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Studies of genetically controlled phenotypic characters in laboratory-reared *Lytechinus variegatus* (Lamarck) (Echinodermata: Echinoidea) from Bermuda and Florida

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1 ABSTRACT

Adult Lytechinus variegatus in Bermuda display consistent morphological differences from adult L. variegatus in Florida and the Caribbean. L. variegatus from Bermuda and from Florida were reared from zygotes through metamorphosis under identical conditions in the laboratory to an age of two years. Post-metamorphic juveniles from each area are distinguishable on the basis of pigmentation patterns. Spine lengths relative to test diameter differ in older juveniles and the gonopores form at different times. As phenetic differences observable in the field are found in laboratory-reared specimens, we conclude that the differences are genetically controlled and not related to environmental conditions. L. variegatus in Bermuda are apparently isolated geographically from Florida and the Caribbean. Recruitment to local populations in Bermuda appears to be entirely internal.

2 INTRODUCTION

The Bermuda Islands, 1000 km to the southeast of Cape Hatteras, N.C., have a geological age of approximately 36 million years (Wilson 1963). Despite its geographic isolation from other landmasses and its properties as a truly oceanic island, Bermuda can scarcely be regarded as isolated in the biological sense. The echinoderms of these islands apparently reached Bermuda by "jump-dispersal" in the sense of Pielou (1979). Larval stages were carried by oceanic currents to Bermuda presumably from the south. Fifty species of shallow-water echinoderms occur around Bermuda (Pawson and Devaney in preparation). All of these species also occur in the Florida-Caribbean area. A feature of the Bermudan echinoderm fauna and other marine invertebrate groups is the complete absence of autochthonously evolved endemic species.

The majority of Bermudan echinoderms have pelagic larval stages, but there is no reliable information on the extent to which Bermuda is colonized by larvae from the Florida-Caribbean area. Since Bermuda has 50 shallow-water species of echinoderms and since there are at least 100 common Florida-Caribbean shallow-water species with pelagic larvae, some species are either not capable of making the journey to Bermuda as larval stages, or they make the journey but are unable to survive. For the echinoderms then, the Bermudan fauna might be regarded as falling somewhere between an impoverished and a harmonic level in the sense of McArthur and Wilson (1967) and Roughgarden (1979). A similar situation exists at Ascension Island, in the South Atlantic (Pawson 1978) but differs in that the process of speciation seems to be proceeding more rapidly at Ascension.

With one conspicuous exception, Bermuda's echinoderm species superficially appear to be morphologically identical to their conspecifics to the south. A genetic continuity is indicated for these phenotypically similar populations. The situation is more complex in the exceptional species, the echinoid Lytechinus variegatus. Adult L. variegatus from Bermuda have purple tests and purple spines; adult L. variegatus elsewhere have red to green tests and red to green spines (Serafy 1973). In one area of Bermuda, Harrington Sound, L. variegatus can have greenish tests and often greenish spines, but they are nonetheless distinguishable as Bermudan forms. Other, less conspicuous, skeletal differences occur

(Pawson, in preparation).

A. Agassiz (1863) referred the Bermudan Lytechinus to a new species, L. atlanticus, on the basis of color and shape of its spines. Agassiz found that the spines of the Bermudan Lytechinus were longer, more slender, and usually of a deep violet color, although light violet or green spines were seen occasionally. Later authors regarded the Bermudan form as L. variegatus, but Jackson (1912) revived the concept of L. atlanticus for he found consistent structural differences in the apical system of Bermudan and Floridan specimens. H.L. Clark (1912) reduced atlanticus to the status of a subspecies of L. variegatus. Most subsequent authors, including Mortensen (1943) and Serafy (1973) followed Clark's subdivision of L. variegatus into three subspecies (or "forms"): atlanticus from Bermuda, carolinus from the southeastern coast of the U.S.A. and the Gulf of Mexico, and the typical subspecies variegatus from southern Florida, the Caribbean and Central and South America (see Serafy 1973).

The purposes of this study was to determine whether these differences are genetically or environmentally induced. In order to eliminate environmental variables, larvae from Bermudan and Floridan populations were raised under identical conditions in the laboratory.

Marcus (1980) used a similar experimental approach for two supposedly isolated populations of the sea urchin Arbacia punctulata. Differences in morphological characters of the offspring were regarded as genetically based, unrelated to environmental parameters.

