

## IS THERE A LINK BETWEEN MORPHOLOGICAL, PHYSIOLOGICAL AND GENETIC VARIABILITY OF THE OPHIUROID *AMPHIPHOLIS SQUAMATA*?

by

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### ABSTRACT

*Amphipholis squamata* is a small ophiuroid considered to be distributed worldwide except in Polar Regions. Numerous colour varieties were reported for this species, which is also bioluminescent. It is hermaphrodite and brood protecting; both selfing and outcrossing occur. A high genetic variability was observed among adult individuals belonging to very close (Mediterranean) local populations. Three distant populations were investigated in order to characterize morphological, physiological and genetic variability. In the population of Normandy (France), *Amphipholis* were collected under stones of tide pools; ophiuroids from Sicily (Italy) were collected in a *Cymodocea nodosa* meadow from a lagoon completely isolated from the sea since 10 years; individuals from Santa Barbara (USA) were sampled in the aquarium system of the Marine Institute. Four colour varieties are studied throughout the sampled populations and each exhibits its own capability to produce light: (1) a spotted variety is present in the 3 populations and produces light of high intensity; (2) the orange, grey and black varieties are only present in the population of Normandy and produce light of lower intensity. Genetic variations were revealed by RAPDs. Preliminary results indicate that genetic structure is homogenous for each colour variety within a population while, as expected, it shows inter-population variations for the same variety. This suggests that polychromatism and bioluminescence might be good indicators of variability of genotypes only at an intra-population level.

KEY WORDS: Echinodermata, *Amphipholis*, distant populations, colour varieties, bioluminescence, genetic structure, PCR-RAPD, intraspecific variability.

### INTRODUCTION

*Amphipholis squamata* is regarded as a cosmopolitan ophiuroid species, hermaphrodite and brood protecting, living in all the oceans except for the

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polar areas, in varied habitats, from the intertidal zone down to 1330 m depth (GAGE *et al.*, 1983; HENDLER, 1995; ALVÀ, 1996). *A. squamata* constitutes dense local populations often occupying microhabitats thanks to its small size (max. 5 mm in disc diameter). The spatial distribution of the species is aggregative and frequently several individuals are observed to occupy the same stone or the same tuft of algae. From a literature survey, it is obvious that variability is the common rule within the species at all studied levels: (i) Numerous colour varieties were reported for this species (BINAUX & BOQUET, 1971; DEHEYN, 1998; DUPONT, 1998). (ii) In *A. squamata* mechanical stimulation induces light production by the arms (BREHM & MORIN, 1977) and large variations of in the capability for luminescence were described (DEHEYN *et al.*, 1997). POULIN *et al.* (1999) have demonstrated the existence of selfing and outcrossing in *A. squamata* by means of genetic markers as well as a great genetic differentiation at a very short distance. At another scale, SPONER *et al.* (1999) showed that global populations are genetically highly differentiated and that different clades were likely to represent different species. The aim of this paper is to evaluate the congruence between morphology (polychromatism), physiology (bioluminescence) and genetic structure.

## MATERIAL AND METHODS

Three distant populations were investigated. A total of 138 ophiuroids were sampled by hand in March–April 1999. In the population of Luc-sur-mer (Normandy, France), *Amphipholis* were collected under stones of tide pools. Ophiuroids from Oliveri-Tindari, lagoon of Porto Vecchio (Sicily, Italy) were collected in a *Cymodocea nodosa* meadow from a lagoon completely isolated from the sea since 10 years. Individuals from Santa Barbara (USA) were sampled in the aquarium system of the Marine Institute.

Four colour varieties were sorted on the coloration of arms and disc: black, orange, grey and spotted, as described in DEHEYN *et al.* (1997). In each individual, two arms were used for studying bioluminescence. Then the animal was frozen at  $-80^{\circ}\text{C}$ . The three other arms were used for RAPD-PCR.

The arms are the only luminescent body parts of *A. squamata* (BREHM & MORIN, 1977) and the five arms from a single individual produce light of the same intensity (MALLEFET *et al.*, 1992). Measurement of on luminescence were carried out in a dark room as describe in MALLEFET *et al.* (1992) using a photomultiplier phototube (PM 270

D, EMI). Maximal light emission was triggered with KCl (200 mM) and registered on a chart recorder. Four parameters were measured to characterize the monophasic light response of *A. squamata* (MALLEFET *et al.*, 1992), the maximal intensity of light expressed in megaquanta per seconds ( $L_{max}$  in  $Mq.s^{-1}$ ) and three kinetic parameters expressed in seconds: (1) the time between the application of the KCl stimulus and the beginning of the light production (Latency time, Lt); (2) the time between the beginning of the light production and the maximum of light production ( $TL_{max}$ ) and (3) the time to reach half extinction ( $T_{1/2ext}$ ). Data were considered as coordinates using the 4 luminous parameters. Euclidian distances were computed and observations were hierarchically cluster using Ward's maximum-variance method (WARD, 1963). Analysis of variance (ANOVA) and *t*-test were used to determine the significance of the observed difference between the groups. Analysis were performed using Statistical Analysis System (SAS institute).

