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Polychromatism and biogeography in the ophiuroid *Amphipholis squamata* (Echinodermata: Ophiuroidea) in the area of Messina (Sicily)

SAMUEL DUPONT*, LORENZA SALPIETRO**°, MARIATERESA COSTANZO°, JÉRÔME MALLEFET*
**Laboratory of Animal Physiology, Catholic University of Louvain, Batiment Carnoy, 5 Place Croix du Sud, B-1348 Louvain-la-Neuve (Belgium)*
e-mail: dupont@bani.ucl.ac.be (Belgium);
and Biodiversity Research Centre, Catholic University of Louvain (Belgium);
***International Research Institute "Fernand Baguet" (Italy)*
°Department of Animal Biology and Marine Ecology, University of Messina (Italy)

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SUMMARY

The cosmopolitan ophiuroid species *Amphipholis squamata* was observed in 10 sampling stations in the area of Messina (Italy). These stations were located in biotope presenting contrasting habitat structures: in gravels, under stones, in the green seaweeds *Chaetomorpha aera* or *C. mediterranea*, in the red seaweeds *Corallina elongata* or *Pterocladia sp.* The species is polychromatic and eight different colour morphs were observed in this area: orange, beige, dark-brown, grey and four spotted forms. Huge differences observed between the populations for the frequencies of these varieties were formalized using the cosinus distance method. Our results suggest that: i) the degree of opening to the sea is a factor that one has to take into account to population studies; and, ii) biotic environment is the principal factor inducing microevolution within this species.

INTRODUCTION

The small brittle-star *Amphipholis squamata* Delle Chiaje has attracted the interest of biologists since more than two centuries. It is the only coastal ophiuroid that presents a cosmopolitan distribution and the species is observed from superficial levels down to 1.330 m (Gage et al., 1983; Jones and Smaldon, 1989). *A. squamata is* also polychromatic and numerous colour morphs were described all over the world (Binaux and Bocquet, 1971; Deheyn et al., 1997). The colour morphs are not randomly distributed in the populations and authors suggest that it could be due to different ecophysiological tolerance between the morphs. Therefore, the distribution of colour morphs between populations might be mainly due to abiotic conditions such as temperature or salinity (Deheyn et al., 2000). Other works demonstrate the importance of other environmental parameters. For example, important differences were observed for the efficiency of predator activity (crustacean and fish) according to the brittle-star colour morph (Dupont et al., 2000a). Therefore, the structure of a population might be a consequence of a complex combination between biotic and abiotic factors.

In *Amphipholis,* an important variability was observed at all the levels: genetics (Poulin et al., 1999; Sponer et al., 1999; Dupont et al., 2000b), physiology (De Bremaeker et al., 1994; Mallefet et al., 1994; Deheyn et al., 1997) and demography (Dupont and Mallefet 1999, 2000c). Polychromatism is the main factor explaining this variability. This species is also bioluminescent and each colour morph possesses its own luminous capabilities (Deheyn et al., 1997) genetically determined (Dupont et al., 2000b). As a consequence, polychromatism and/or bioluminescence properties constitute good genetic markers.

The aim of this work was to use polychromatism as an indirect tool for studying population genetics and microevolutive processes. This original approach was used to compare geographically close populations, presenting similar physicochemical conditions but with substantial differences in the structure of the biotope and in the degree of connection with the open sea. This could enable the identification of the influence of these factors on the genetic structure of the populations.

MATERIALS AND METHODS

Specimens of *A. squamata* were sampled in ten different stations in the area of Messina (Italy) (Fig. 1). In the Oliveri-Tindari system (38°108'N 15°103'E), the species is present in four of the six lagoons. These lagoons were completely isolated from the open sea at different periods (Crisafi et al., 1981; Costa et al., 1996). In Lago Mergolo (LM) and Fondo Porto (FP), isolated from the sea 30 and 10 years ago respectively, ophiuroids were found on gravel substratum when in Lago Nuovo (LN) and Porto Vecchio (PV), isolated since 20 and 10 years respectively, animals were found in the green seaweed *Chaetomorpha mediterranea* Kutzing located in a meadow of *Cymodocea nodosa* (Ucria) Ascherson. Three different stations were chosen in Lago Faro, a lagoon in the zone of Torre Faro (38°117'N, 15°139'E). In the first station (LFI), brittle-stars were located in the seaweed *Chaetomorpha aera* (Dillwyn) Kutzing; in the second station (LFII), animals were

