

# Evidence from polychromatism and bioluminescence that the cosmopolitan ophiuroid *Amphipholis squamata* might not represent a unique taxon

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**Abstract** – Individuals of the cosmopolitan ophiuroid *Amphipholis squamata* were collected from eight stations. Eleven colour varieties were described and their distribution was non-random among stations. This suggests that the varieties differ in ecophysiological tolerance and that their geographical distribution is modulated by environmental conditions. Varieties also differed in bioluminescence. Contrary to kinetics, intensity of light production varied among co-occurring varieties, meaning that they have similar bioluminescent reactions but a different amount of bioluminescent reagent. Light intensity differed in absolute value among stations but the rank position of each variety relative to others remained constant from one station to another. The 'colour–bioluminescence' link appeared clearly fixed (the same level of bioluminescence for the same variety) and is suggested to be of genetic origin. The species '*A. squamata*' may then be a mosaic of genetically different entities (the varieties) rather than a unique cosmopolitan taxonomic entity. © 2000 Académie des sciences/Éditions scientifiques et médicales Elsevier SAS

***Amphipholis squamata* / Echinodermata / bioluminescence / polychromatism / colour varieties / cosmopolitism / sibling species**

**Résumé** – Évidences basées sur le polychromatisme et la bioluminescence que l'ophiure cosmopolite *Amphipholis squamata* pourrait ne pas représenter un taxon unique. L'ophiure cosmopolite *Amphipholis squamata* collectée dans huit stations présente des individus de onze variétés de couleur. Leur distribution entre stations n'est pas fortuite, suggérant des différences de tolérance écophysiological entre variétés, et donc une influence des conditions environnementales sur leur distribution. Les variétés coexistantes sont différentes s'agissant de la bioluminescence. Les différences s'observent pour l'intensité et non la cinétique, indiquant que la réaction lumineuse est similaire entre les variétés alors que la quantité de réactif lumineux est différente. L'intensité lumineuse varie en valeur absolue entre stations mais l'ordre relatif des variétés classées entre elles en fonction de l'intensité reste constant d'une station à

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l'autre. Le lien « couleur–bioluminescence » apparaît clairement établi (toujours le même potentiel lumineux pour la même variété) et serait d'origine génétique. L'espèce « *A. squamata* » pourrait être constituée d'une mosaïque d'entités génétiquement distinctes (les variétés) plutôt que d'être une entité taxinomique unique et cosmopolite.  
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***Amphipholis squamata* / Echinodermata / bioluminescence / polychromatisme / variétés de couleur / cosmopolitisme / espèces sœurs**

## Version abrégée

*Amphipholis squamata* est une ophiure répandue dans toutes les mers et océans, à toutes les profondeurs. Actuellement, sur base de la géométrie des caractères morphologiques, rien ne permet de différencier les individus qui sont originaires de stations éloignées ou différentes par l'habitat, et l'espèce est donc considérée comme représentant un seul et unique taxon. Ce résultat va toutefois à l'encontre d'analyses moléculaires effectuées à partir d'individus de populations distantes montrant que des populations de l'ophiure peuvent être génétiquement distinctes. Dans ce cas cependant, de manière caractéristique et inexplicable, une forte variabilité génétique est exprimée au sein des populations étudiées. Toute analyse moléculaire supplémentaire apparaît donc prématurée à ce stade-ci de la recherche étant donné que la variabilité génétique pourrait être due soit à un polyallélisme prononcé de chaque individu de l'espèce, soit, au contraire, à une variabilité génétique entre les individus mêmes. Dans ce cas, chaque population pourrait être constituée de divers groupes génétiquement distincts (e.g. des espèces sœurs) qu'il faudrait avant tout pouvoir identifier de visu. Le présent travail s'est appliqué à déterminer au sein de populations de l'ophiure si des groupes d'individus pouvaient être identifiés sur base d'un caractère somatique (la couleur des individus) et d'un caractère neurophysiologique (leur capacité à produire de la lumière visible ; i.e. leur capacité de bioluminescence). Il s'agissait de comparer des populations proches ou distantes, présentant un habitat identique ou différent, et de déterminer si les groupes identifiés pour une population se retrouvent dans d'autres. Si les groupes se retrouvent uniformément dans les populations, alors il est peu probable qu'ils constituent des entités génétiquement distinctes et soient responsables de la variabilité génétique intra- et/ou inter-populations mentionnées par d'autres travaux. Si par contre les groupes peuvent être différents entre stations, alors il est possible qu'ils participent à la variabilité génétique intrapopulation qui caractérise la plupart des populations de l'ophiure, tout en participant aux différences génétiques qui peuvent s'observer entre certaines populations.

