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Morphological, physiological and genetic variability of the ophiuroid *Amphipholis squamata* from the lagoon system of Oliveri-Tindari (Sicily)

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ABSTRACT: *Amphipholis squamata* is considered to be the only echinoderm distributed world-wide, excepted in polar regions. This vast distribution area contrasts with a low dispersal potential due to the lack of a pelagic larval stage and an aggregative spatial distribution.

Previous works have shown strong inter- and intra-population variability among adult individuals for both phenotype and genotype, although the species seems to be anatomically uniform. The aim of this work was to understand these individual variations at three different levels : (a) the morphological differences characterized by the colour of arms and disc; (b) the physiological variations estimated by luminous capabilities and (c) the genetic variations as revealed by RAPDs.

Four populations were sampled in the system of Oliveri-Tindari in Sicily which consists of several lagoons completely isolated from the open sea and from each other for various periods. This system constitutes a suitable model for studying differentiation between closely neighbouring demes. Our results confirm that each colour variety possesses its own luminous capabilities; they also indicate that colour varieties are genetically differentiated.

1 INTRODUCTION

In spite of the absence of a larval phase, the simultaneous hermaphrodite (Nisolle, 1990) and brood protecting ophiuorid *Amphipholis squamata* is regarded as a cosmopolitan species. This species lives in all the oceans except Antarctica and, in varied habitats, from tidal zone down to 1330 m depth (Gage et al. 1983, Hendler 1995, Alvà 1996). *A. squamata* constitutes dense local populations often occupying microhabitats. There is a paradox of an extremely wide distribution of a species that lacks a larval stage and often shows an aggregative spatial distribution. The reproductive biology of the species can explain a high potential of colonization, *e.g.* by means of rafting (Mortensen (1933, 1941), recently confirmed by Highsmith (1985), Alvà & Vadon (1989) and Murray(1989)), but it is still difficult to understand how it could maintain regular gene flow between distant populations (Poulin et al. 1999).

Variability seems the rule within *Amphipholis*. Numerous colour varieties were reported for this species (Binaux & Boquet 1971, Deheyn 1998, Dupont 1998, Deheyn & Jangoux 1999, Dupont et al., *this volume*). *A. squamata* is luminescent (Brehm & Morin 1977) and important infraspecific variations of luminous capabilities were described (Deheyn et al. 1997). Poulin et al. (1999) have demonstrated the co-existence of selfing and outcrossing in this species (a result different from Murray (1989) who hypothetized that only outcrossing was occurring). They also demonstrated a great genetic differentiation of *A. squamata* at a very short distance. At another scale, Sponer et al. (1999) have showed that global populations are genetically highly differentiated and hypothetized that different clades were likely representing different species.

The results of Deheyn and Jangoux (1999) discussing the inheritance of body colour and bioluminescence characters and those of Poulin et al. (1999), Sponer et al. (1999) and Dupont et al. (*this volume*) discussing the relationships between phenotypic characters and genetic structuring questioned about the taxonomic status of *A. squamata*.

The aim of this paper is to characterize the intra- and inter- population variability at 3 levels, morphological, physiological and genetic. It is also to evaluate the congruence between polychromatism, bioluminescence and genetic structure of very close local populations of *A. squamata*, recently separated from the open sea and from each other.

2 MATERIAL AND METHODS

Four populations were sampled in Sicily, from the lagoon system of Oliveri-Tindari (Figure 1): this recent system of lagoons (about one century old) is in permanent change due to the action of wind and currents. These 4 populations have been completely isolated from the open sea for various periods: Lago Nuovo and Lago Mergolo della Tonnara were isolated from Fondo Porto since respectively 30 and 20 years ; Fondo Porto and Porto Vecchio are connected to each other by a small canal, were both isolated from open sea 10 years ago (Crisafi et al. 1981, 1987. Dupont 1998). Therefore, this lagoon system *a priori* constitutes a suitable model for studying differentiation between closely neighbouring separated demes.