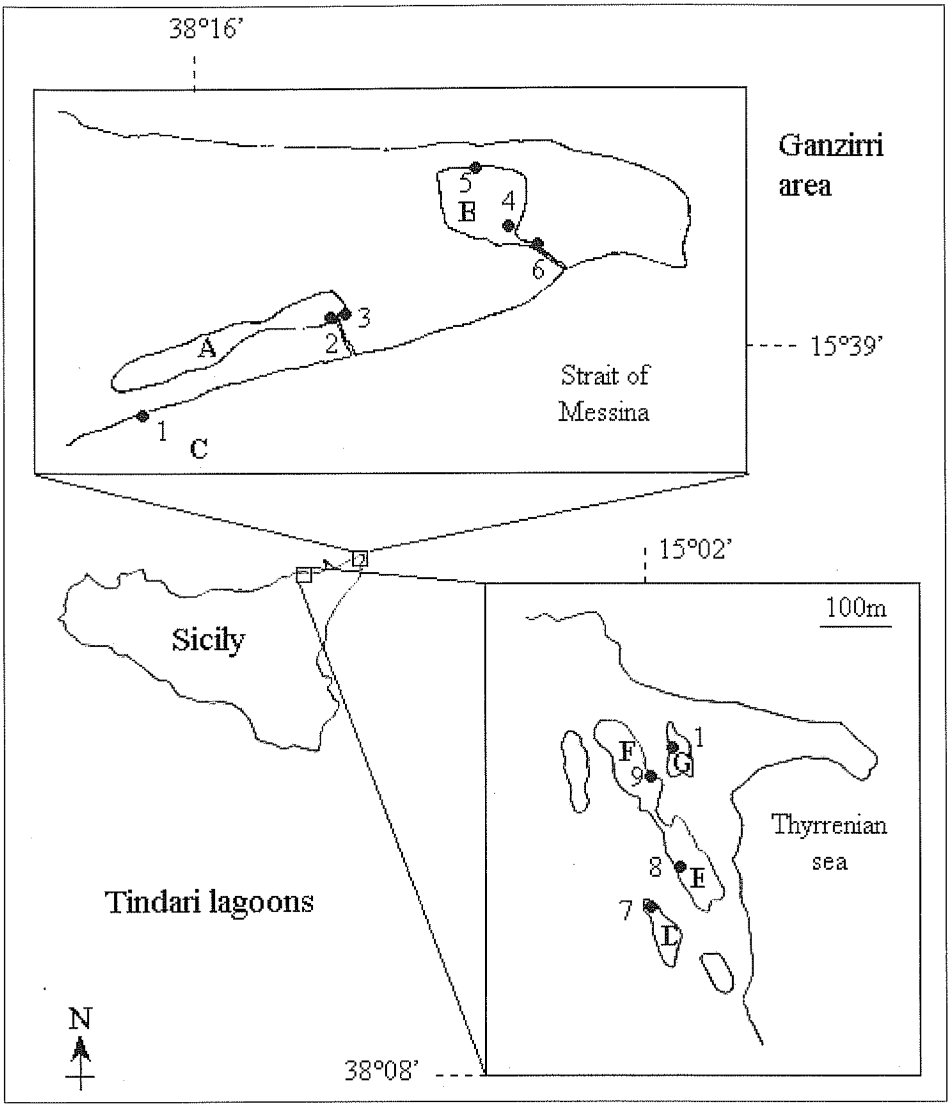

Fig. 1 - Sampling stations. *Ganzirri area*: A, Lungo Lago; B, Lago Faro and C, Strait of Messina. *Oliveri-Tindari lagoon system*: D, Lago Mergolo, E, Porto Vecchio, F, Fondo Porto et G, Lago Nuovo. Black spots correspond to the sampling sites: 1, Strait; 2, LLI; 3, LLII; 4, LFI; 5, LFII; 6, LFIII; 7, LM; 8, PV; 9, FP et 10, LN

found under stones; and, in the third station (LFIII), *Amphipholis* were associated with the red seaweed *Pterocladia sp.* In the lagoon Lungolago of the Ganzirri area (38°116'N, 15°138'E), two stations were sampled (LLI and LLII) where ophiuroids live in close association with the green seaweed *C. aera.* Both lagoons of Torre Faro and Ganzirri are not completely isolated from the open sea since they are connected to the strait of Messina by several channels. In the last station, ophiuroids were sampled in the red calcareous seaweed *Corallina elongata* Ellis and Solander located 1 to 2 m depth in the strait of Messina at Ganzirri. In all the studied stations, similar abiotic conditions were observed (temperature, salinity, pH and photoperiod).