3 MATERIALS AND METHODS

3.1 Larvae

Adult Lytechinus variegatus were collected at Mullet Bay, Bermuda and Hobe Sound, Florida during September, 1979. Mature gametes were obtained from several specimens of each population by injection of 0.2-0.4 ml of 0.5M KCl through the peristomial membrane. After rinsing with filtered sea water, eggs were fertilized with a diluted sperm suspension. Six purebred cultures (3 Florida ♀♀X♂♂ ; 3 Bermuda ♀♀X♂♂) were maintained under identical conditions of temperature, salinity, illumination and food.

Developing pluteus larvae were raised in 2-liter beakers at 22° (±2°C), at a salinity of 34°/$_{oo}$ (±1°/$_{oo}$). Culture seawater was Millipore filtered at 0.45µm and constantly stirred at 15 RPM with a simple stirring device (Figure 1). Larval densities in the cultures varied from 8-10 larvae/ml for young larvae to 1-4 larvae/ml for mature larvae with developing echinus rudiments. A mixture of the chlorophytes Dunaliella salina, Platymonas sp. and Stephanoptera sp. were used as food. Algal cells were extracted from the algal culture medium by centrifugation at 3000 RPM for 3 minutes before adding to larval cultures. Food concentrations were maintained at 5-6x10^4 cells/ml.

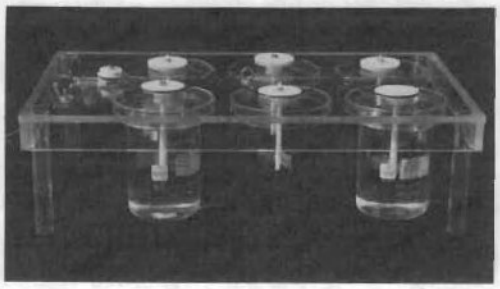

Figure 1. Culture table used in rearing larvae of Lytechinus variegatus.

3.2 Metamorphosis

Larvae competent to metamorphose were placed in finger bowls containing seawater and a small quantity of naturally occurring shell fragments from a variety of mollusks taken from aquaria containing adult L. variegatus. Initiation of metamorphosis usually occurred within several minutes. Complete metamorphosis required 20 minutes to one hour.

3.3 Juveniles and adults

Juveniles were placed in plastic culture dishes containing the surface-dwelling diatom, Nitzchia sp. Fresh Nitzchia sp. dishes were supplied weekly. Juveniles with tests approximately 1mm in horizontal diameter (HD) were placed in a divided 5-gallon aquarium which had a thick layer of algae (species unknown) growing on the walls and sediment. Specimens with a HD greater than 10mm were maintained in 30-gallon aquaria at 26-28°C and were fed shoots of the turtle grass Thalassia testudinum. Specimens were regularly examined and measurements of HD and longest spine (LS) were made. Specimens less than 20mm HD were measured with a binocular microscope equipped with ocular micrometer; larger specimens were measured with vernier calipers. Records were kept of pigmentation patterns, time of formation of the genital

Table 1. Comparison of laboratory-reared Lytechinus variegatus from Florida and Bermuda; Larvae and Juveniles.

Larvae	Florida			Bermuda		
Pluteus morphology	Larvae similar			Larvae similar		
Developmental time to metamorphosis	28-50 days			23-53 days		
Percent metamorphosis	17% (1500 larvae; 252 metamorphosed larvae)			28% (700 larvae; 199 metamorphosed larvae)		
Juveniles						
F_1 population	N = 22 specimens			N = 20 specimens		
Horizontal diameter of test	Range (mm)	$\bar{X}$(mm)	SD	Range(mm)	$\bar{X}$(mm)	SD
2 days	0.38- 0.46	0.42	-	0.34- 0.40	0.36	-
26 weeks	3.2 -16.7	5.6	3.25	2.3 -15.0	5.4	2.71
30 weeks	3.9 -20.0	6.8	4.15	2.9 -19.3	6.0	3.49
33 weeks	4.2 -26.7	8.5	5.52	4.6 -20.0	7.2	3.79
36 weeks	4.2 -25.0	8.4	5.36	3.5 -22.3	8.1	4.51
Longest spine / Horizontal diameter	Range of means = 0.41-0.43 (26-36 weeks)			Range of means = 0.51-0.56 (26-36 weeks)		
Pigmentation						
2-3 days	Dark purple pigment patch covers presumptive periproct; purple pigment band on proximal 1/3 of each 1° spine; no concentration of pigment over presumptive peristome.			Scattered red pigment on aboral surface; no pigment on 1° spines; star-shaped concentration of pigment over presumptive peristome.		
7 weeks	Test pigmentation similar to Bermuda specimens; purple pigment band remains evident on 1° spines.			Test pigmentation similar to Florida specimens; some 1° spines with faint brown pigment bands.		
17 weeks	Test brownish-red with dark red sutures; spines white to greenish-yellow; banding of 1° spines no longer evident; tube feet lacking pigment; jaws of globiferous pedicellariae white or pink.			Test red with dark red sutures; spines tan proximally, purple distally; tube feet pigmented; jaws of globiferous pedicellariae dark red.		

