causal relationship between mtDNA polymorphism and orientation performance is by no means demonstrated by our experiments.

Even so, the significance of H on orientation needs to be discussed in light of the possible role played by local differences in the shoreline dynamics in affecting the demography of each subpopulation. At point C3, the genetic diversity was the lowest, while orientation was the most scattered. The lack of mtDNA variation at this point might well be due to the limited number of individuals tested as compared to the other points included in the study. We cannot rule out the occurrence of such a sample size-related bias in our data. On the other hand, it is worth noting that this subpopulation, given the local conditions, is presumably temporally unstable. Here the beach is

strongly eroding; thus it represents quite an unfavourable environment for sandhoppers (SCAPINI 2006). This beach dynamic is exacerbated by high density trampling during the peak tourist season (FANINI et al. 2005), and could cause cyclical population crashes, which, in turn, could be responsible for the lack of genetic variability. The idea of an ephemeral subpopulation fits well with the strong male bias in the sex ratio found at this location (SCAPINI et al. 2005). This bias is also the most likely explanation for the significant effect detected by multiple regression analyses of sex on orientation (see Results). Males are more mobile than females along the shoreline and tend to move more often in directions other than the TED than do females, thus determining an overall difference in orientation between sexes. Interestingly, a survey on the diversity of beach arthropods conducted at the very same points at which the orientation experiments were performed revealed that arthropod diversity in general is the least at point C3 (COLOMBINI et al. 2005). At point C4, most of the samples were concentrated around a direction slightly different from the TED (Table 2). This homogeneity, yet relatively low adaptiveness in the orientation of sandhoppers, could be explained both in light of the dynamic equilibrium of the coastline at this point and of the sudden change in the past demography of the subpopulation as detected at the mtDNA level. At C6 the orientation was more scattered than at C4 and C5 (compare circular dispersions, CDs, in Table 2), but the genetic diversity was the highest found along the beach (Table 4). The beach here is under accretion; dunes are of new formation and are backed by marshes. We have two not mutually exclusive hypotheses to explain these results. First, the presence of marshes behind the dune offers the possibility to sandhoppers of finding strips of wet sand both seaward and landward. Second, migrants are more likely to contribute to this subpopulation. The finding of the highly divergent haplotype 17 only at C5 and C6 supports the hypothesis of immigration events (though sporadic) at these locations. Given the number of substitutions among haplotype 17 and the others, the four individuals bearing it (2 each at C5 and C6) could represent occasional long-distance migrants that appeared in our sampling. Alternatively, we should invoke the amplification and sequencing of nuclear mitochondrial DNA (Numt) to explain our results. However, we tend to exclude this hypothesis, as the highly divergent sequences do not differ structurally from the other haplotypes. As a matter of fact, haplotype 17 has exactly the same reading frame as all the other haplotypes found in the study and we did not need to postulate indels to include it in the final alignment. Nonetheless, it is evident that a larger number of individuals from the same as well as from different locations should be sequenced to properly address the issue. Whatever the explanation for the finding of this divergent haplotype might be it is evident that C6 is behaviourally more heterogeneous than C5. Given the data at our disposal, we cannot tell whether this heterogeneity is due to individuals coming from the marsh area mixing up with those living more close to the shoreline or rather this is an adaptation evolved in response to the environmental heterogeneity of that particular section of the beach. It is unlikely that a sub-population of *T. saltator* may establish in the marsh area, which is ephemeral and subject to high salinity changes. However, it would be extremely interesting to run a set of experiments designed ad hoc to discriminate the "marsh" sandhopper signal from that of "shoreline" sandhoppers. This would not only allow a more complete interpretation of the results presented in this study but, more generally, would also provide us with a better understanding of the species ecology. In particular, if the hypothesis of a recent immigration of individuals from the marsh area were true, this would imply that the species is able to move across ecologically different habitats. An indirect confirmation to the hypothesis of an ongoing immigration of individuals at point C6 comes from the finding of a significant effect of

daytime on orientation. We found a remarkable difference between the behaviour in the morning and afternoon at this location. This result implies that a fraction of the individuals tested here express a positive phototactic behaviour toward the sun. This is considered a basic behavioural strategy observed in laboratory-reared individuals and expected in colonising individuals, both coming from a near shore and from the sea (transported by currents). A resident population would rather use a sun compass to compensate for sun movements and to orient towards a specific direction (SCAPINI 2006). Through a process of operant learning these (immigrants?) individuals will eventually correct the positive phototactic behaviour to adapt to the local shoreline orientation (SCAPINI et al. 1988). The global radiation is a comprehensive variable, which includes sky cover and sun visibility, both relevant to sun compass. Thus a high significance of these effects to the model was expected.