*A. squamata* est une espèce polychromatique, c'est-à-dire que les individus appartiennent à différentes

variétés de couleur. Onze variétés ont été reconnues et décrites dans huit stations choisies en Europe et dans la région Indo-Pacifique (Nouvelle-Zélande et Papouasie-Nouvelle-Guinée). Il apparaît que les onze variétés ne sont jamais trouvées coexistantes dans une même station et que l'espèce consiste en fait en une mosaïque de différents groupes de variétés, chacun de ces groupes ayant une distribution géographique distincte qui résulte vraisemblablement d'exigences environnementales différentes pour chaque variété. C'est ainsi que des stations proches mais différentes par l'habitat présentent différents groupes de variétés, et inversement des stations éloignées mais ayant un habitat semblable ont en commun la plupart des variétés. Il semble que des propriétés écophysiologiques spécifiques à chacune des variétés soient à l'origine de différences dans leur distribution géographique. *A. squamata* est une espèce bioluminescente, c'est-à-dire que les individus sont capables de produire de la lumière visible. Ce caractère a été décrit pour les différentes variétés de chacune des stations et les résultats obtenus renforcent l'idée selon laquelle la différenciation écophysiologique des variétés est d'origine génétique. Au sein de chaque station, chaque variété présente sa propre intensité de lumière produite. Ce caractère variété-spécifique de la bioluminescence devient évident quand les stations qui ont le même groupe de variétés sont comparées en classant les variétés entre elles par ordre croissant d'intensité de lumière. Les variétés se succèdent alors selon un ordre qui est maintenu d'une station à l'autre, indiquant que ce sont toujours les mêmes variétés qui ont la bioluminescence la moins intense et les mêmes la bioluminescence la plus intense. Il faut noter toutefois qu'en valeur absolue, l'intensité de bioluminescence de chaque variété peut changer d'une station à l'autre, ce qui probablement exprime une influence des conditions physicochimiques de l'environnement sur la chimie de la bioluminescence. Cette influence ne participe que dans une faible proportion (10,1 %) dans la variabilité d'intensité lumineuse observée entre stations, et c'est en fait la composante « variété » qui est responsable, dans une majeure proportion (80,1 %), de cette variabilité en production de lumière. Il ressort du présent travail que les caractères « bioluminescence » et « variétés de couleur » ont leur expression totalement corrélée et dépendante, ce qui leur attribue une base génétique. La variabilité

génétique décrite au sein de populations de l'ophiure pourrait être liée au fait qu'elles regroupent différentes variétés de couleur, et celle décrite entre populations liée au fait que les groupes de variétés peuvent être différents d'une population à l'autre. L'espèce « *A.*

*squamata* » apparaîtrait ainsi comme une mosaïque géographique d'entités génétiquement distinctes (les variétés de couleur) qui pourraient être des espèces sœurs dont la distribution serait notamment modulée par les conditions environnementales.

## 1. Introduction

The ophiuroid species *Amphipholis squamata* is distributed worldwide with collections of individuals reported from diverse habitats in intertidal and subtidal zones (down to 1 400 m in depth [1]), and in temperate and tropical waters (e.g. [2, 3]). The species is small in size; the disc of adult specimens measures ca 3 mm in diameter and the arms measure ca 15 mm in length. Overall morphology of individuals (geometry of their morphological characters) is so consistent that taxonomists have considered the species a worldwide taxon [4]. It has been shown, however, that specimens from different areas may be genetically different, and that individual genotype may be particularly variable within a single area [5, 6] with up to 14 different alleles for some loci [7]. The variability is substantial enough that multiple primers have been used for single loci to process genetic analysis from distinct populations [6], which thus makes the molecular genetic analysis problematic. The genetic variability in a discrete area could be explained by high individual genomic variability (genes having two or more alleles) or, alternatively, by high pairwise genetic difference between individuals. In other words, different groups of individuals with distinct genetic identity might be found in one area and be responsible for the genetic variability. The goal then is to determine whether such groups can be identified by analysing phenotypic or somatic characters of the ophiuroid.

The second hypothesis (*A. squamata* as a group of distinct genetic entities) is supported by the fact that the species is polychromatic. Indeed, individuals in a defined area belong to different varieties according to the colour patterns of their disc and arms [8], which might be the expression of a genetic variability among individuals [9, 10]. It has long been known that *A. squamata* is bioluminescent [11] and the intensity of light produced was recently shown to be significantly different among six colour varieties co-occurring in the same area, with light intensity of the more luminous variety 500 times higher than that of the least luminous one [12]. Thus, in the aforementioned area, two characters (body colour and intensity of produced light) can be used to define each variety. This combination could, however, be a local coincidence and the present study addresses whether varieties are recognizable by body colour alone, with the level of bioluminescence being determined by exogenous factors, or by both body colour and the intensity of produced light with which it would be specifically associated. In the second scenario varieties could be considered as distinct genetic groups.

The present study investigates these characteristics in populations of *A. squamata* from different geographical areas and habitats. The body colour pattern and the bioluminescence of individuals of each population were characterized and the degree to which bioluminescence changed according to the geographical locality of the given variety was determined.