Investigations on the genetic structure of *A. squamata* were performed by Random amplified polymorphic DNAs (RAPD). Total genomic DNA extractions for adults were done with the CTAB protocol (WINNEPEN-NINCKX *et al.*, 1993). Only the arms were used for the DNA extraction to avoid contamination by the DNA of the brooded juveniles (POULIN *et al.*, 1999). RAPD technique provides a rapid detection of genomic polymorphism (WILLIAMS *et al.*, 1990). A decamer oligonucleotide primer of arbitrary sequence (5'-d[GGTGCGGGAA]-3') was used in a polymerase chain reaction (PCR). The PCR reaction was carried out under low stringency conditions to generate a reproducible array of strain-specific products that were subsequently analysed by agarose gel electrophoresis. The gels were stained with ethidium bromide and then photographed under UV illumination. Images of each gel were scanned and the DNA profiles analyzed using SCION IMAGE software v.beta3b (1998). The RAPDistance programs (ARMSTRONG *et al.*, 1994) were used to record and analyse the DNA fragment data, which were encoded as presence/absence of bands. The primary data were then used to calculate pairwise distances between the samples using Pearson's Phi coefficient (SOKAL & SNEATH, 1963). Phenetic trees were build using the neighbor-joining agglomeration procedure (NJTREE programme of the RAPDistance package).

## RESULTS

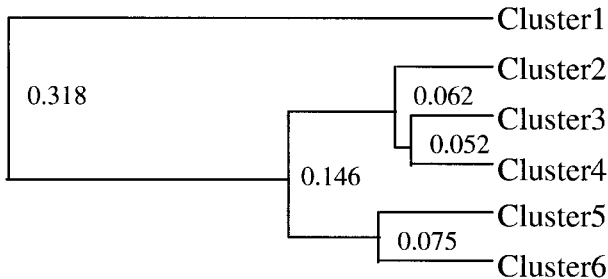
### *Morphology and physiology*

*Amphipholis squamata* is polychromatic. For practical reasons (abundance and ease of recognition), only four varieties have been chosen

from the six described by DEHEYN *et al.* (1997). The spotted variety occurs in all three local populations while the black, grey and orange varieties are only present in the population of Luc-sur-mer.

*A. squamata* from the three localities are bioluminescent. A tree was built from an euclidian distance matrix calculated using the four parameters describing bioluminescence (fig. 1A). Ward clustering method revealed six clusters separated by a minimal distance of 0.01. Cluster 1 corresponds to the orange and to the grey ophiuroids from Luc-sur-mer. Cluster 2 represents the black *Amphipholis* from Luc-sur-mer. Clusters 3 to 6 are the spotted individuals from the three local populations. The composition of clusters 3 to 6 is given in figure 1B, which shows that each of these clusters contain spotted individuals from the three distant

1A



1B

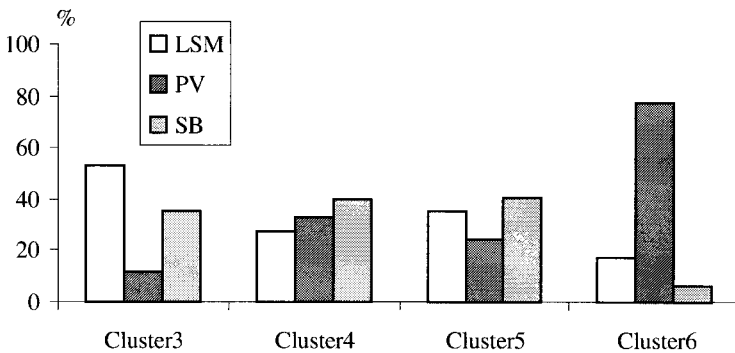


Fig. 1. Tree inferred from euclidian distances between bioluminescence parameters (1A) and percentage of the spotted variety at each site present in cluster 3 to 6 (1B). Distances within a cluster are less than 0.01. LSM: Luc-sur-mer, PV: Porto Vecchio, SB: Santa Barbara.

TABLE 1

Luminous parameters for the 4 varieties from the three studied populations (LSM, Luc-sur-mer; SB, Santa Barbara and PV, Porto Vecchio). Mean value  $\pm$  standard error of mean; n = number of ophiuroids.