pores and pedicellariae numbers and types. Mean values for the ratios of LS to HD were calculated for each group and compared at each census by Student's t-distribution (Sokal and Rohlf 1973).

4 RESULTS

4.1 Larvae and metamorphosis (Table 1)

No difference in skeletal morphology, pigmentation patterns, body length or

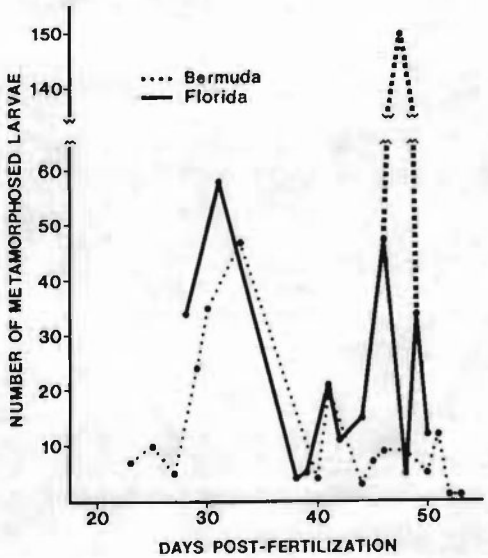

Figure 2. Time to metamorphosis of larvae in Lytechinus variegatus. Dashed line for Florida (day 48) refers to larvae which settled but which did not complete metamorphosis.

behavior occurred between larvae from Bermuda and Florida. Mazur and Miller (1971) found the shortest time required to reach the competent (to metamorphose) stage to be 33 days at 23°C. The time required to reach the competent stage in this study ranged from 23 to 53 days for the Bermudan larvae and from 28 to 50 days for the Floridan larvae. Both groups reached a peak level of competence at approximately 32 days (Figure 2). Additional peaks occurred at 41 days for both groups and at 45 and 49 days for the Floridan larvae. Percent metamorphosis was calculated by dividing the total number of metamorphosed larvae by the number of larvae surviving in the culture of the first day metamorphosis occurred. The percent of larvae attaining metamorphosis is similar (approximately 28%) for both groups if one takes into account the incompletely metamorphosed juveniles from Florida (Figure 2) day 48. A stable F_1 population of 22 Floridan urchins and 20 Bermudan urchins were raised to maturity from approximately 450 metamorphosed larvae (250 Florida, 200 Bermuda).

4.2 Juveniles (Table 1)

Differences between the Floridan and Bermudan Lytechinus became apparent at 2-3 days

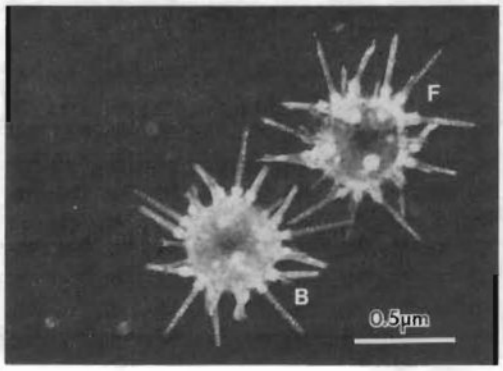

Figure 3. Juveniles of Lytechinus variegatus two days post-metamorphosis. B, Bermuda; F, Florida.