## 2. Materials and methods

Individuals of *Amphipholis squamata* (Delle Chiaje, 1828) were collected from eight different locations in France (two stations), United Kingdom (four stations), New Zealand (one station), and Papua New Guinea (one station). All sampling took place between April 1994 and July 1995, in the intertidal zone of each station. The habitats were described and water temperature and salinity noted for each site (table 1). Individuals were collected randomly, regardless of their colour, and collecting was always carried out by hand and using fine forceps by a single collector. Individuals were transferred to the laboratory in small containers filled with sea water and algae; the transport under such conditions has been shown to induce no change in individuals' bioluminescence [13].

Colour patterns of disc and arms of specimens from various stations were determined under a compound microscope by the collector. Only adult specimens were considered (disc diameter  $\geq 1.7$  mm [14]). They were sorted according to colour variety and the proportion of each colour variety was calculated relative to the total number of individuals collected at each station [from 273 individuals at Portobello (NZ) to 1 986 at Langrune-sur-Mer (Fr)]. Individuals were kept in an open circuit aquaria for no longer than 3 days before their bioluminescence was measured. The light measurement procedure was identical for all individuals whatever the variety and the geographic origin. First they were anaesthetized by immersion (3 min) in a 3.5 % w/w  $MgCl_2$  sea-water solution. Then, after their length was measured (in mm), arms were isolated from the disc using a fine scalpel. Arms are the only bioluminescent body parts in *A. squamata* and their light production was recorded following stimulation by injection into the chamber of 200 mM KCl [12]. Once in KCl, arm tissues are instantly and maximally depolarized (such stimulation avoids individual differences in transduction-diffusion of neurophysiological stimulus through tissues). The bioluminescence reaction involves the whole luminous system, the measured light production corresponds to the maximal bioluminescence ability of individuals [12, 15]. Light measurement was carried out

**Table 1.** *Amphipholis squamata*: geographical localization, period of collection, water temperature, salinity, and type of habitat for each station (PSU: Practical Salinity Units).

	Cold-temperate North Atlantic*						Cold-temperate sub-Antarctic*	Tropical Indo-West-Pacific*
	France		UK				New Zealand	Papua-New-Guinea
	Langrune-sur-Mer	Roscoff	Wembury	Prawle Point	Newhaven	Millport	Portobello	Laing Island
Coordinates	49°19' N 0°22' W	48°43' N 3°59' W	50°19' N 4°05' W	50°12' N 3°44' W	50°47' N 0°03' E	55°46' N 4°55' W	45°51' S 170°39' E	4°10' S 144°52' E
Marine area	North Sea (English Channel)	North Sea (English Channel)	North Sea (English Channel)	North Sea (English Channel)	North Sea (English Channel)	Irish Sea (North Channel)	South Pacific Ocean	Bismarck Sea
Period of collection	April 1994	April 1994	April 1995	April 1995	May 1995	May 1995	June 1995	July 1995
Water temperature (°C)	10	10	10	10	11	7	7	29
Salinity (PSU)	32	33	32	32	35	33	33	29
Habitat	Mid-intertidal; under stones in muddy areas	Mid-intertidal; under pebbles in <i>Zostera marina</i> beds and sandy detritic areas	Upper-intertidal; under stones in sandy detritic areas	Upper-intertidal; in tide pools, on <i>Corallina</i> <i>officinalis</i>	Upper-intertidal; on byssal nets of mussels	Lower-intertidal; on byssal nets of mussels or under stones in muddy areas	Upper to lower- intertidal; under stones in sandy detritic areas	Upper-intertidal; under blocks of death madreporas on flat reef

\* According to [48].

in a dark room at constant temperature (from 18 to 25 °C, depending on the station) and light production was measured for up to 12 individuals (i.e. 60 arms) representing each of the coexisting varieties at each station, depending on abundance of the variety. The light measurement apparatus was calibrated at each temperature and every week using a tritium-phosphor source, which produces a known value of light quanta per second representing the light intensity of reference [12].

Two characteristics, the maximum light intensity (LMax) and the time to reach the maximum light intensity (TLMax), were used to describe light production in the ophiuroid [12, 15]. LMax represents the maximal flux of quanta energy associated with the emitted photons, and it is expressed in Megaquanta per second normalized per millimeter of arm ( $\text{Mq}\cdot\text{s}^{-1}\cdot\text{mm}^{-1}$ ); when arms are KCl stimulated, LMax gives information about total amount of the luminous system in the arms, and thus in individuals. TLMax is expressed in seconds (s); it is a kinetic value that indicates the time for the reagents of the luminous system to maximally react, which depends on both the nature of the reagents and the pathway of the chemical reaction.

The statistical significance of the differences in bioluminescence between varieties and stations was tested using one- and two-factor nested analyses of variance (arms being nested within individual) and multiple mean comparison (Tukey test). When individuals in a single station were of several varieties, they were ranked according to the values of their intensity character (LMax values). For each station, changes in bioluminescence when going from one variety to another were represented by a linear slope calculated from all light measurements for each variety. Stations with at least two varieties in common were then compared for variation of bioluminescence when going from one variety to another. Change in the bioluminescence was tested for concordance between the different stations using the Spearman rank correlation method [16, 17]. All data were  $\log(X + 1)$  transformed, as indicated when heterocedasticity (non-homogeneity of variance range) occurs among values [17]. The significance level was limited to 5 % of the probability ( $P < 0.05$ ) and all statistical analyses were performed using SuperAnova 1.11 and Statistica software.

