

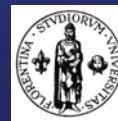
# Beach dynamics have an impact on the variation of genetic and behavioral traits in the sandhopper *Talitrus saltator* (Crustacea, Amphipoda): a study case in Southern Tuscany.

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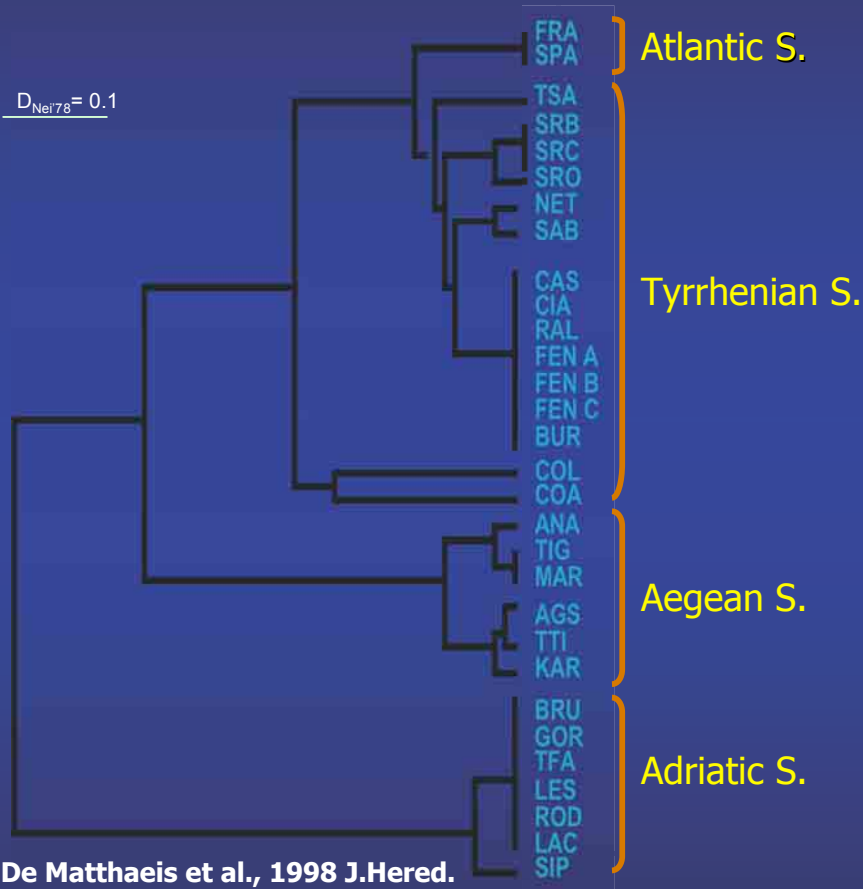
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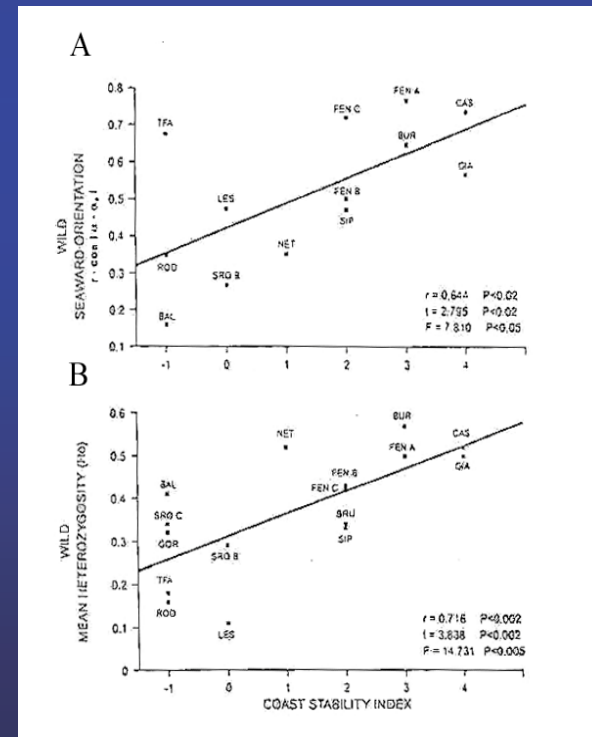
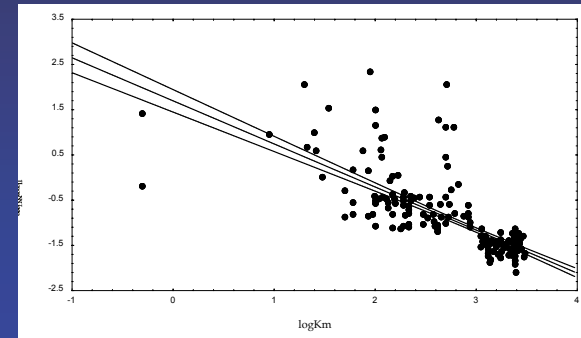