CONCLUSIONS

In this study we found variation along the Collelungo beach in both the behavioural and genetic traits we analysed. Patterns of variation were not completely alike and did not thoroughly parallel the coastal erosion/accretion trend. Genetic polymorphism was the highest on the accreting sections (C5 and C6) of the beach with respect to the sections under erosion (C3) or in dynamic equilibrium (C4). On the other hand, the highest concentration around the TED was observed at C5. A good adherence to the TED can be achieved in the short and medium terms. During a sandhopper life span (short term, months), individuals may learn which is the most appropriate zone of the beach where to burrow and/or escape. This will allow the population to persist over generations (medium term, years). On the other hand, genetic polymorphism is likely to be more dependent on long-term (decades to centuries) factors such as the environmental stability. For the beach under study, changes in the shoreline dynamics have been documented from historical times (INNOCENTI & PRANZINI 1993; CIAMPI 2005). Our data suggest that only in a shoreline in equilibrium or gradually accreting for a long time relatively to the generation turnover of the population, would a well-adapted behavior be coupled with genetic diversity.

In conclusion, the result of this study supports the hypothesis of a complex relation between population genetics, orientation and beach geo-morphological trends (SCAPINI et al. 1995). It is evident that the beach dynamics have short-term effects on the behaviour of sandhoppers. This is because the animals have continuous experiences of the characteristics of the surrounding environment. Consequently, variation in a crucial adaptive trait like the orientation performance tested in the present study (adherence to TED) is essential to guarantee the maintenance of populations in time. On the other hand, the intrinsic characteristics of the species are likely to play a role in determining its genetic structure. The low potential for long distance dispersal and the differences in the daily surface activity between sexes are both reflected in the mtDNA pattern we obtained. Variation of behaviour does not parallel the erosion/accretion trend of the shoreline, while a linear relationship is apparent between distances among locations along the beach and levels of genetic polymorphisms. This is most likely due to the different time scales acting on these traits. Given our results, the sandhopper *T. saltator* could be proposed as a bio-indicator to monitor changes of beach features granted that an accurate multidisciplinary approach is adopted.

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REFERENCES

- AKAIKE H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.
- ANASTÁCIO P.M., GONÇALVES S.C., PARDAL M.Â. & MARQUES J.C. 2003. A model for amphipod *Talitrus saltator* population dynamics. *Estuarine, Coastal and Shelf Science* 58 (S): 149–157.
- BORGIOLO C., MARCHETTI G.M. & SCAPINI F. 1999. Variation in zonal recovery in four *Talitrus saltator* populations from different coastlines: a comparison of orientation in the field and in an experimental arena. *Behavioral Ecology and Sociobiology* 45: 79–85.
- CIAMPI G. 2005. Some new knowledge about Ombrone river delta (Grosseto, Italy) dynamics inferred through maps. Abstracts of the MEDCORE International Conference. The Mediterranean coastal areas from watershed to the sea: interactions and changes. Florence, 10–14/11/2005. *Firenze: Firenze University Press*.
- CLEMENT M., POSADA D. & CRANDALL K.A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1659.
- COLOMBINI I., CHAOUTI A., FALLACI M., GAGNARLI E., SCAPINI F., BAYED A. & CHELAZZI L. 2005. Effects of freshwater discharge on terrestrial arthropods on Atlantic and Mediterranean sandy shores, pp. 233–264. In: Scapini F., Ed. Proceedings of the MEDCORE International Conference. *Firenze: Firenze University Press*.
- DE MATTHAEIS E., COBOLLI M., MATTOCCIA M., SACCOCCIO P. & SCAPINI F. 1994. Genetic divergence between natural populations of Mediterranean sandhoppers, pp. 15–29. In: Beaumont A.R., Ed. Genetics and evolution of aquatic organisms. *London: Chapman and Hall*.
- DE MATTHAEIS E., COBOLLI M., MATTOCCIA M. & SCAPINI F. 1995. Geographical variation in *Talitrus saltator* (Crustacea, Amphipoda): biochemical evidence. *Bollettino di Zoologia* 62: 77–84.
- DE MATTHAEIS E., DAVOLOS D. & COBOLLI M. 1998. Genetic divergence between populations and species of talitrids from Aegean islands. *Journal of Heredity* 89: 37–43.
- DE MATTHAEIS E., DAVOLOS D., COBOLLI M. & KETMAIER V. 2000. Isolation by distance in equilibrium and non-equilibrium populations of four talitrid species in the Mediterranean sea. *Evolution* 54: 1606–1613.
- ELGTARI M., CHARFI-CHEIKHROUHA F., MARCHETTI G.M. & SCAPINI F. 2000. Behavioural adaptation of *Talitrus saltator* populations to beaches with different dynamics and degree of human disturbance along Tunisian coasts. *Polskie Archiwum Hydrobiologii* 47: 643–650.
- EXCOFFIER L., LAVAL L.G. & SCHNEIDER S. 2005. Arlequin Ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* 1: 47–50.
- EXCOFFIER L., SMOUSE P.E. & QUATTRO J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.
- FALLACI M., COLOMBINI I., LAGAR M., SCAPINI F. & CHELAZZI L. 2003. Distribution patterns of different age classes and sexes in a Tyrrhenian population of *Talitrus saltator* (Montagu). *Marine Biology* 142: 101–110.

