Bayed A. & Scapini F. (éditeurs). Ecosystèmes côtiers sensibles de la Méditerranée : cas du littoral de Smir
Travaux de l'Institut Scientifique, Rabat, série générale, 2005, n°4, 55-59

Genetic resources and molecular markers in *Talitrus saltator* (Amphipoda, Talitridae) from the beach of Smir

Valerio KETMAIER, Vanessa IURI & Elvira DE MATTHAEIS

Università di Roma "La Sapienza", Dipartimento di Biologia Animale e dell'Uomo, V. le dell'Università 32, I-00185 Roma, Italy - e-mail: valerio.ketmaier@uniroma1.it

Abstract. A case study on the genetic divergence between North African and Polish populations of the sandhopper *Talitrus saltator* is presented. We studied allozymic variation at 23 enzymatic loci and we sequenced a fragment (397 bp) of the mitochondrial gene encoding for subunit I of cytochrome oxidase (COI). Genetic divergence deduced from allozymes and mtDNA were not always consistent with each other. This partial incongruence is likely related to differences in the characteristics of the collecting sites and in the pattern of surface marine currents. Overall, we found a negative trend between the mean level of genetic variability of populations and the degree of disturbance by human activities on beaches where the populations were collected. This is in agreement with extensive previous results obtained in our laboratory on this species at the scale of the whole Mediterranean Sea.

Key words: *Talitrus saltator,* allozymes, mitochondrial DNA, genetic variability, genetic divergence.

Ressources génétiques et marqueurs moléculaires chez Talitrus saltator (Amphipoda, talitridae) de la plage de Smir

Résumé. Nous avons étudié la divergence génétique entre des populations de *Talitrus saltator* récoltées sur la côte baltique et sur la côte nord-africaine du Maroc et de la Tunisie. Les valeurs de distance génétique ont été obtenues en utilisant deux groupes différents de marqueurs moléculaires : les allozymes à 23 loci enzymatiques et un fragment (397 bp) de l'ADN mitochondrial (COI). Les résultats obtenus avec les allozymes sont peu différents de ceux obtenus avec l'ADN mitochondrial, mais les différences peuvent être expliquées en considérant les moyens de dispersion des talitres, qui sont entraînés par les courants marins superficiels. Le niveau moyen de la variabilité génétique des populations étudiées est lié au degré de changement du milieu par les actions humaines. Ces résultats s'accordent avec les résultats obtenus sur la même espèce à l'échelle de la Mer Méditerranée.

Mots clés *: Talitrus saltator,* allozymes, ADN mitochondrial, variabilité génétique, divergence génétique.

INTRODUCTION

The conservation of genetic resources has a primary importance in biodiversity conservation plans and molecular genetic research programs are worldwide acquiring a new role in conservation and genetic resource evaluation. Population genetics plays an important role in all of genetic resource programs through its focus on describing and monitoring genetic variation (Loeschcke *et al*. 1994). Brown & Schoen (1994) addressed the problem on the several levels of choice in conservation taking into account the fundamental question: "should one maximize the diversity of the products of evolution, or attempt to conserve the potential for future diversification, the process of evolutionary change"? Anyway, the study of the genetic diversity of natural populations is the most basic level of studies on biodiversity and studies dealing with genetic variation within species focus on describing patterns of variation in space and time. Conservation programs are aware of the fact that species are non random distributed and that there is a consistent degree of genetic variation within each species. How species maintain and distribute genetic variation is a book-length subject (Gilpin 1991). Moreover, species are often constituted by an array of semi-isolated populations and this aspect leads to consider the metapopulation model (a set of local populations which interact via individuals moving among populations) as well as the role played by migration together with natural selection and genetic drift in shaping the pattern of genetic variation within a species. We have chosen to analyse these problems within the family Talitridae (Crustacea, Amphipoda), which has a worldwide distribution and is well represented in the Mediterranean area in the marine and freshwater supralittoral zone.

In the framework of the MECO project (ERB-IC-18-CT-98-0270) funded by the European Commission we have described and monitored the genetic variation of the *Talitrus saltator* population from the beach of Smir utilising two different molecular markers: allozymes and COI fragment (mtDNA). The population of Smir has been compared with other North-African populations and with a population from a Baltic locality. Such a sampling strategy will allow us to discriminate between the action of geographic distance and environmental perturbations in determining the genetic structure of *T. saltator*. Allozymic markers resulted to be highly informative to analyse the level of genetic divergence among Smir population towards other three North-African populations of the same species as well as to ascertain the degree of geographic isolation of Smir population from Turkish, Aegean, Adriatic, Tyrrhenian and Baltic populations of *T. saltator* (Ketmaier & De Matthaeis 2002, Ketmaier *et al*. 2003). The analysis of COI fragment was applied to individuals from Smir,

Tunisia and Poland to test the usefulness of this mitochondrial marker in revealing the pattern of genetic structuring of *T. saltator* on different geographic scales. Combining nuclear (allozymes) and mitochondrial (COI) genes will allow us to evaluate the differences in the performance of two classes of markers with different evolutionary properties.