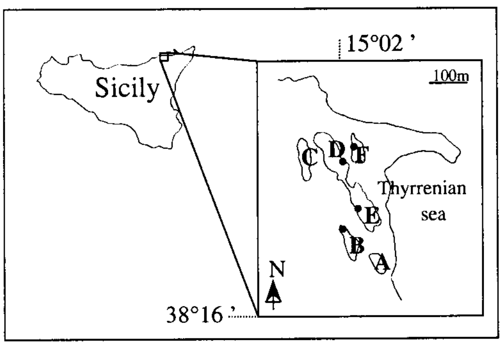

Figure 1. Oliveri-Tindari lagoons: Lago Marinello (A), Lago Megolo (B), Lago Verde (C), Fondo Porto (D), Porto Vecchio (E), Lago Nuovo (F); point of sampling (●)

183 individuals were collected (78 in Lago Nuovo, 31 in Lago Mergolo, 42 in Fondo Porto and 32 in Porto Vecchio) in shallow phanerogam meadows (*Cymodecea nodosa*) or in algae (*Cheatomorpha mediterranea*). Ophiuroids were sorted on the colouration of arms and disc. For each individual, two arms were used for studying bioluminescence, then the 3 other arms were frozen at -80°C and used for RAPD-PCR.

Methods used for studying bioluminescence and for evaluating the genetic structuring of *Amphipholis squamata* are given in Dupont et al. (*this volume*). DNA profiles were analysed using SCION IMAGE software v.beta3b (1998). Cluster analysis of RAPD-PCR patterns in individuals were performed with RAPDPLOT program, using Nei and Li's genetic similarity index, developed for RAPD markers (Black 1995). Cluster analysis with nearest neighbor procedures was done in conjunction with the NEIGHBOR program in PHYLIP 3.5c (Felsenstein 1993). A single unrooted tree was generated and plotted using DRAWGRAM in the same package.

3 RESULTS

3.1 *Morphological data (colour varieties)*

Great differences were observed among the lagoons. Three colour varieties were sorted on the colouration of arms and disc: dark-brown, orange, and spotted (see Deheyn et al. 1997) . Figure 2 gives the frequency of these colour varieties in each lagoon. Spotted *Amphipholis* were found in all lagoons, however, the spotted individuals from Lago Mergolo were clearer than all the others. They are referred to as the spotted "a" variety. The others individuals form the spotted "b" variety. A dark-brown variety was found in Fondo Porto and in Lago Nuovo. Orange ophiuroids were only found in Lago Nuovo.

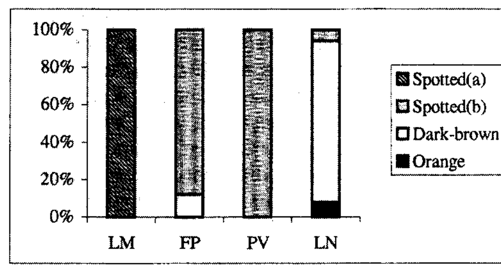

Figure 2. Frequency of the colour varieties in the lagoons of Lago Mergolo (LM), Fondo Porto (FP), Porto Vecchio (PV) and Lago Nuovo (LN). The spotted variety is split into 2 subcategories.

3.2 *Physiological data (bioluminescence)*

When stimulated by KCl, the arm response is a monophasic light emission characterized by the maximal intensity (Lmax in Mquanta.s^{-1}), and by 3 kinetic parameters (expressed in seconds), namely, the latency time (Lt), the time to reach the maximal intensity (Tlmax) and the time to reach half- extinction ($T_{1/2}$ext). Data were considered as coordinate using the 4 luminous parameters. Euclidian distances were computed and observations were hierarchically clustered using Ward's maximum-variance method (1963).Using this method, 12 groups were isolated (Figure 3). The three colour varieties are clearly separated: cluster 1 consists of orange ophiuroids, clusters 2 to 4 consist of the dark-brown variety and clusters 5 to 12 include the spotted individuals. Two groups are distinguishable among the spotted ophiuroids, one of them consisting mostly on *Amphipholis* of Lago Mergolo, the second of all the spotted "b" individuals from the other sites. Nevertheless, no