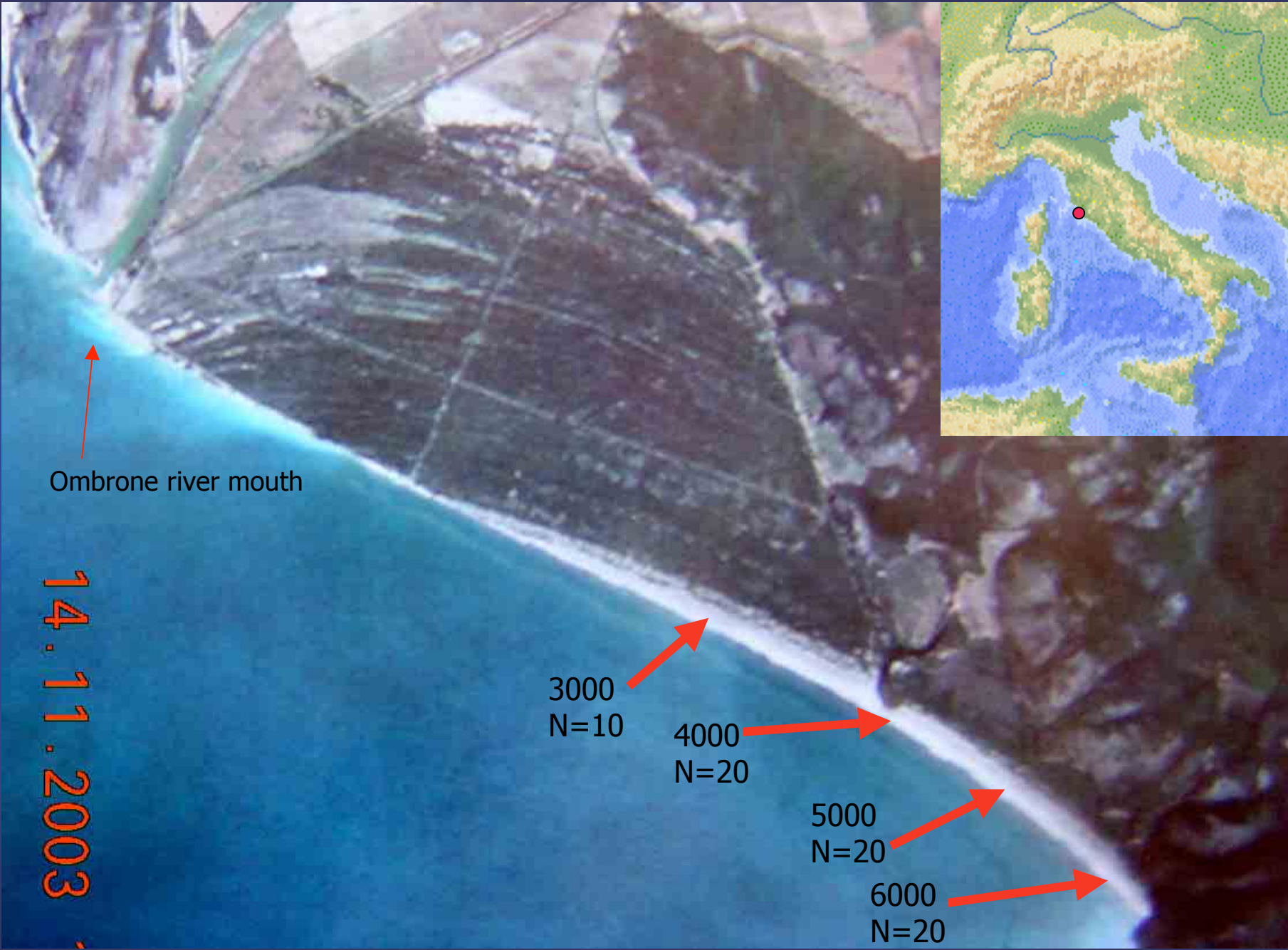
The sandhopper *T. saltator* has been the object of several research projects