MATERIALS AND METHODS

Sampling

The following populations of *T. saltator* were analysed for genetic variation; for each population are given the collecting site, code and sample size for allozymes (N-all.) and mtDNA (N-COI); collecting sites are shown in figure 1:

1. Poland, Hel Gdansk, POL, N.-all.=18, N-COI=3
2. Morocco, Kabyla beach, MAR, N.-all.=60, N.-COI=3
3. Tunisia, Tabarka, TAB, N.-all.37
4. Tunisia, Zouaraa, ZOU, N.-all-=27
5. Tunisia, Rtiba, TUN, N.-COI=3
6. Tunisia, Korba, KOR, N.-all=68

Tabarka, Korba and Kabyla are placed within large bays, Rtiba is in a protected bay whereas Zouara and Hel Gdansk are exposed beaches. Rtiba, Hel Gdansk and Korba are not impacted by constructions; at Kabyla and Tabarka there are constructions on the dune. Kabyla, Hel Gdansk and Tabarka are also highly affected by trampling by tourists during summer period. Finally, Rtiba is a stable beach, while the degree of erosion increases progressively in Korba, Hel Gdansk, Kabyla, Tabarka and Zouara beaches.

The number of individuals per populations used in mtDNA analysis is small because for the region of the COI gene we sequenced the amount of intra-population polymorphism is expected to be low relative to the extent of inter-population divergence (Lunt *et al*. 1996). Animals used for allozymes were transported alive to the laboratory and then stored at –80°C; animals used for mtDNA were preserved in EtOH 90-100%.

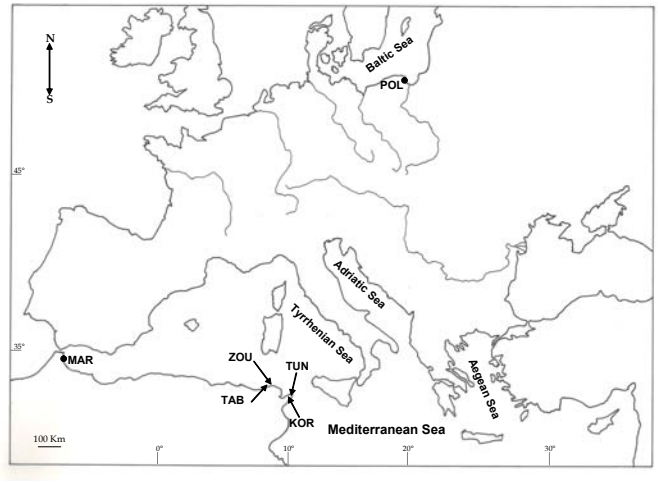

Figure 1. Map of sample localities for the six populations of *Talitrus saltator* included in the study.

Allozymes

The following 13 enzymatic proteins, coded by 23 presumptive gene loci, were tested in each population (the scored loci are given in parentheses): Acph (*Acph-1; Acph-2; Acph-3*); Ao (*Ao*); Aph (*Aph-1; Aph-2*); Ca (*Ca-1; Ca-2*); Est (*Est-1; Est-2, Est-3*); Got (*Got-1; Got-2*); Hk (*Hk*); Lap (*Lap-1; Lap-2*); Ldh (*Ldh*); Mpi (*Mpi*); Pep (*Pep-1; Pep-2; Pep-3*); Pgm (*Pgm*); Phi (*Phi*). Details of the protein codes and electrophoretic procedures are given elsewhere (De Matthaeis *et al.* 1994, 1995; see also the MECO Project Web Site at http://www.Meco.unifi.it). The genetic variability of samples was estimated by H_e (expected mean heterozygosity under Hardy-Weinberg equilibrium); H_o (observed mean heterozygosity); P (proportion of polymorphic loci according to the 0.99 criterion) and A (mean number of alleles per locus). The genetic relationships among populations were estimated on the basis of Nei's (1978) genetic distance (D) values; an UPGMA dendrogram (Sneath & Sokal 1973) was plotted on the basis of these values. These calculations were carried out using BIOSYS-1 (Swofford & Selander 1981).