- FANINI L., CANTARINO C.M. & SCAPINI F. 2005. Relationships between the dynamics of two *Talitrus saltator* populations and the impacts of activities linked to tourism. *Oceanologia* 47: 93–112.
- FELSENSTEIN J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- FISHER N.I. 1993. Statistical analysis of circular data. *Cambridge: University Press*.
- FOLK R.L. & WARD W.C. 1957. Brazos river bar: a study of significance of grain size parameters. *Journal of Sedimentary Petrology* 27: 3–26.
- HANSKI I. 1998. Metapopulation dynamics. *Nature* 396: 41–49.
- HUELSENBECK J.P. 2000. Mr Bayes: Bayesian inference of phylogeny. (Distributed by the author). Rochester, NY: Department of Biology, University of Rochester.
- INNOCENTI L. & PRANZINI E. 1993. Geomorphological evolution and sedimentology of the Ombrone River delta, Italy. *Journal of Coastal Research* 9: 481–493.
- KETMAIER V., IURI V. & DE MATTHAEIS E. 2005. Genetic resources and molecular markers in *Talitrus saltator* (Amphipoda: Talitridae) from the beach of Smir. Ressources génétiques et marqueurs moléculaires chez *Talitrus saltator* (Amphipoda, Talitridae) de la plage de Smir, pp. 55–60. In: Bayed A. & Scapini F., Eds. *Université Mohammed V, Agdal: Special Issue of Série des Travaux de l'Institut Scientifique*.
- KETMAIER V., SCAPINI F. & DE MATTHAEIS E. 2003. Exploratory analysis of talitrid population genetics as a sandy beach quality indicator. *Estuarine, Coastal and Shelf Science* 58S: 159–167.
- LARGET B. & SIMON D. 1999. Markov chain Monte Carlo algorithm for Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16: 750–759.
- LUNT D.H., ZHANG D.X., SZYMURA J.M. & HEWITT G.M. 1996. The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology* 5: 153–165.
- MARCHETTI G.M. & SCAPINI F. 2003. Use of multiple regression models in the study of sandhopper orientation under natural conditions. *Estuarine, Coastal and Shelf Science* 58S: 207–215.
- MARDULYN P. & MILINKOVITCH M.C. 2005. Inferring contemporary levels of gene flow and demographic history in a local population of the leaf beetle *Gonioctena olivacea* from mitochondrial DNA sequence variation. *Molecular Ecology* 14: 1641–1653.
- MARQUES J.C., GONÇALVES S.C., PARDAL M.Â., CHELAZZI L., COLOMBINI I., FALLACI M., BOUSLAMA M.F., ELGTARI M., CHARFI-CHEIKHROUHA F. & SCAPINI F. 2003. Biology, population dynamics and secondary production of the sandhopper *Talitrus saltator* (Montagu) (Amphipoda, Talitridae) at Lavos (Western coast of Portugal), Collelungo (Western coast of Italy) and Zouaraa (North-Western coast of Tunisia). A comparative study of Atlantic and Mediterranean populations. *Estuarine, Coastal and Shelf Science* 58 (S): 127–148.
- MAU B. & NEWTON M. 1997. Phylogenetic inference for binary data on dendrograms using Markov chain Monte Carlo. *Journal of Computational and Graphical Statistics* 6: 122–131.
- MAU B., NEWTON M. & LARGET B. 1999. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometrics* 55: 1–12.
- NARDI M., MORGAN E. & SCAPINI F. 2003. Seasonal variation in the free-running period in two *Talitrus saltator* populations from Italian beaches differing for morphodynamics and human uses. *Estuarine, Coastal and Shelf Science* 58 (S): 199–206.
- NAYLOR E. 1989. Temporal aspects of adaptation in the behavioral physiology of marine animals, pp. 123–135. In: Klekowski R.Z. et al., Eds. *Proceedings of the 21st European Marine Biology Symposium. Warszawa: Polish Academy of Science*.
- POSADA D. & CRANDALL K. 1998. Modeltest: testing the model of DNA substitutions. *Bioinformatics* 14: 817–818.
- RANNALLA B. & YANG Z. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304–311.
- SAITOU N. & NEI M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.
- SCAPINI F. 1997. Variation in scototaxis and orientation adaptation of *Talitrus saltator* populations subjected to different ecological constraints. *Estuarine, Coastal and Shelf Science* 44: 139–146.