Population	Variety	n	Lmax	TL	TLmax	T1/2ext
LSM	Orange	7	1.80 $\pm$ 0.56	1.57 $\pm$ 0.16	6.78 $\pm$ 0.94	9.08 $\pm$ 0.97
LSM	Grey	30	21.51 $\pm$ 5.06	1.69 $\pm$ 0.15	5.48 $\pm$ 0.69	6.76 $\pm$ 0.61
LSM	Black	11	545.27 $\pm$ 82.03	1.37 $\pm$ 0.17	3.49 $\pm$ 0.91	4.12 $\pm$ 0.77
LSM	Spotted	30	898.16 $\pm$ 164.29	1.44 $\pm$ 0.16	11.46 $\pm$ 1.06	3.31 $\pm$ 0.44
SB	Spotted	30	1094.57 $\pm$ 106.06	1.32 $\pm$ 0.13	11.38 $\pm$ 0.76	3.15 $\pm$ 0.38
PV	Spotted	30	2211.99 $\pm$ 187.16	1.01 $\pm$ 0.01	7.98 $\pm$ 0.75	1.76 $\pm$ 0.19

populations. This analysis based on luminous capabilities clearly separate the colour varieties (excepted for orange and grey which are clustering) whereas it does not show differences between populations. Variance analysis of the four parameters (Table 1) reveals that each variety exhibits its own maximal light intensity ( $p < 0.01$ ); a difference of two orders of magnitude being observed between the orange and the spotted variety; the grey and black varieties emit intermediate maximal light intensity. Although significant differences for the kinetic parameters were found, it must be pointed out that no clear distinction can be made between the colour varieties.

#### *Genetic differentiation*

The analysis of the DNA profiles of individuals from Normandy, where all the colour varieties observed in our study co-exist, shows three groups (fig. 2): Group I: spotted (LT), Group II: black (LN) and orange (LO) and Group III: gray (LG). Despite the presence of a similar colour variety at all the considered sites, there is an important genetic distance between the spotted individuals of the 3 local populations (fig. 3). The population of Luc-sur-mer (Group A) is the most separated, while the populations of Porto Vecchio (Group C) and of Santa Barbara (Group B) appear to be closer to each other. The genetic variability appears to be different in the lagoon of Tindari (C), where the individuals are quite homogenous, and at Santa Barbara (B) and Luc-sur-mer (A) where the genetic diversity is much higher.

## DISCUSSION

The cosmopolitan ophiuroid species *Amphipholis squamata* presents a great morphological homogeneity throughout the world (HYMAN, 1955;

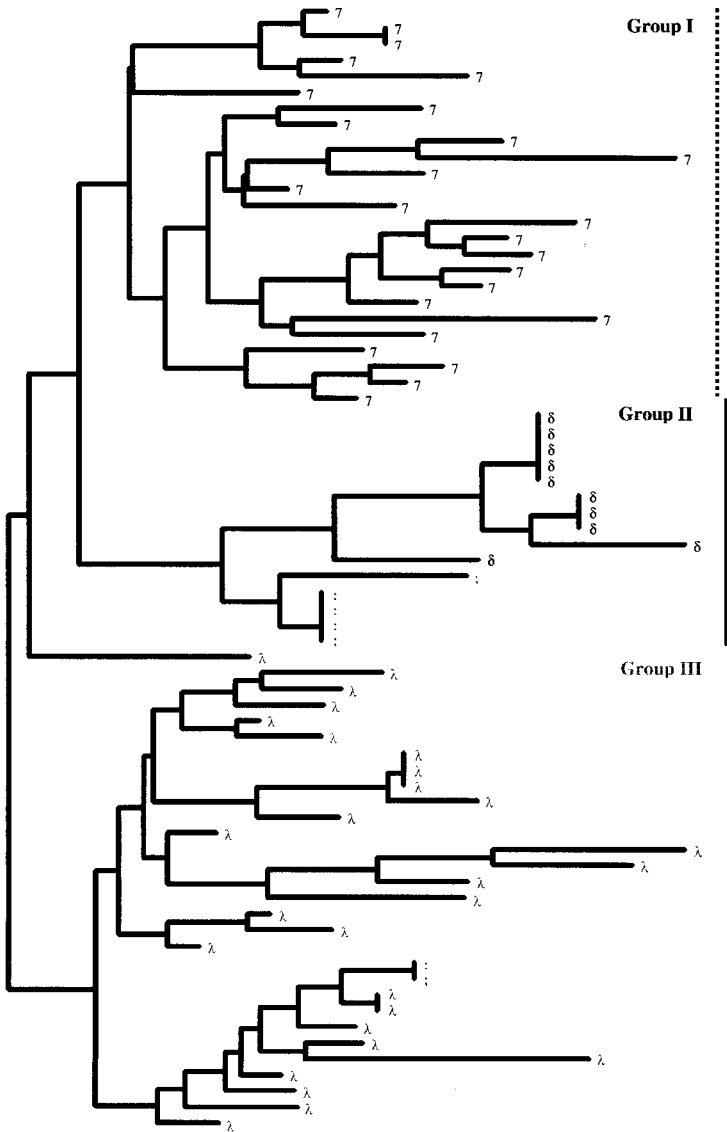


Fig. 2. Inferred tree from a distance (Pearson's coefficient) matrix showing the genetic diversity and structure of the local population from Luc-sur-mer. Group I represents the spotted (7) *Amphipholis*; black ( $\delta$ ) and orange ( $\lambda$ ) ones are clustered in group II, group III consists of the grey ( $\lambda$ ) *Amphipholis*.