mtDNA

We sequenced 3 individuals per population. Total DNA was extracted from frozen and/or ethanol-preserved specimens using the Easy-DNA kit from Invitrogen (Invitrogen, CA). PCR amplifications of a 397-bp (base pair) fragment of the cytochrome oxidase I (COI) gene were carried out using the primers COIf (5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and COIr (5'-TAA AGA AAG AAC ATA ATG AAA ATG-3'). Double-stranded amplification was performed with a Hybaid Omn-E thermal cycler in 25 µl of a solution containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, each dNTP at 2.5 mM, each primer at 1 mM, genomic DNA (10-100 ng) and 1 unit of Amplitaq-Gold (Perkin-Elmer). After a 10 min. denaturation step (94°C), each cycle of the polymerase chain reaction consisted of denaturation for 1 min. at 93°C, annealing for 1 min. and 15 sec. at 52°C, and extension for 1 min. and 15 sec. at 65°C. This cycle was repeated 40 times and followed by a 7 min. incubation step at 68°C. PCR fragments were purified with the GenEluteTM PCR DNA Purification Kit from Sigma. Sequences were determined with an automated sequencer (Applied Biosystems 373A) following the manufacturer's protocols. Strands were sequenced in both directions for each individual. Sequences were edited using Sequencher 3.1.1 (Gene Code Corporation, Ann Arbor, MI) and aligned by eye following the guide provided by the reading frame. Base composition, number of transversions (Tv) and transitions (Ti) and genetic divergence among populations (uncorrected p) were calculated with PAUP* 4.0B10 (Swofford 2002).

RESULTS

Allozymes

Allele frequencies are available upon request. *Est-3* and *Pep-1* were fixed for the same allele in all populations; *Ao* was fixed for alternative alleles between POL-MAR and KOR-ZOU-TAB; *Got-2* and *Pep-3* discriminate between

POL and KOR-ZOU-TAB-MAR. These populations were also characterised by strong differences in allele frequencies at several additional loci (*Acph-2, Mpi, Pgm, Phi*). Among North-African populations, MAR showed the presence of private alleles at *Mpi.* Table I reports genetic variability estimates. *P* ranged from 13.0 in POL to 30.4 in MAR. POL also had the lowest H_o (0.019 ± 0.011) and H_e (0.022 ± 0.013) values whereas ZOU was characterised by the highest values of H_o (0.053 ± 0.027) and H_e (0.085 ± 0.040). We generally detected a deviation from Hardy-Weinberg expectations in the majority of samples; all these deviations were heterozygote deficiencies (results not shown). Table II also reports the matrix of genetic distances *D* (Nei, 1978): *D* ranged from 0.009 (KOR vs TAB) to 0.277 (POL vs TAB). There were three different levels of genetic divergence: 0.258 ± 0.025 (POL vs North-African populations); 0.065 ± 0.025 (MAR vs Tunisian populations) and 0.017 ± 0.012 (among Tunisian populations). Genetic distances among study populations are summarised by the UPGMA dendrogram shown in figure 2.

mtDNA

Different individuals of the same population had identical sequences for the COI fragment studied. All mutational changes were base pair substitutions; no indels were found. Table II reports the average frequencies of A+T and G nucleotides on each codon position and on all position; these were 0.676 (A+T) and 0.171 (G) in all positions and 0.855 (A+T) and 0.038 (G) when only third codon positions were considered. In table III are reported the uncorrected p distances together with the absolute number of substitutions (Tv+Ti) and the number of Tv changes between each population pair. Percentages of sequence divergence (uncorrected p) ranged from 10.2% (MAR vs TUN) to 12.8% (POL vs MAR); Ti's were generally more numerous than Tv's.

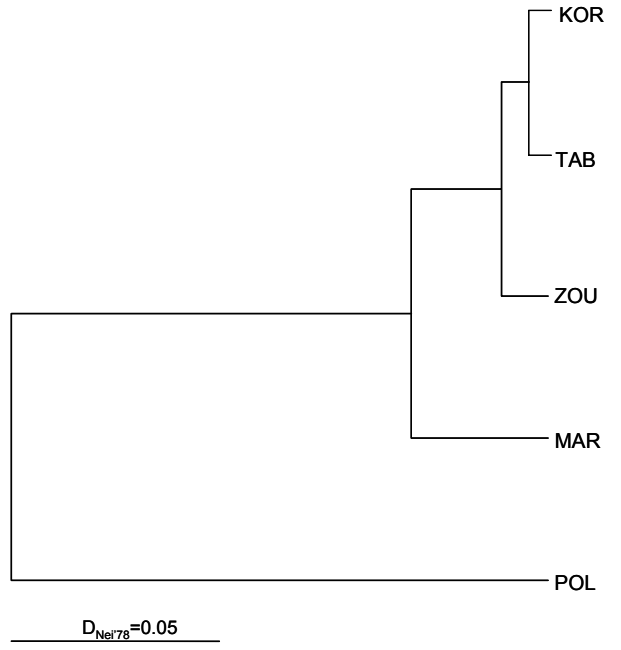

Figure 2. UPGMA dendrogram based on the Nei (1978) genetic distance values reported in table I.