### 3. Results

#### 3.1. Colour patterns and geographical distribution of varieties

Eleven body colour patterns were distinguished from the eight stations investigated, each defining a particular variety of *A. squamata* (figure 1). Of the eleven varieties described, six (orange, beige, dark brown, grey, black and spotted varieties) were previously identified [8, 12] while the five others (the black–brown, ochre, speckled, brown–grey and pale beige varieties) are reported for the first time.

Individuals of the orange variety are pale orange with the disc having a brown–red tint (figure 1.A). Individuals of

the beige variety have beige disc and arms with the disc radial shields possibly being slightly darker (figure 1.B). Individuals of the dark brown variety have a dark brown disc and arms which vary from beige to brown with a few segments that are not or only slightly coloured (figure 1.C). Individuals of the grey variety have a dark to pale grey disc and pale grey arms and often have a greenish tint except for a few segments which are almost colourless (figure 1.D). Individuals of the black variety have a deep black–coloured disc and arms with the aboral surface black and the oral and lateral surfaces yellowish, only a few segments being pale and almost colourless (figure 1.E). Individuals of the spotted variety have a beige or grey disc and arms (the disc being darker than arms), frequently have a greenish tint and always have black and orange spots (figure 1.F). Individuals of the black–brown variety have a dark disc (black or grey) with pale radial shields, and pale brown arms with a yellowish tint, except for a few pale segments (figure 1.G). Individuals of the ochre variety have a brown disc tinted with orange, and rusty-orange arms (figure 1.H). Individuals of the speckled variety have a disc and arms pale brown to black depending on the number and expanse of black spots spread over the epiderm. The disc of this variety appears darker than the arms as the latter have numerous pale segments (figure 1.I). Individuals of the brown–grey variety have a dark brown disc and pale grey arms with a greenish tint but for a few pale segments (figure 1.J). Individuals of the pale beige variety have a whitish to pale beige disc and arms with the arms displaying fine and light pigmentation (small black spots), especially in the aboral and proximal areas of each segment (figure 1.K).

A complete representation of all eleven varieties together was never observed at a single station, the maximum being six varieties found simultaneously. Each station had multiple varieties with the exception of Laing Island. Some varieties were common to several stations though their abundance varied from one station to the next. (Table II) summarizes the respective abundance for each of the eight investigated stations. Six varieties (orange, beige, black–brown, grey, black and spotted) were found in Langrune-sur-Mer, Roscoff and Wembury while only four (beige, black–brown, ochre and speckled) were found at Prawle Point and Millport. Out of these latter four varieties, two (the beige and black–brown) were identified at Newhaven. These two varieties and a third one (brown–grey) were collected at Portobello. Finally, a unique and exclusive variety (pale beige) was described at Laing Island table II.

#### 3.2. Bioluminescence character

Every investigated individual produced light in response to KCl stimulation and the light production under such conditions was monophasic lasting for around 180 s after KCl addition. Figure 2 gathers the values of bioluminescence characteristics for the eleven varieties collected from the eight stations investigated. The bioluminescence varied significantly according to both varieties and stations ( $P < 0.0001$ ). It appeared from the two-factor