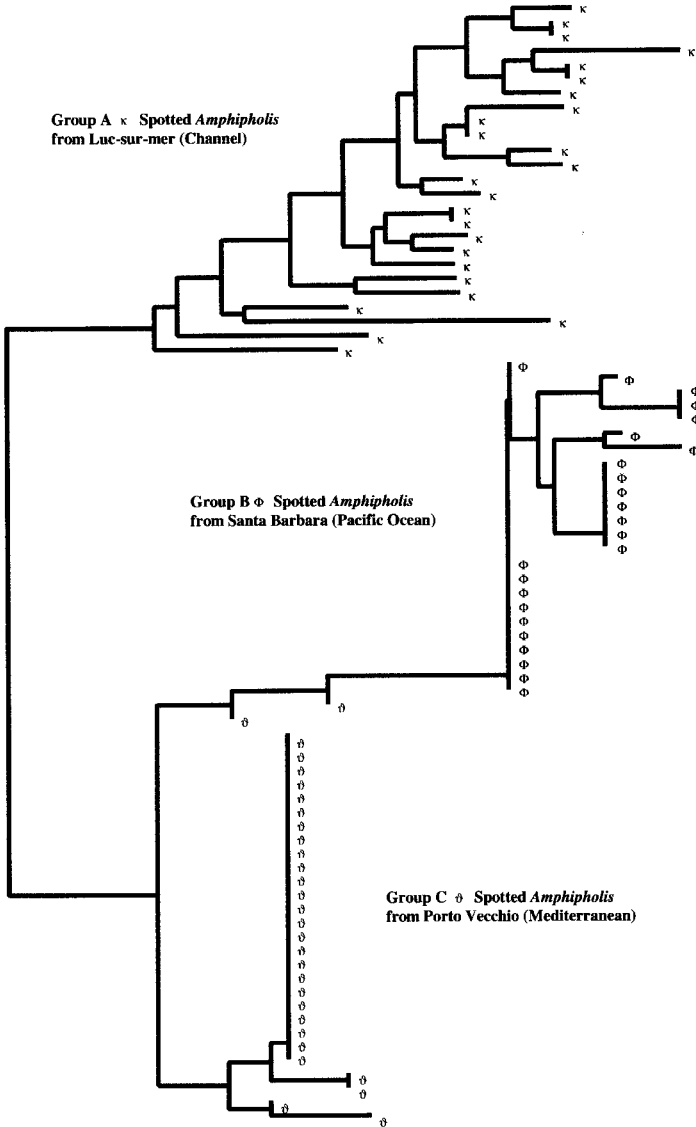


Fig. 3. Inferred tree from a distance (Pearson's coefficient) matrix showing the genetic diversity and structure of the spotted variety of *Amphipholis* in the 3 studied sites: Luc-sur-mer ( $\kappa$ ), Santa Barbara ( $\Phi$ ) and Porto Vecchio ( $\vartheta$ ).

SPONER *et al.*, 1999). Nevertheless, it is well documented that numerous features exhibit high variability: colour patterns, bioluminescent capabilities and control mechanisms, life-history traits and genetics (DEBREMAEKER *et al.*, 1994; MALLEFET *et al.*, 1994; DEHEYN *et al.*, 1997; DUPONT & MALLEFET, 1999; POULIN *et al.*, 1999; SPONER *et al.*, 1999). Our results (1) confirm and generalise the existence of a link between polychromatism and bioluminescence, already observed for the local population of Luc-sur-mer by DEHEYN *et al.* (1997) and for the local population of Tindari by DUPONT (1998) and (2) demonstrate the existence of a link between polychromatism, bioluminescence and genetics within a local population. However, distant local populations of the same colour variety are genetically strongly differentiated. Polychromatism (and/or bioluminescence) are parameters that one has to take into account to study the spatial and genetic structure of the species and are good indicators of genetic variability only within a population. Our results, and those of DEHEYN & JANGOUX (1999) discussing about the inheritance of body colour and bioluminescence characters, question the taxonomic statute of *Amphipholis squamata*, a problem which is under study in our laboratories.

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