Table I. Nei genetic distance values *D* (Nei 1978) between study populations of *T. saltator* above the diagonal. Variability estimates for each population (± SD) are also given; for variability estimate codes see the Materials and Methods section.

	POL	KOR	ZOU	TAB	MAR
POL	*****				
KOR	0.266	*****			
ZOU	0.269	0.012	*****		
TAB	0.277	0.009	0.032	*****	
MAR	0.220	0.057	0.082	0.057	*****
	Variability estimates (SD)				
A	1.1 (.1)	1.3 (.1)	1.4 (.2)	1.4 (.2)	1.4 (.1)
*P**	13.0	26.1	17.4	21.7	30.4
H_O	.019 (.011)	.046 (.021)	.053 (.027)	.031 (.019)	.032 (.022)
H_E**	.022 (.013)	.072 (.031)	.085 (.040)	.057 (.027)	.063 (.028)

* a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99
** Unbiased estimate (see Nei 1978).

Table II. Percent of A+T and G by codon positions for *Talitrus saltator* populations.

Codon positions	% A+T	% G	N° of sites
First	57.1	31.3	132
Second	59.9	16.2	132
Third	85.5	0.38	133
All	67.6	17.1	397

Table III. Transition/transversion number (above the diagonal) and uncorrected p distance values (below the diagonal) for the COI fragment analysed in this study for three populations of *Talitrus saltator*.

	POL	MAR	TUN
POL	*****	29/21	26/21
MAR	0.128	*****	24/16
TUN	0.118	0.102	*****

DISCUSSION

From the data in table I the mean value of genetic distance between MAR and POL is lower than values obtained in comparisons between POL and each of the other three North-African populations of *T. saltator*. This result is due to the contribution of two loci: at the *Ao* locus MAR and POL share the same unique D allele, at the *Est-1* locus the two populations share the B allele, which lacks in any other population. Mean levels of genetic divergence detected trough the use of mtDNA did not follow the same trend: from data in table III TUN population is closer to POL population. The particular way of dispersal of supralittoral talitrid could help explaining these results. Talitrids as all peracarid crustaceans lack larval stages. Active dispersal capability is very low and restricted to small-scale movements. Over long distances dispersal would be mainly passive and occasional, via animals attached to drifting

wrack and wood. It has been observed that the genetic similarity between populations of *T. saltator* is strongly related to the pattern of sea surface currents either at local geographic scale or at the scale of whole Mediterranean Sea (De Matthaeis *et al*. 1998, 2000).

The particular position of the collecting sites seems to be very important too (De Matthaeis et al. 1998): along the North-Africa coast the principal surface current comes from the strait of Gibraltar, so it seems likely to expect a relatively higher genetic similarity between MAR and POL than POL versus the more western Tunisian populations, as revealed by allozymic markers. On the other hand, the Tunisian population of Rtiba, analysed only by mtDNA marker, was collected along the promontory of Cap Bon where a particular circulation of surface currents is reported (Istituto Idrografico della Marina 1982). In addition, TAB and ZOU populations were collected from sites under coastal erosion; TAB is within a sort of large bay, whereas ZOU may be considered an exposed beach. Results obtained in the present work are consistent with previous data, confirming our hypothesis on the great importance within talitrid species of the process of colonization in maintaining a large population size. Problems arise when human activities (as tourism infrastructures, permanent buildings) interfere in the natural process of colonization of beaches by the supralittoral community. The natural process of local extinction could predominate lowering the population size of different species. In these cases genetic drift would act and one of the first effects could be read in the reduction of the mean level of genetic variability of the local populations. The mean value of observed heterozygosity (H_o) obtained trough the allozymic study could be utilized as a rough indicator of the levels of genetic polymorphism of natural populations.