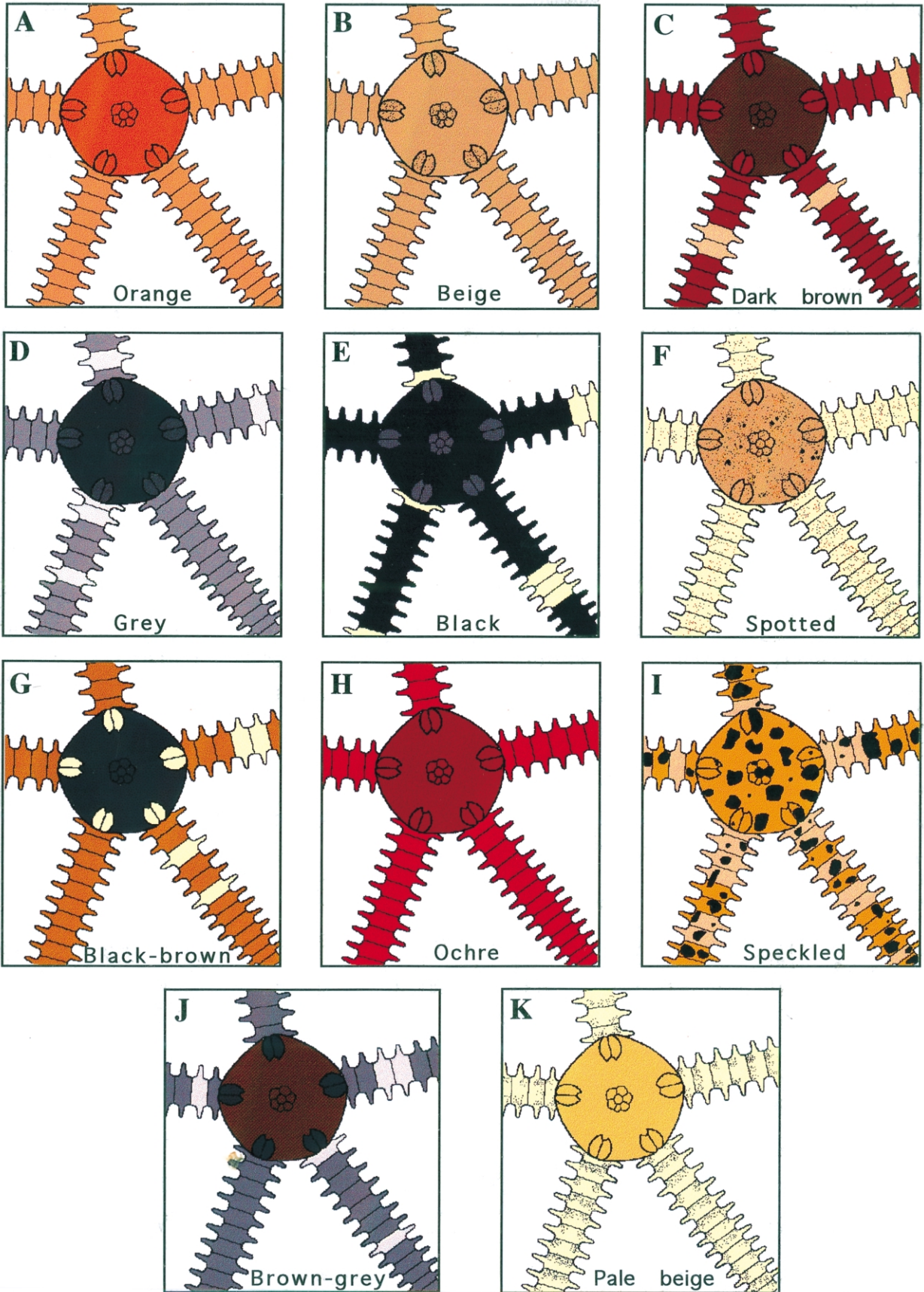


Figure 1. *Amphipholis squamata*: diagrammatic representation of the eleven observed body colour patterns.

**Table II.** *Amphipholis squamata*: occurrence (number of individuals and the corresponding frequency in %) of the different varieties in the eight investigated stations.

Stations of collection	Varieties*											Total
	Orange	Beige	Dark Brown	Grey	Black	Spotted	Black–brown	Ochre	Speckled	Brown–grey	Pale Beige	
Langrune/Mer (FR)	603	675	278	655	219	99	–	–	–	–	–	1986
	34 %	14 %	33 %	11 %	5 %	–	–	–	–	–	–	100 %
Roscoff (FR)	181	465	521	614	56	186	–	–	–	–	–	1860
	18.1 %	46.5 %	28 %	33 %	3 %	10 %	–	–	–	–	–	100 %
Wembury (UK)	8	64	85	101	97	48	–	–	–	–	–	403
	2 %	16 %	21 %	25 %	24 %	12 %	–	–	–	–	–	100 %
Prawle Point (UK)	–	583	–	–	–	–	249	38	87	–	–	957
	–	61 %	–	–	–	–	26 %	4 %	9 %	–	–	100 %
Newhaven (UK)	–	620	–	–	–	–	431	–	–	–	–	1051
	–	59 %	–	–	–	–	41 %	–	–	–	–	100 %
Millport (UK)	–	627	–	–	–	–	435	192	26	–	–	1280
	–	49 %	–	–	–	–	34 %	15 %	2 %	–	–	100 %
Portobello (NZ)	–	142	–	–	–	–	112	–	–	19	–	273
	–	52 %	–	–	–	–	41 %	–	–	7 %	–	100 %
Laing Island (PNG)	–	–	–	–	–	–	–	–	–	–	387	387
	–	–	–	–	–	–	–	–	–	–	100 %	100 %

\* For the definition of varieties, see text and figure 1.