*T. saltator* (28 pops.)  $Nm_{\text{mean}} = 0.046$   
 $R^2 = 0.586$  ( $p < 0.01$ );  $R = 0.697$  ( $p < 0.01$ )



De Matthaëis et al., 1998 J.Hered.  
 De Matthaëis et al., 2000 Evolution  
 Ketmaier et al., 2003 Est.Coast.Shelf Sci.  
 Scapini et al., 1995 J.Evol. Biol.





## The four collecting sites differ in the erosion/accretion dynamics:

1. At point 3000 the dune was severely eroded
2. At point 4000 the dune was well developed but recently has been subjected to erosion
3. At point 5000 the dune had an accreting foredune
4. At point 6000 the dune was of new formation and backed by marshes

The four collecting sites also differ in the distribution, abundance and diversity of terrestrial beach arthropods. In particular, Colombini *et al.* (2003; *Small-scale spatial distribution of terrestrial macro-invertebrates at increasing distances from the Ombrone river delta- Maremma Regional Park, GR, Italy- Technical report*) demonstrated that:

1. There was a linear trend in the abundance of the arthropods along the beach with the higher number of individuals at the last station
2. Indices of species richness and diversity increased with increasing of the distance from the river mouth

## *Talitrus saltator*



### Aims:

1. Compare levels of genetic variability at the DNA level with previous allozyme (protein) data.
2. Reveal subtle genetic differences among different sub-populations of the species sampled along qualitatively different sites of the dynamic Uccellina beach.
3. Compare genetic data with behavioral ones to obtain a multidisciplinary picture of the relationships of the species with the quality of the environment. It is important to emphasize that the sampling of the animals has been contemporary with the acquisition of behavioral data.

We amplified by Polymerase Chain Reaction (PCR) and sequenced both strands of a 407 bp fragment of the mitochondrial gene encoding for the first subunit of the cytochrome oxidase (COI) for each individual included in the study.

For this study, we have generated about 57 kb of new mtDNA data.

We also included in the study samples from Tunisia, Morocco and Poland in order to obtain information at the COI level on the pattern of genetic differentiation of the species on a macro-geographical scale.

# Results

We revealed 17 haplotypes in the *T.saltator* populations from the Uccellina beach

We found a progressive increase in the level of genetic variability from point 3000 to point 6000

Standard diversity indices	3000	4000	5000	6000
$\pi^*$	-	2.621±1.461	15.421±7.191	15.578±7.261
$\pi_n^{**}$	-	0.006±0.004	0.037±0.019	0.038±0.019

\* mean number of pair-wise differences between all pairs of haplotypes

\*\* nucleotide diversity: probability that two randomly chosen homologous nucleotides are different

Haplotype: 3000 4000 5000 6000  
(10) (20) (20) (20)

1	1	0.25	0.25	0.1
2	0	0.2	0	0
3	0	0.3	0	0
4	0	0.15	0	0
5	0	0.1	0	0
6	0	0	0.1	0
7	0	0	0.15	0
8	0	0	0.2	0
9	0	0	0.2	0
10	0	0	0	0.15
11	0	0	0	0.1
12	0	0	0	0.1
13	0	0	0	0.15
14	0	0	0	0.15
15	0	0	0	0.1
16	0	0	0	0.05
17	0	0	0.1	0.1

## Comparison with allozymes (previous data based on 22 enzymatic loci)

Pop.	Mean number of alleles per locus	Heterozygosity	
		observed	HW-expected
UCC'92	1.3±0.2	0.064±0.034	0.087±0.046
1000*	1.3±0.1	0.018±0.011	0.079±0.031
4000	1.3±0.2	0.052±0.031	0.072±0.036
6000	1.4±0.2	0.039±0.021	0.065±0.034