There are some differences among the North-African populations for what concerns H_o values (Tab. II): H_o is about 0.03 in MAR and TAB, while H_o is about 0.05 in KOR and ZOU. If we compare these values with data obtained by Scapini *et al*. (1995) we could use the results obtained with the North-African group of *T. saltator* to understand the role that human disturbance could have on the genetic resources of a species. From the results of Scapini *et al.* (1995) western Italian populations of *T. saltator* appeared to be more polymorphic (average H_o= 0.05) than the Adriatic Italian group (average H_o= 0.03) and the mean values of H_o per population resulted to be correlated with the coast stability of the collecting sites. Coastal zones are characterised by natural dynamics, but in the last years the anthropic pressure is strongly affecting the natural dynamic process of sandy beaches all over the Mediterranean. The MAR population was collected in a coastal zone highly affected by human activities (constructions on dunes) as well as the site of TAB population.

It is evident that both classes of molecular markers detected a certain degree of genetic heterogeneity among the populations of *T. saltator* included in the study (though allozymes and mtDNA results were not always consistent with each other) and such an heterogeneity can only be partially explained in the light of the geographic distance separating the different collecting sites. In particular, the MAR and TAB populations (collected in highly impacted beaches; see materials and methods) showed some peculiar traits in their genetic structure that could trace the impact of environmental alteration on the populations of this sand-hopper. In conclusion, the characterization of the distribution of genetic variation of species trough space could be used (1) to understand how different are the populations belonging to the same species and (2) as a useful biological indicator of environmental impact.

Acknowledgements

We wish to thank F. Scapini, F. Charfi, M. F. Bouslama and M. El Gtari for their help in the sampling. F. Scapini provided valuable comments on a first draft of the paper.
This research was been done in the framework of the MECO Project supported by the European Union Programme INCO-DC 4th FP Contract ERB IC 18-CT98-0270.

References

Brown A.H.D. & Schoen D.J. 1994. Optimal sampling strategies for core collections of plant genetic resources. *In*: Loeschcke, V., Tomiuk, J., Jain, S.K. (Eds.) - *Conservation Genetics*. Birkhäuser Verlag, Basel, 357-370.

De Matthaeis E., Cobolli M., Mattoccia M., Saccoccio P. & Scapini F. 1994. Genetic divergence between natural populations of Mediterranean sandhoppers. *In*: Beaumont, A.R. (Ed.): - *Genetics and evolution of aquatic organisms*. Chapman & Hall, London, 15-29.

De Matthaeis E., Cobolli M., Mattoccia M. & Scapini F. 1995. Geographical variation in *Talitrus saltator* (Crustacea, Amphipoda): biochemical evidence. *Boll. Zool*., 62, 77- 84.

De Matthaeis E., Davolos D. & Cobolli M. 1998. Genetic divergence between populations and species of talitrids from Aegean Islands. *J. Hered*., 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.

Gilpin M. 1991. The genetic effective size of metapopulation. *In* Gilpin, M., Hanski, I. (Eds.): - *Metapopulation dynamics: empirical and theoretical investigation*. Academic Press, London, pp. 165-175.

Istituto Idrografico Della Marina. 1982. *Atlante delle correnti superficiali dei mari italiani*. I.I. 3068. Istituto Idrografico della Marina, Genova, pp. 24, 12 maps.

Ketmaier V. & De Matthaeis E. 2002. A preliminary phylogenetic study on the Mediterranean Talitrid Amphipods based on mitochondrial and nuclear DNA sequences. *In*: Fourth European Crustacean Conference Abstracts, Lodz, 22-26/7/2002, 55-56.

Ketmaier V., Scapini F. & De Matthaeis E. 2003. Exploratory analysis of Talitrid population genetics as an indicator of the quality of sandy beaches. *Estuar. Coastal Shelf Sci.*, 58S, 159-167.

Loeschcke V., Krebs R.A. & Barker, J.S.F. 1994. Genetic variation and acclimation to high temperature stress in *Drosophila buzzatii*. *Biol. J. Linn. Soc*., 52, 83-92.

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 Mol. Biol.*, 5, 153-165.

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

Scapini F., Buiatti M., De Matthaeis E. & Mattoccia M. 1995. Orientation behaviour and heterozygosity of sandhopper populations in relation to stability of beach environments. *J. Evol. Biol*., 8, 43-52.

Sneath P.H.A. & Sokal R.R. 1973. *Numerical Taxonomy*. Freeman W.H. and Company, San Francisco, 575 p.

Swofford D. 2002. PAUP* ß-version. 4.10. Sinauer Associates, Sunderland. http://paup.csit.fsu.edu/

Swofford D.L. & Selander R.B. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.,* 72, 281-283.

Manuscrit reçu le 30 mai 2003