More than 200 brittle-stars were collected at each stations between June 1997 and September 2000. Sampled specimens were transported to the University of Messina and maintained in aerated aquaria. The colour morph was determined for each specimen on the basis of the colour of arms and disc. Then, the frequencies of each colour morph were calculated in each station. On the basis of these frequencies, a distance matrix was computed using the cosinus distance method:

$$di,j = 1 - \sqrt{\sum_{morphi} fi \times fj}$$

where the distance between the populations i and j (di, j) was calculated using the frequencies of each morphs in the two stations (fi and fj respectively). Tree was built using the neighbour-joining method. All data analyses were performed using Statistical Analysis System (SAS Institute).

RESULTS

Eight different colour morphs of *A. squamata* were observed in the 10 sampled stations: orange, beige, dark-brown, grey and spotted as described by Deheyn et al. (1997). The spotted morph was separated in four sub-morphs (a, b, c and d) on the basis of the coloration of the arms. Frequencies of these morphs in the various populations are presented in the Table I. Whereas in Lago Mergolo, only specimens of the spotted "a" were observed, the other stations are constituted of two to six colour morphs coexisting in sympatry. Moreover, some morphs were present in more stations than the others. For example, the orange and the spotted "d" morphs were only present in Lago Nuovo and in the strait of Messina respectively. The other morphs were observed in most of the sampled stations. For example, the spotted "a" and "b" morphs were present in eight of the ten stations. The distances were calculated between the populations using the cosinus method on the frequencies of the colour morphs (Tab. II). These data were presented as a tree (Fig. 2). The strait of Messina was isolated from all the other stations since it was composed by more than 90% of a rare variety (spotted "d"). The Lago Faro stations I and II are clustered with the station II of Lungolago since they shared 4 colour morphs (Grey, Dark-brown, Spotted "a" and "b") with a dominance of the grey morph. The station Porto Vecchio and Lago Mergolo were clustered and were characterized by a dominance of the spotted "a" morph. The last cluster was composed by the station I of Lungolago, the station III of Lago Faro and the stations from Fondo Porto and Lago Nuovo. It constituted the most heterogeneous group but with a dominance of the spotted "b" morph.

Tab. I - Frequencies of colour morphs in the different sampled stations

	Orange	Beige	Grey	Dark-brown	Spotted a	Spotted b	Spotted c	Spotted d
Porto Vecchio	0	0	0	0.07	0.6	0.33	0	0
Lago Mergolo	0	0	0	0	1	0	0	0
Lago Nuovo	0.12	0	0	0.44	0.2	0.24	0	0
Fondo Porto	0	0	0.04	0.04	0	0.92	0	0
Strait	0	0	0	0	0	0	0.09	0.91
Lungo Lago I	0	0	0.13	0.24	0.24	0.39	0	0
Lungo Lago II	0	0	0.64	0.04	0.06	0.26	0	0
Lago Faro I	0	0.26	0.25	0.05	0.14	0.2	0.1	0
Lago Faro II	0	0	0.61	0.11	0.01	0.06	0.21	0
Lago Faro III	0	0.04	0	0	0.02	0.78	0.16	0

Tab. II - Matrix of distance between Stations calculated using the cosinus distances on frequencies of colour morphs

	PV	LM	LN	FP	Strait	LLI	LLII	LFI	LFII	LFIII
PV	0									
LM	0.2254	0								
LN	0.5204	0.5528	0							
FP	0.4495	1	0.5142	0						
Strait	1	1	1	1	0					
LLI	0.4619	0.5101	0.5028	0.3920	1	0				
LLII	0.6470	0.7551	0.6967	0.4864	1	0.5433	0			
LFI	0.6082	0.6258	0.6870	0.5595	0.5641	0.6049	0.5284	0		
LFII	0.8170	0.9	0.7454	0.7551	0.8625	0.6374	0.3589	0.7161	0	
LFIII	0.4810	0.8586	0.5627	0.1575	0.88	0.4441	0.5483	0.5697	0.5614	0