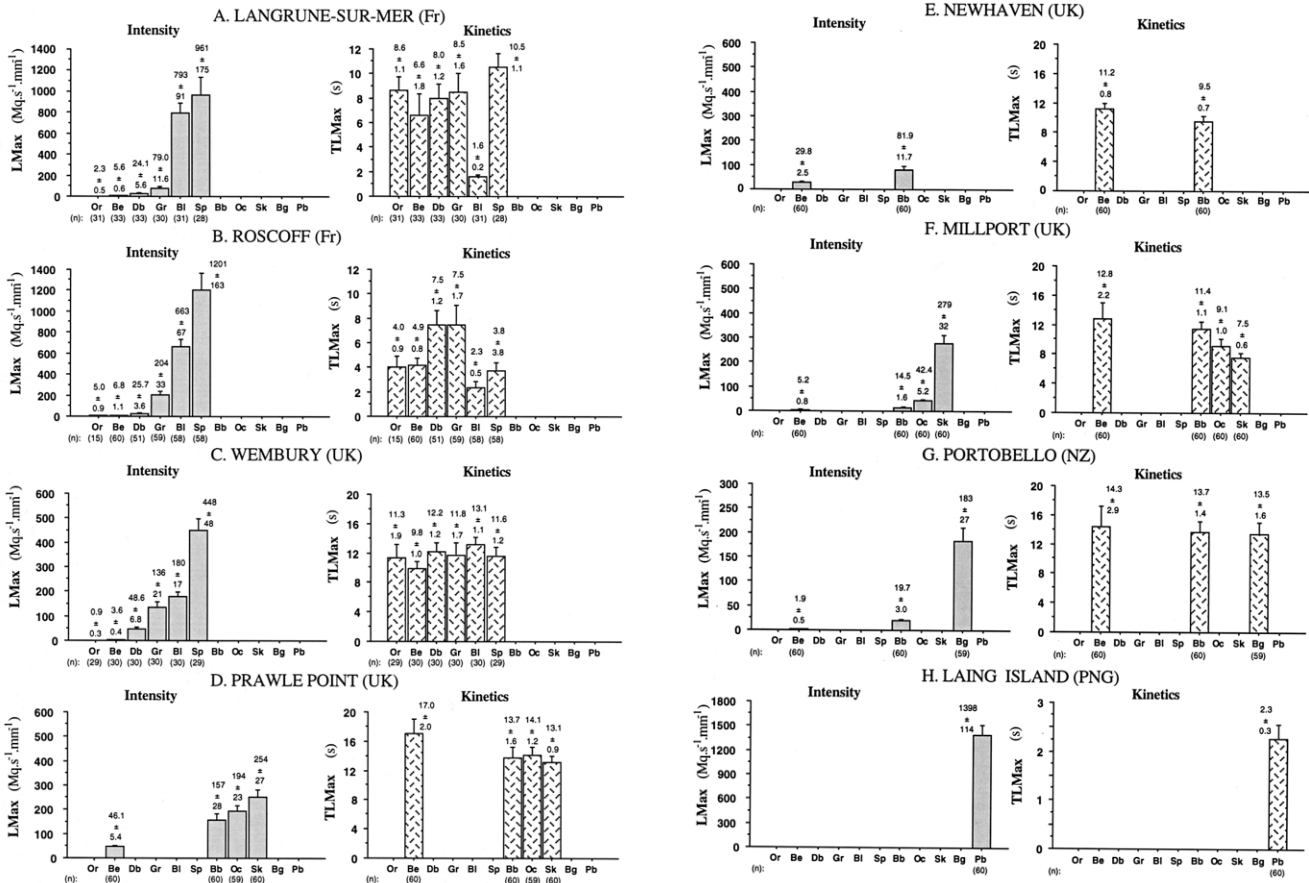
ANOVA that both variety and station affect light intensity (LMax) and kinetics (TLMax), but to different extents. The factor variety explained 80.1 % of the variation in light intensity and 29.2 % of the variation in light kinetics, whereas only 10.1 % of the variation in light intensity and 22.8 % of the variation in kinetics were explained by the factor station. The interaction factor variety–station contributed to 3.4 and 5.8 % of the variations in light intensity and kinetics, respectively. Thus, the two investigated factors together, viz. colour variety and geographical location, could explain 93.6 % of the individuals' variation in light intensity, and 57.8 % of that in kinetics.

The grouping of individuals according to their variety and independent of the station showed that light intensity varied significantly ( $P < 0.0001$ ) among varieties (figure 3.A). This means that the bioluminescence depends on the variety. Conversely, the kinetic characteristic appeared much more uniform and its values were only significantly different for the black and the pale beige varieties (figure 3.B).

As shown in table II, some varieties were found in two or more sampling stations and the value of LMax consistently differed from one variety to the next ( $P < 0.0001$ ). Moreover, when two or more varieties co-occurred in a station, their relative level of LMax remained similar compared to other co-occurring varieties ( $P < 0.0001$ ) although the raw value of LMax differed from station to station [Spearman coefficient ranged from  $r = 0.98$  (98 % of concordance between Roscoff and Wembury; figure 4.A) and  $r = 0.88$  (88 % of concordance between Prawle Point and Millport; figure 4.C)]. Conversely, changes in TLMax values were not always concordant for all stations [(Spearman coefficient ranged from  $\rho = 0.84$  (84 % of concordance between Prawle Point and Millport; figure 4.D) and  $\rho = 0.22$  (22 % of concordance between Roscoff and Wembury; figure 4.B)].