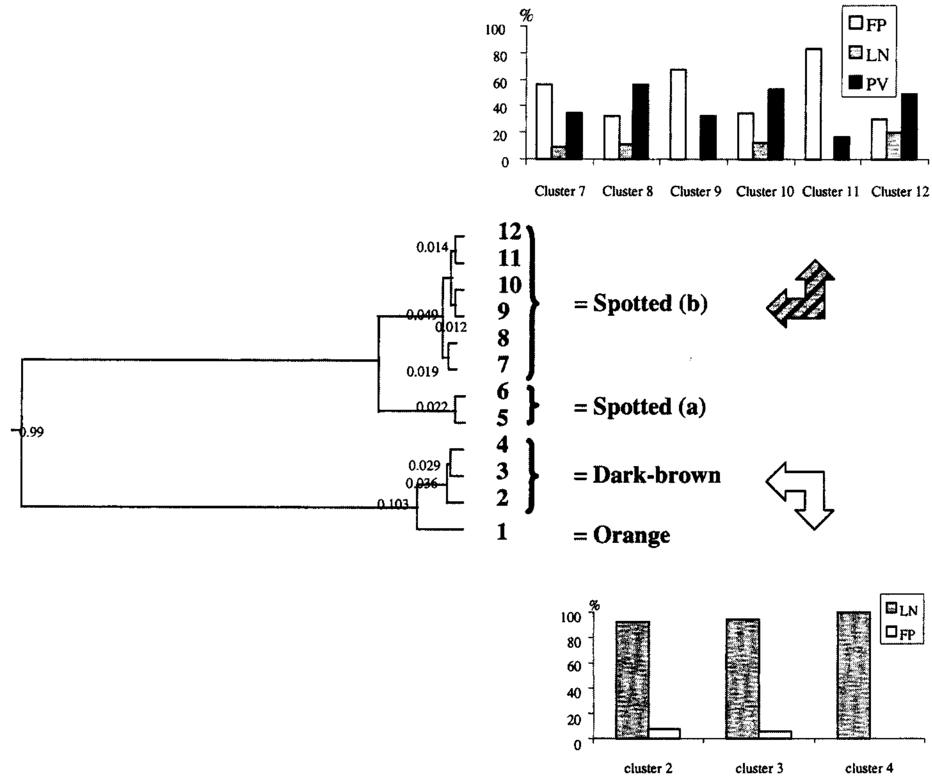

Figure 3. Tree inferred from Euclidian distances between bioluminescence parameters (distances within a cluster is inferior to 0.01). The frequency of individuals from the different demes is given for the spotted "b" variety (clusters 7 to 12 - up right) and for the dark-brown variety (clusters 2 to 4 - bottom right).

clear differences among the lagoons were observed using this method.

Canonic discriminant analysis was used on luminous parameters to summarize between-class variations. When a geographical key was taken as the qualitative variable, it does not give clear pattern; there is no discrimination of bioluminescence between the lagoons (Figure 4-left). However, using a morphological key (colour varieties) 3 groups of ophiuroids can be identified according to their colour (Figure 4-right). Spotted "a" and "b" are clustering together. They are not distinguishable by this

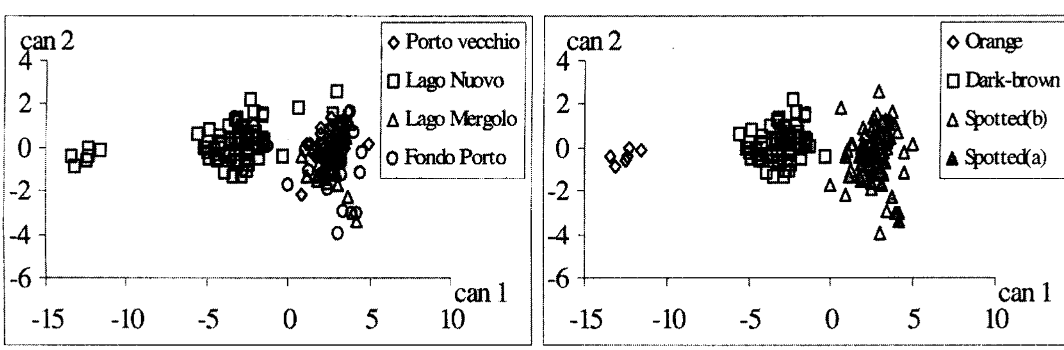

Figure 4. Canonic discriminant analyses lead to define 3 groups which are not interpretable when using a geographical key (on the left). However, when using a morphological key (on the right) the 3 groups are cor-responding to the colour varieties. It is to be noticed that spotted "a" and spotted "b" groups are not separated by this method.