\*this subpopulation is now extinct

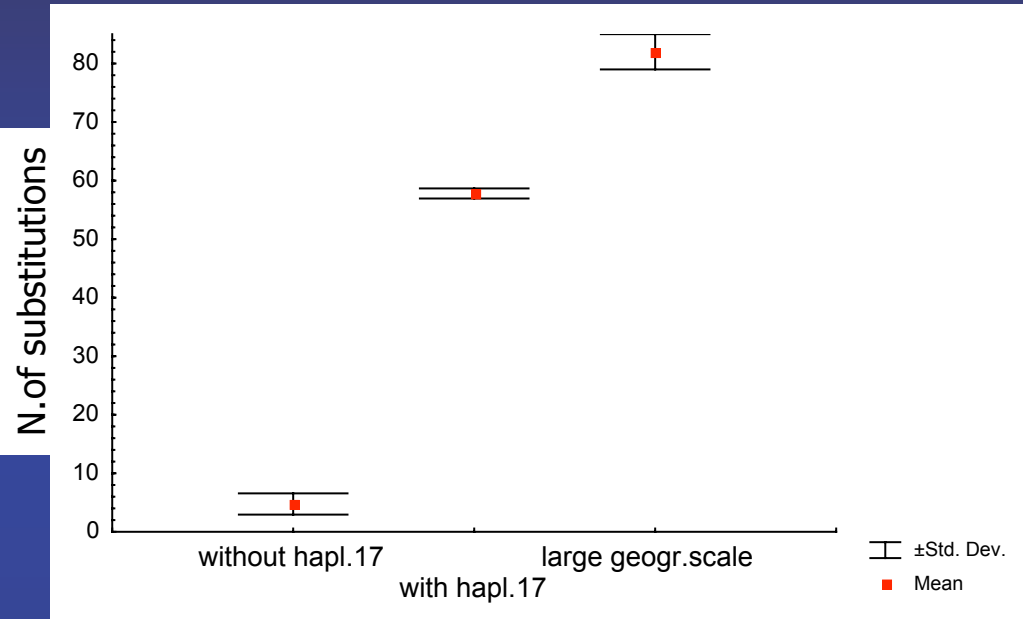
- There is a variation in the observed heterozygosity which is consistent with the estimates of genetic variability based on mtDNA
- The number of alleles per locus is too low and does not allow subtle discrimination among different sub-populations



Haplotype: 3000 (10) 4000 (20) 5000 (20) 6000 (20)

Haplotype	3000 (10)	4000 (20)	5000 (20)	6000 (20)
1	1	0.25	0.25	0.1
2	0	0.2	0	0
3	0	0.3	0	0
4	0	0.15	0	0
5	0	0.1	0	0
6	0	0	0.1	0
7	0	0	0.15	0
8	0	0	0.2	0
9	0	0	0.2	0
10	0	0	0	0.15
11	0	0	0	0.1
12	0	0	0	0.1
13	0	0	0	0.15
14	0	0	0	0.15
15	0	0	0	0.1
16	0	0	0	0.05
17	0	0	0.1	0.1

