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

by

SAMUEL DUPONT\textsuperscript{1}, STEPHEN CHAUFER\textsuperscript{2}, ÉLIE POULIN\textsuperscript{2,3}, JEAN-PIERRE FÉRAL\textsuperscript{2} and JÉRÔME MALLEFET\textsuperscript{1}

(\textsuperscript{1}Laboratoire de Physiologie Animale, Université catholique de Louvain, Louvain-la-Neuve, Belgium; \textsuperscript{2}Observatoire Océanologique, UMR CNRS 7628, BP 44, Banyuls-sur-mer, France; \textsuperscript{3}Departamento de Ecologia, P. Universidad Católica de Chile, Alameda 340, Santiago, Chile. E-mail: dupont@bani.ucl.ac.be)

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
polar areas, in varied habitats, from the intertidal zone down to 1330 m depth (GAGE et al., 1983; HENDLER, 1995; ALVÁ, 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 $-80^\circ$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