Table 1. Mean values (±S.D.) of the bioluminescent parameters. *N=number of ophiuroids, see abbreviations in the legend of figure 2 and in the text.*

Population	Variety	N	Lmax	Lt	TLmax	T1/2ext
LN	Orange	6	0.68±0.09	2.83±0.29	5.67±0.49	7.23±0.68
LN	Dark-brown	67	104.33±7.47	1.33±7.47	5.20±0.33	3.70±0.15
FP	Dark-brown	5	134.76±29.21	1.08±0.18	3.63±0.76	3.07±0.96
LN	Spotted (b)	5	1097.50±205.54	0.72±0.18	9.66±1.67	2.75±0.37
FP	Spotted (b)	37	2757.49±300.51	1.06±0.08	4.77±0.41	1.77±0.41
PV	Spotted (b)	32	1910.48±187.37	0.78±0.06	5.50±0.39	1.85±0.15
LM	Spotted (a)	37	3119.86±304.26	0.82±0.05	1.49±0.10	1.09±0.10

method.

Table 1 gives the average values of the bioluminescent parameters for the colour varieties from the 4 sites. ANOVA on luminous parameters show that there is no difference of intensity between the lagoons for the same colour variety (dark-brown and spotted "b"), but that the differences among varieties are highly significant ($F=439.27$, $p<0.01$). Each colour variety produces light at a specific intensity (Figure 5). They also show that if one can distinguish the colour varieties on the basis of emitted light intensity; the kinetic parameters are less clear.

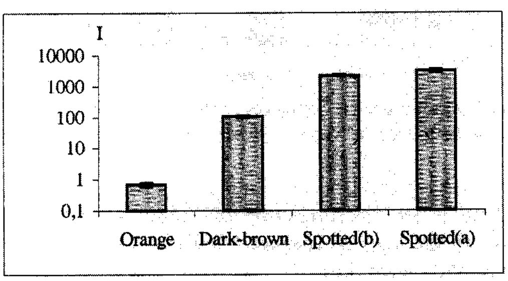

Figure 5. Intensity average value (log) in the 4 colour varieties.

3.3 *Genetic data*

Patterns obtained by RAPD were quite different between the sites. Figure 6 represents the genetic relationships among the 183 individuals.

When using a geographical key, Lago Mergolo is quite identifiable, as Porto Vecchio is. Only one colour variety is living in each of these two sites. However, no clear pattern appears for Lago Nuovo and Fondo Porto is split into at least 4 groups. None of them corresponding to a particular variety.

Using a morphological key gives a more ordered picture. Four main clusters appear based on the genetic distance:

- One orange group which consists of *Amphipholis squamata* of Lago Nuovo.
- Two spotted groups: one consisting mostly of individuals referred to the spotted "a" variety of Lago Mergolo and some individuals of Fondo Porto. A second spotted group corresponds to the spotted "b" variety. It includes ophiuroids from Porto Vecchio and Fondo Porto.
- One dark-brown group consists of *A. squamata* of Lago Nuovo.

4 DISCUSSION

Amphipholis squamata presents a great anatomical homogeneity in Sicily and throughout the world (Hyman 1955). However it shows a great variability of colours, bioluminescent capabilities and genetics (Murray 1989, DeBremaeker et al. 1994, Mallefet et al. 1992, Deheyn et al. 1997, Dupont & Mallefet 1999, Poulin et al. 1999, Sponer et al. 1999). The present results, obtained on very close local populations, confirm the link between polychromatism and bioluminescence, already observed for the population in Normandy by Deheyn et al. (1997). Each colour variety possesses its own luminous capabilities. It also demonstrates the existence of a link between polychromatism and genetics within local populations. However, no clear genetic differences were demonstrated between colour varieties in the different lagoons. One explanation is that these varieties surely originated before the formation of the lagoons. These results complete those of Deheyn and Jangoux (1999) discussing about the inheritance of body colour and bioluminescence characters.