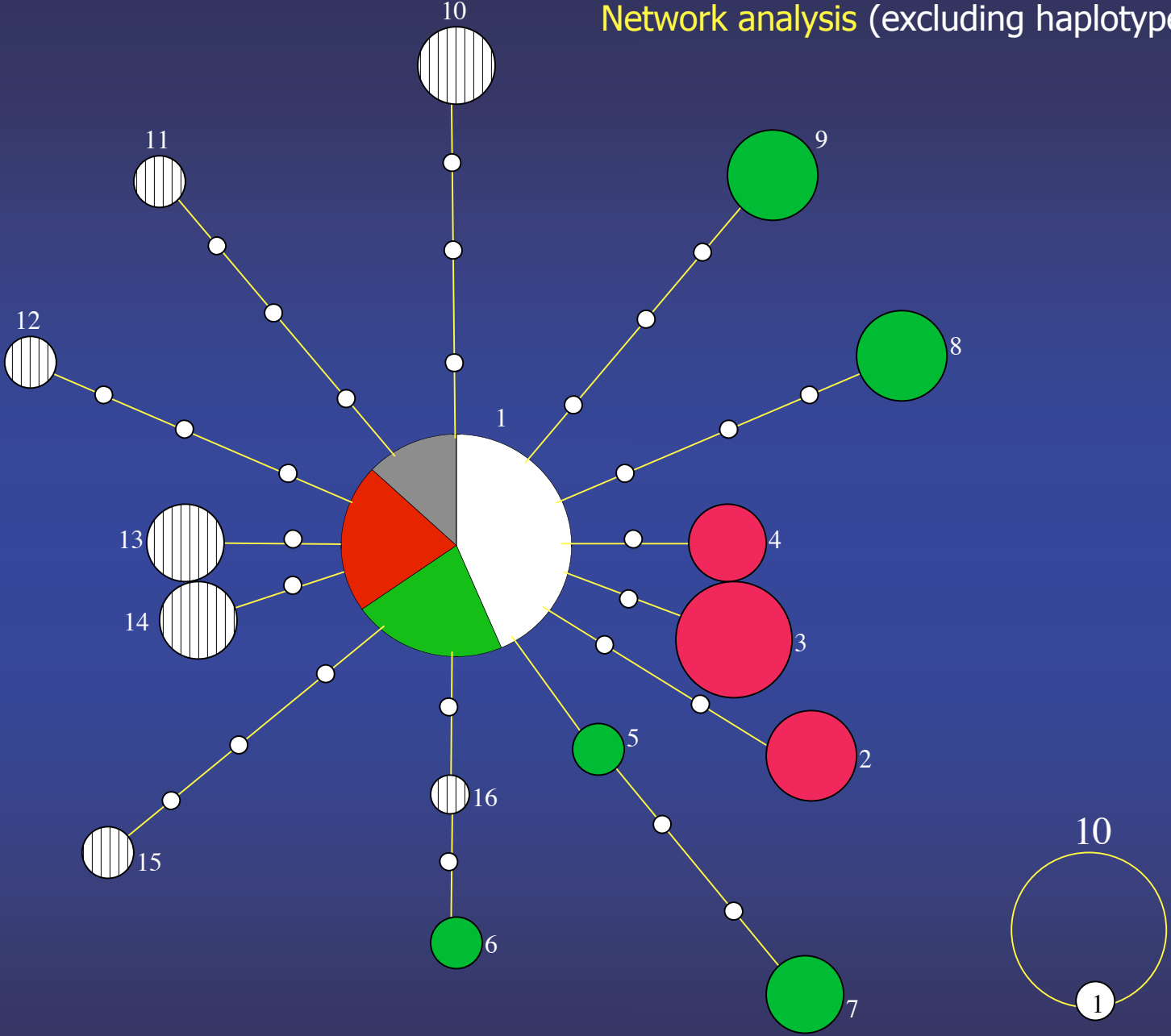
(4 individuals)



**Hypothesis:** the 4 individuals with haplotype 17 could represent occasional long-distance migrants that appeared in our sample.

Network analysis (excluding haplotype 17)