DISCUSSION

In *Amphipholis squamata,* the observed genetic variability as revealed by RAPDs markers, is correlated to the phenotypic plasticity at both physiological (bioluminescence properties) and morphological (polychromatism) levels (Deheyn et al., 1997; Dupont et al., 2000b). As a consequence, the polychromatism is a good genetic marker for population genetic studies. In Sicily, eight colour morphs were observed, reflecting an important inter and intrapopulation genetic variability. Moreover, important differences were observed in the frequencies of these morphs in sampled stations. Our analysis indicates that both geographical position and degree of connection to the open sea have effects on the population structure: i) the strait of Messina population is the only one completely opened to the sea and is well separated from the others

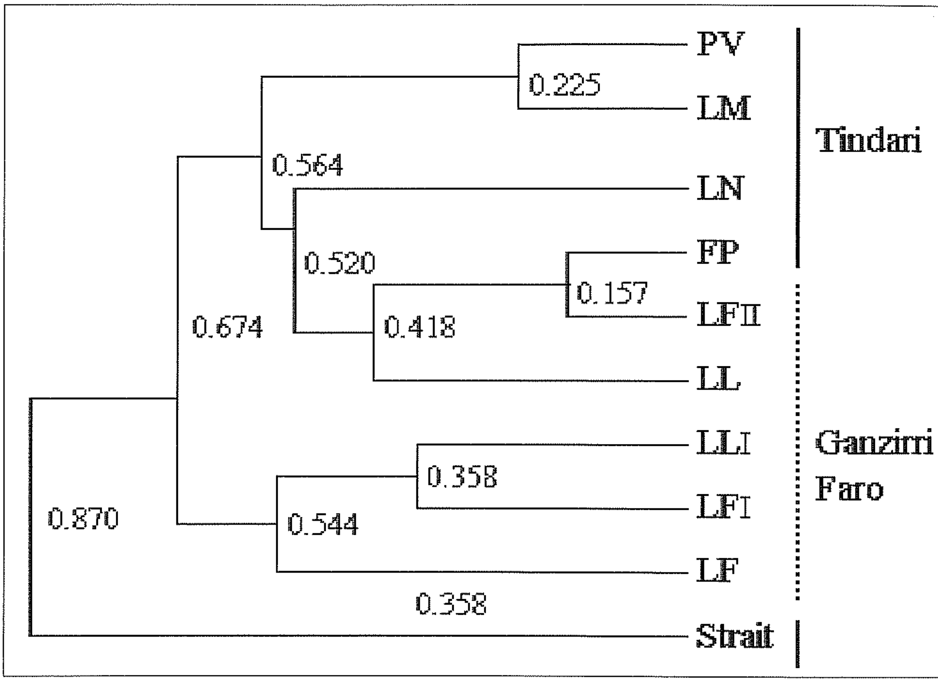

Fig. 2 - Tree inferred from cosinus distances between frequencies of colour morphs. PV, Porto Vecchio; LN, Lago Nuovo; FP, Fondo Porto; LM, Lago Mergolo; Strait, strait of Messina; LLI-II, Lungo Lago stations I-II; LFI-III, Lago Faro stations I-III

by the analysis; ii) Tindari's stations (Lago Mergolo Lago Nuovo, Fondo Porto and Porto Vecchio), isolated from the open sea since more than 10 years, are clustered together; and iii), three of the five stations connected to the sea by small channels (Lungolago II and Lago Faro III) are grouped in the same cluster.

Two factors have been proposed to explained differences between populations: differences between morphs in the sensibility to the abiotic conditions (Deheyn et al., 2000) and/or the influence of the biotic conditions (mainly predation, Dupont et al., 2000a). Large differences were observed between the sampling stations even though abiotic conditions are similar (temperature, salinity, pH and photoperiod). Therefore, these differences cannot be explained by a differential sensibility to abiotic conditions. We suggest that the degree of opening to the sea is a factor that one has to take into account to explain the population structure. This parameter could influence the biotic composition of an environment: the presence of a parasite, the abundance of a predator, etc. We can then conclude that the population structure is mainly determined by the biotic conditions. Experimental works are currently in progress in our laboratories to confirm this hypothesis.

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