Two main hypotheses arise from our results:

- Are the observed genetic patterns the result of a founder effect, due to the "subsampling" of genes triggered by the history of the lagoons ? We are going to use other decaprimers for RAPD and other "rapid" genetic markers, such as VNTR, to confirm genetic patterns and find out relationships among populations between the lagoons.
- Are the observed patterns characterized by important differences between the frequencies of colour varieties among demes are the result of natural selection due to differential parasitism (*e.g.* the

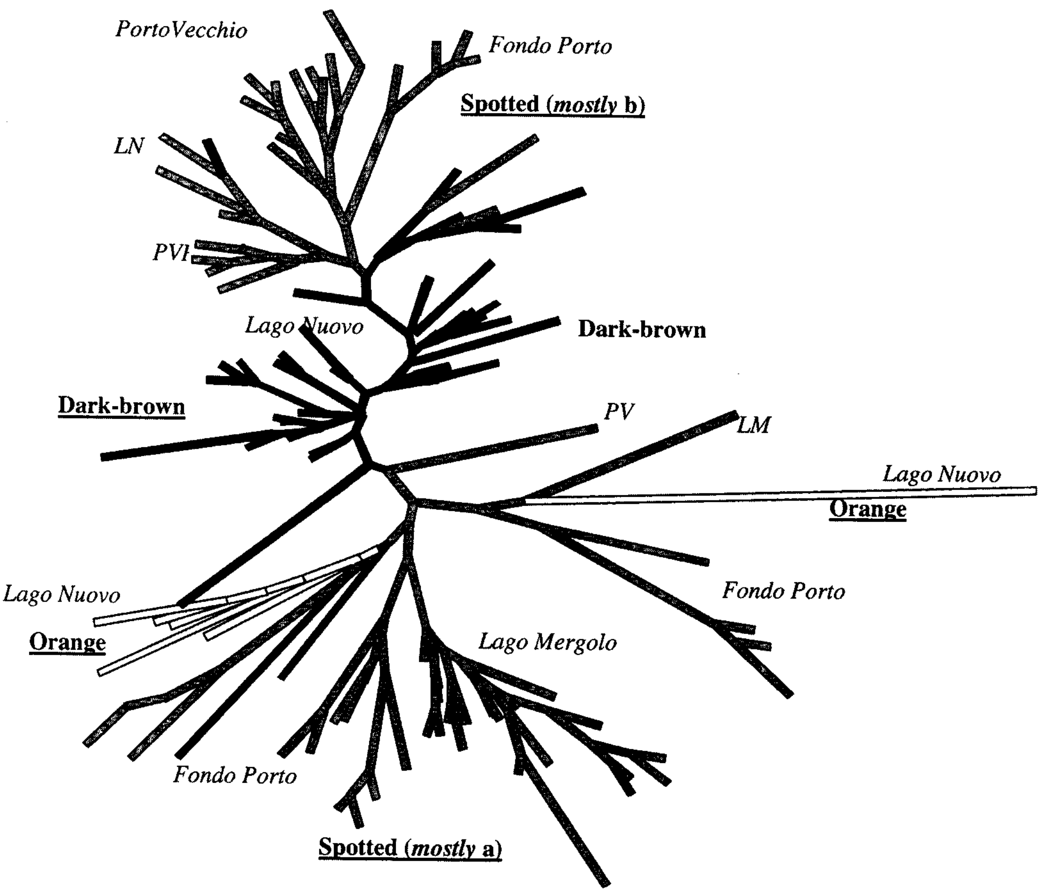

Figure 6. Inferred tree from a distance matrix (Li & Nei) showing the genetic diversity and structure of the four colour varieties of *Amphipholis squamata* from the lagoon system of Oliveri-Tindari.
Colour varieties: *gray* = spotted "a" and "b", *black* = dark-brown, *white* = orange.

Ciliate *Rhopalura ophiocomae* specifically castrates the spotted variety) and/or to differential predation by fishes (*e.g. Gobius niger*) and/or shrimps (*e.g. Palaemon serratus*) ?

All these aspects are currently under study.

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