- SCAPINI F. 2002. Baseline research for the integrated sustainable management of Mediterranean sensitive coastal ecosystems. A manual for coastal managers, scientists and all those studying coastal processes and management in the Mediterranean. *Firenze: Istituto Agronomico per l'Oltremare, Società Editrice Fiorentina*.
- SCAPINI F. 2006. Keynote papers on sandhopper orientation and navigation. *Marine and Freshwater Behaviour and Physiology* 39: 73–85.
- SCAPINI F., ALOIA A., BOUSLAMA M.F., CHELAZZI L., COLOMBINI I., EL GTARI M., FALLACI M. & MARCHETTI G.M. 2002. Multiple regression analysis of the sources of variation in orientation of two sympatric sandhoppers, *Talitrus saltator* and *Talorchestia brito*, from an exposed Mediterranean beach. *Behavioral Ecology and Sociobiology* 51: 403–414.
- SCAPINI F., AUOGLIO M., CHELAZZI L., COLOMBINI I. & FALLACI M. 1997. Astronomical, landscape and climatic factors influencing oriented movements of *Talitrus saltator* in nature. *Marine Biology* 128: 63–72.
- SCAPINI F., BUIATTI M., DE MATTHAEIS E. & MATTOCCIA M. 1995. Orientation behavior and heterozygosity of sandhopper populations in relation to stability of beach environments. *Journal of Evolutionary Biology* 8: 43–52.
- SCAPINI F., CHELAZZI L., COLOMBINI I. & FALLACI M. 1992. Surface activity, zonation and migrations of *Talitrus saltator* on a Mediterranean beach. *Marine Biology* 112: 573–581.
- SCAPINI F., CHELAZZI L., COLOMBINI I., FALLACI M. & FANINI L. 2005. Orientation of sandhoppers at different points along a dynamic shoreline in southern Tuscany. *Marine Biology* 147: 919–926.
- SCAPINI F. & FASINELLA D. 1990. Genetic determination and plasticity in the sun orientation of natural populations of *Talitrus saltator*. *Marine Biology* 107: 141–145.
- SCAPINI F., UGOLINI A. & PARDI L. 1985. Inheritance of Solar Direction Finding in Sandhoppers: II Differences in arcuated coastlines. *Journal of Comparative Physiology (A)* 156: 729–735.
- SCAPINI F., UGOLINI A. & PARDI L. 1988. Aspects of direction finding inheritance in natural populations of littoral sandhoppers (*Talitrus saltator*), pp 93–103. In: Chelazzi G. & Vannini M., Eds. Behavioral adaptation to intertidal life. *New York: Plenum Publishing Corporation*.
- SCHNEIDER S. & EXCOFFIER L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 152: 1079–1089.
- SLATKIN M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47: 264–279.
- SWOFFORD D. 2003. PAUP* β -Version 4.10. *Sinauer, Sunderland*.
- TAFANI B., UGOLINI A., BAZZICALUPO M., MENGONI A. & RUFFO S. 2004. Phylogenetic relationships among Mediterranean sandhoppers. *Journal of Natural History* 38: 499–508.
- TAMURA K. & NEI M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512–526.
- TEMPLETON A.R., CRANDALL K.A. & SING C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132: 619–633.
- WILLIAMS J.A. 1987. The relationship between the antennal segment number and molting in *Talitrus saltator* (Montagu, 1808) (Amphipoda Talitridae). *Crustaceana* 53: 313–316.