- 3000
- 4000
- 5000
- 6000



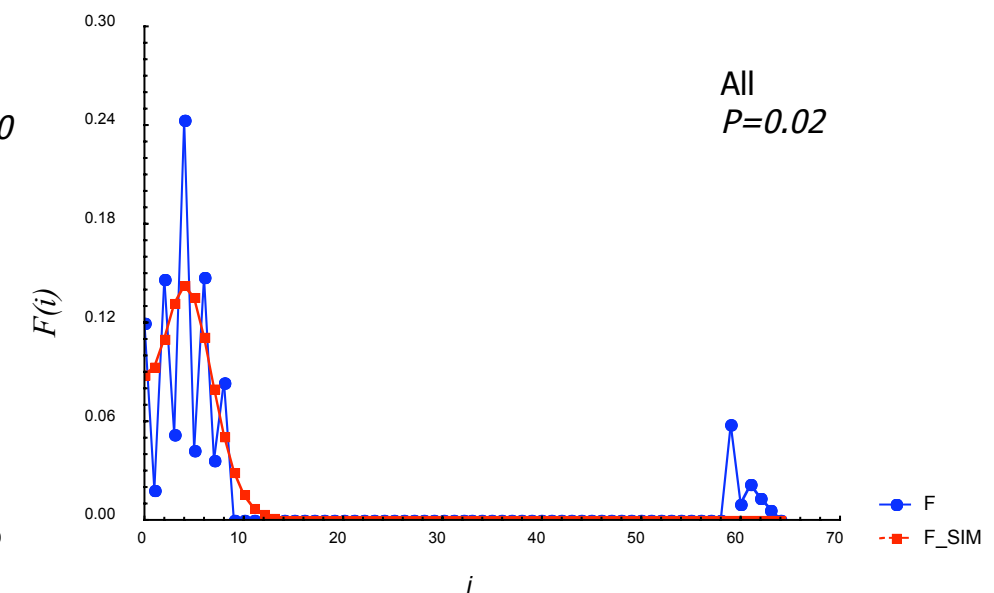
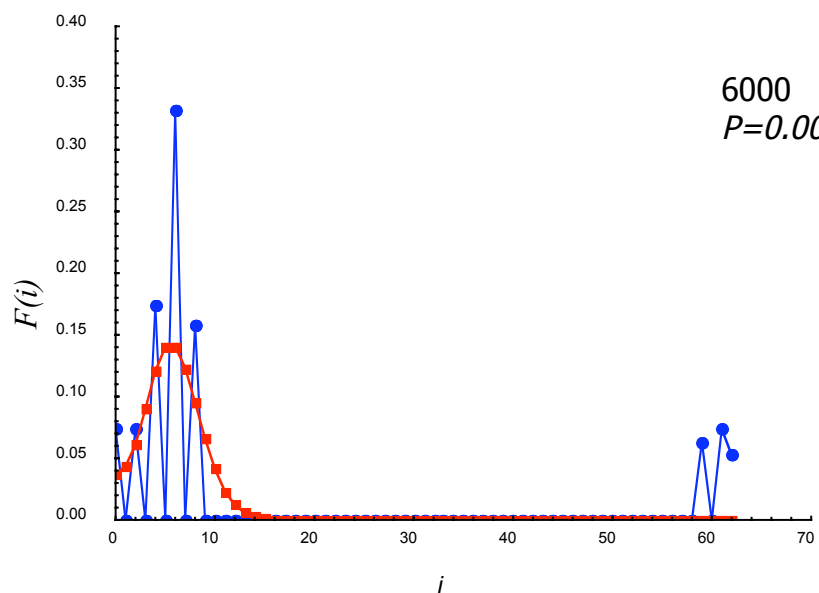
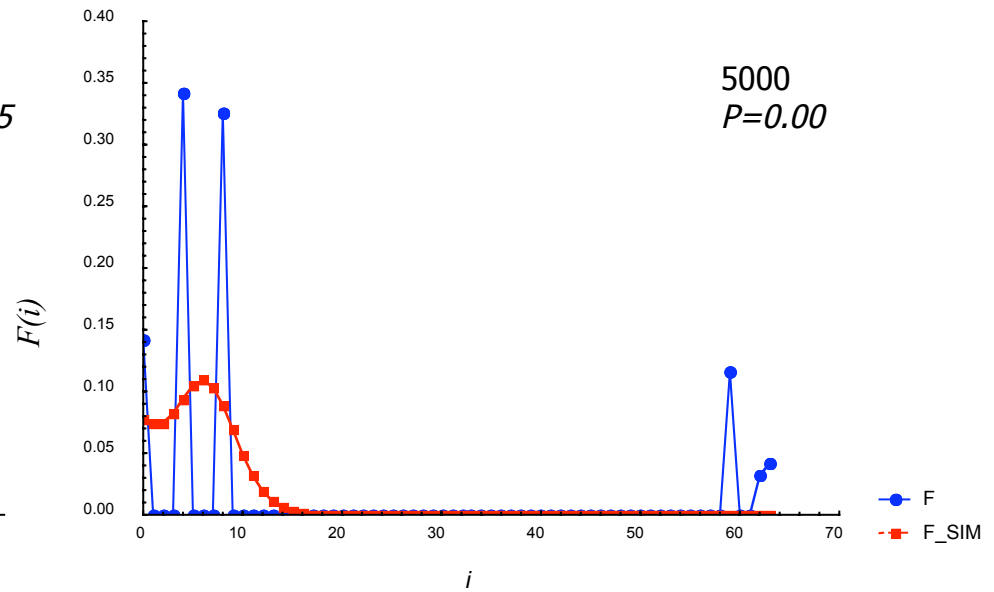
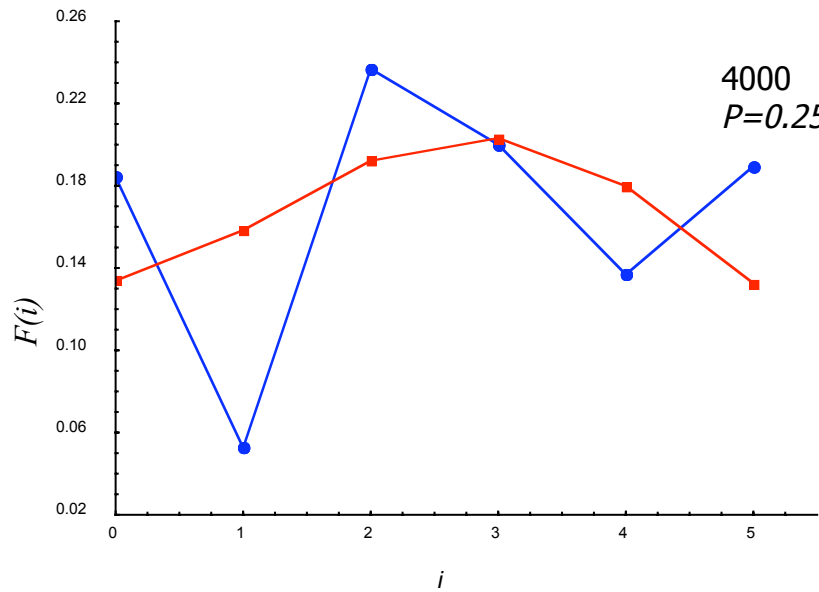
Network analysis revealed a star-like pattern of relationships among haplotypes, with a single haplotype shared among the different sub-populations and a large number of haplotypes unique to single sub-populations.