## 4. Discussion

Individuals of *Amphipholis squamata* from eight different stations from temperate and tropical regions were investigated for colour pattern and bioluminescence ability. In every station but one (Laing Island, PNG), polychromatism was the rule with up to six colour varieties co-occurring per site. The intensity of light production (LMax) was significantly different between colour varieties independently of the stations (figure 3.A), meaning that at stations sharing varieties the same varieties produced either a weak bioluminescence or an intense bioluminescence. This suggests that the amount of luminous reagent is directly related to the individual variety rather than being modulated by environmental conditions. Conversely, the kinetics of light production (TLMax) was not always significantly different between varieties and does not appear variety-dependent (figure 3.B). This supports the hypothesis that the chemical pathway of the bioluminescence reaction is similar across varieties, yet intensity of the light produced appears variety-dependent. The link between body colour and bioluminescence does not appear to be due to exogenous factors since LMax showed the same link with varieties independently of environmental conditions (figure 4). This suggests that body colour and bioluminescence ability are biological characters absolutely correlated (dependence of genetic expression between the colour and the bioluminescence character) [18–20]. The fact that LMax is variety-specific does not imply, however, that the bioluminescence for a given variety is fixed or constant. Indeed, for a given variety LMax values differed according to station and clearly disparities in the environment and/or the geographical location could lead to variation in light production (figure 4.A, B) [21]. The environmental determinism of bioluminescence seems to be the rule in luminous organisms [22, 23], and the present study



**Figure 2.** *Amphipolis squamata*: values of light intensity (LMax) and light kinetics (TLMax) for each variety (mean values  $\pm$  95 % confidence limit). (n) = number of stimulated arms. Abbreviations: Or = orange; Be = beige; Db = dark brown; Gr = grey; Bl = black; SP = spotted; Bb = black–brown; Oc = ochre; Sk = speckled; Bg = brown–grey; Pb = pale beige. Note that the absolute scale for intensity and kinetic values differ according to the station.

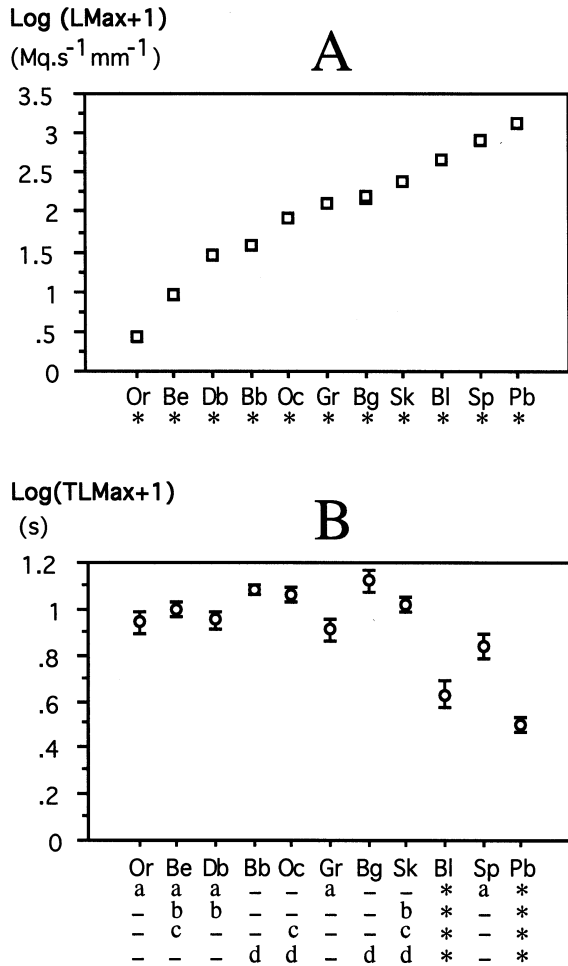
is no exception. Yet, when varieties are graded by LMax values, the rank each displays in one station is identical to the rank they hold across all stations, whatever their absolute LMax value (figure 4.A, B).

Thus, what are referred to as varieties in *A. squamata* can be systematically differentiated by at least two distinct biological characters (body colour and bioluminescence) which supports the contention that the varieties are genetically distinct [19, 20, 24]. This agrees with the findings of Deheyn and Jangoux [25] showing that body colour and bioluminescence characters are identical in a parent individual and its offspring when the parental individual co-occurs with individuals of both the same or different varieties. The absence of other colour varieties in the offspring suggests that each colour variety is genetically distinct. In the Deheyn and Jangoux's study, there was seemingly no successful cross-fertilization between individuals of different varieties [25].

The geographic distribution of varieties is not random. Some varieties were never found together, whereas some others co-occurred often. They can be grouped into three subsets of six varieties (the orange, beige, dark brown, grey, black and spotted), four varieties (the beige, black–brown, ochre and speckled), and three varieties (the beige,

black–brown and brown–grey). Only two varieties (the beige and the brown–grey) were found in more than one subset. In addition, when different subsets of varieties occurred in the same area they were in different habitats. For example, Wembury and Prawle Point sites are in close proximity to each other, yet individuals live either under stones of the intertidal zone or in tide pools, respectively. These two stations support different variety subsets (six varieties for Wembury and four for Prawle Point). The Wembury varieties were also found along the coast of France in a similar habitat, while the Prawle Point varieties were seen only in the UK stations (tables I and II).