The presence of these unique haplotypes suggests reduced mtDNA gene flow among different sub-populations.

Genetic data contain information not only about relationships among individuals but also about the demography of a population. In particular, the shape of the distribution of pairwise nucleotide site differences between individuals (mismatch distribution) is informative about past changes in the population size.

A sudden population expansion will produce a unimodal mismatch distribution; a population with a stable size will produce a bi- or multimodal mismatch distribution.

We tested the hypothesis of a sudden population expansion in our study population, considering each sub-population separately and pooling all the sub-populations together.



The hypothesis of a sudden population expansion is always rejected except for sub-population at point 4000. Data indicate a population stasis with only slight signs of population expansion.

## Compare genetic data with behavioural ones

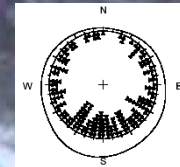
The rationale for this approach comes from a work conducted by Scapini *et al.* (1995) that showed how populations living on stable beaches are, on average, more genetic polymorphic than populations living in changing beaches.

Previous genetic surveys based on allozymes failed in revealing statistically significant differences in the level of genetic polymorphism among different sub-populations sampled at the Uccellina beach.

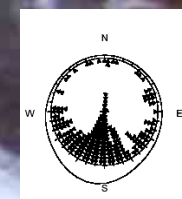
In this study we used a more variable marker and we have had the possibility, for the first time, to analyze simultaneously genetic and behavioral traits in sub-populations living on qualitatively different points along a dynamic beach.

Ombrone river mouth

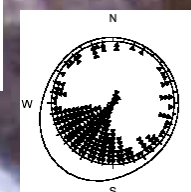
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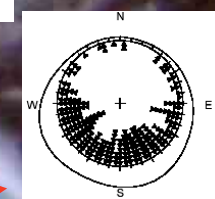
3000  
 $\pi = 0$



4000  
 $\pi = 0.006 \pm 0.004$



5000  
 $\pi = 0.037 \pm 0.019$



6000  
 $\pi = 0.038 \pm 0.019$

Orientation data from Scapini *et al.* Marine Biology, in press

Samples from the eroded point 3000 showed no genetic variability and a poor adaptation to the orientation of shoreline.

At point 4000 (beach with well developed dune and recent signs of slight erosion) we observed an increase in the mean level of genetic variability and samples begin to show a good adaptation to the orientation of shoreline.

Genetic variability detected at points 5000 and 6000 (accreting beaches) is significantly higher ( $t$ -test;  $p < 0.05$ ) than genetic variability found at point 4000. At point 5000 sandhoppers showed a good adaptation to the orientation of shoreline; at point 6000 the adaptation was less precise because of the heterogeneity of the environment (i.e. presence of marshes behind the low dune).

# Conclusions

COI proved to be a highly informative marker

We found parallel trends in variation of genetic and behavioural traits

A protocol that would include a combination of both analyses could be proposed as a sensitive bioassay to assess the stability of shoreline