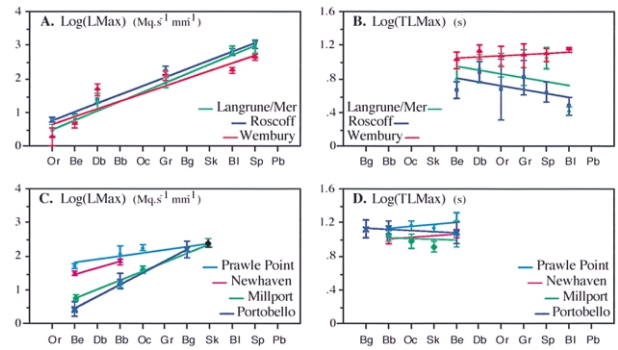
Occurrence of varieties therefore seems to be modulated by local environment. For instance, Newhaven individuals occur in the upper-intertidal zone and only two of the four varieties found elsewhere in UK stations exist here. Differences in the distributions of varieties within the tidal zone seems to be common. In the French population at Roscoff, only three of the six colour varieties, i.e. the beige, dark brown and grey varieties, are found in the supratidal [26]. Conversely, widely distant stations with similar habitat may show similar subsets of varieties, such as Newhaven (UK) and Portobello (NZ). We consequently suggest the species *A. squamata* consists of subsets of



**Figure 3.** *Amphipholis squamata*: mean values ( $\pm$  95 % confidence) of light intensity (LMax, A) and light kinetics (TLMax, B) for each variety ranked according to increasing light intensity. \* Significantly different from all others varieties ( $P < 0.0001$ ); varieties sharing the same letter (see light kinetics) are not significantly different. Values were expressed after log (X + 1) transformation to avoid heterocedasticity.

colour varieties with biogeographical distributions dependent on the environment. This suggests that the varieties could have different ecophysiological tolerances, a possibility supported by differences in genetic composition postulated as occurring between individuals from different varieties [8, 25].

*Amphipholis squamata* can therefore be considered as a geographic mosaic of so-called colour varieties that might actually be genetically distinct (even for those occurring sympatrically) and be either cryptic or sibling species [18, 24, 27, 28]. Thus, two components are proposed to influence ophiuroid speciation: 'geographic' and 'variety' components. The geographic component (the factor 'site' or 'station'; see [7]) depends upon physico-chemical conditions of the environment. However, since the ophiuroid broods juveniles to subadult stages [2, 29] and the dispersion of specimens is presumed to be limited only to floating and rafting [30, 31], the geographic component



**Figure 4.** Changes in light intensity (A, C) and light kinetics (B, D) values according to variety in selected stations of collect (i.e. in stations sharing at least two varieties). Slopes were calculated from all values. Mean values  $\pm$  95 % confidence limit were indicated for each variety at each station.

also depends upon distance occurring between stations. If these two elements (physico-chemical conditions and distance) approximate the main pressure of speciation, then the species *A. squamata* can be considered a philopatric species that forms 'sets of closed and relatively inbred local populations' [32]. This is also supported by the fact that the ophiuroid has a tendency to form aggregates [33]. If geographic location (i.e. distance) is the main component that approximates genetic variability, then polymorphism is expected to be weak among individuals of the same area [32]. This has not been observed for *A. squamata* [6, 7], which indicates that there are multiple components that promote genetic variability within each population. We suggest that the second component, variety, might be responsible for genetic variability between individuals of the same area. This component was never considered in genetic analyses [5–7], which may explain high genetic variability found for the *A. squamata* populations.

Polychromatism is common in many marine invertebrates, including echinoderms where it is well-documented for echinoids (e.g. [10, 34]), but only suggested for ophiuroids [8, 28, 35]. Echinoderms of different colour varieties often also vary in behaviour, morphology or physiology, and local distribution. Differences in local distributions correspond to heterogeneities either in the local salinity, water temperature or wave exposure conditions of the biotope, or in the biocoenosis within which the population density of conspecifics or the density of predators are decisive factors (e.g. [36–38]). As for *A. squamata*, individuals from different geographic regions have been shown to differ by ecological traits [3] and those from different varieties by neurophysiological properties [39–41]. The present study showed that individuals from different varieties had different abilities to produce light. Assuming that the luminous system is endogenous in *A. squamata* [12, 25], the difference in bioluminescence among varieties should have a genetic basis. This result also supports the ecological distinction of varieties with distribution and relative abundance determined by environmental factors.

The proposition that *A. squamata* is a composite assemblage of genetically distinct entities (i.e. the colour varieties) rather than a single unique taxon is a matter for discussion for many cosmopolitan organisms [42]. Individuals from closely related species often display morphological variation that is too continuous to discriminate the taxa, yet they are then considered sibling species in differentiation, distinct forms of a cryptic species, or distinct ecotypes [18, 24, 27, 28, 43]. In such circumstances, it is important to consider combined physiological, behavioural and ecological characters to identify in situ differences among individuals closely related but assumed to be genetically distinct [18, 19, 43–47]. These data along with our observations invite the consideration of *A. squamata* as an assemblage of genetically distinct entities rather than as a unique cosmopolitan taxonomic entity.

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