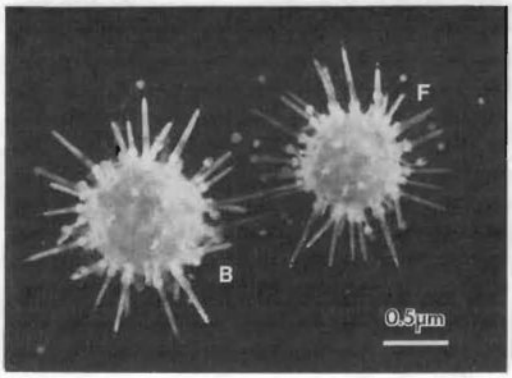

Figure 4. Juveniles of Lytechinus variegatus 23 days post-metamorphosis. B, Bermuda; F, Florida.

post-metamorphosis. The most obvious difference at this stage was in the pigmentation of the aboral surface (Figure 3). The pigmentation in the urchins became similar and the juveniles are virtually indistinguishable at an age of approximately 3-4 weeks (Figure 4). By 17 weeks, the two groups are again easily distinguishable on the basis of pigmentation.

The relationship of longest spine length (LS) and horizontal test diameter (HD) differed between the Floridan and Bermudan forms (Figure 5) throughout development of the juvenile and adult urchins. A statistical comparison of the means for LS/HD indicates that the Floridan and Bermudan juveniles were significantly different (Student's t-distribution, $p<.01$) at each census. The primary spines of the

Table 2. Comparison of laboratory-reared Lytechinus variegatus from Florida and Bermuda: Adults.

	Florida			Bermuda		
Horizontal diameter of test (HD)	Range(mm)	$\bar{X}$(mm)	SD	Range(mm)	$\bar{X}$(mm)	SD
39 weeks	4.2-25.2	8.7	5.45	5.2-21.0	8.1	4.5
44 weeks	5.2-28.4	10.7	6.1	7.5-21.8	8.0	10.3
49 weeks	5.6-28.0	11.5	6.0	8.0-21.7	11.4	3.6
56 weeks	6.8-28.0	13.0	5.9	8.7-21.8	12.9	3.3
64 weeks	9.7-27.9	17.1	5.6	14.3-22.5	18.7	2.5
84 weeks	16.2-33.8	22.7	6.9	18.0-33.0	26.5	3.4
Longest spine / Horizontal diameter	Range of means = 0.36-0.43 (39-84 weeks)			Range of means = 0.41-0.50 (39-84 weeks)		
Genital pore formation						
Percent of F_1 population mature (5 genital pores open) at 39 weeks	45%(N = 10; HD range = 6.8-25.2 mm; $\bar{X}$ = 12.4 mm; SD = 6.3). 64 weeks were required for 100% maturation.			100% (N = 20; HD range = 5.2-21 mm; $\bar{X}$ = 8.1 mm; SD = 4.5)		
Minimum HD of a mature specimen	6.1 mm			4.4 mm		
Pigmentation	Test pink to red; 1° spines commonly pink, occasionally white or red; 2° spines olive green.			Test grayish red; 1° spines dark purple; 2° spines olive green.		

Bermudan specimens were consistently longer than those of Floridan specimens.

4.3 Adults (Table 2)

Table 2 shows that at various post-metamorphic ages (39 weeks, 44 weeks, etc.), the specimens of the Floridan and Bermudan L. variegatus varied considerably in HD, but average growth over time was continuous, and occurred at approximately the same rate in both cultures.

Specimens which had formed five genital pores were regarded as adults. The smallest mature adult from Bermuda measured 4.4mm HD; the smallest from Florida measured 6.1mm HD. The Bermudan specimens reached maturity more rapidly than the Floridan specimens (Figure 6). These differences may be related in some way to the cooler temperature regimes and a shorter reproductive season at Bermuda.

Pigmentation patterns became increasingly distinctive with increasing age as the laboratory-reared urchins resembled the parental stocks. This difference is consistent through most of the juvenile and early adult lives of these urchins.

An examination of the calcareous jaws from pedicellariae and the endplate ossicles from tube-feet, using the scanning electron microscope, failed to reveal any differences between the Floridan and Bermudan urchins.

Bermudan juveniles and adults were more active than the Floridan specimens. Spine movement and tube-foot extension and contraction were always more rapid in Bermudan specimens. A righting-reaction experiment was conducted at an aquarium temperature of 26.5°C. Five Floridan urchins remained inverted in excess of five minutes with no inclination to right themselves; the other seven specimens righted in 78-151 seconds. The Floridan specimens, reared in the laboratory at average temperatures lower than those normally experienced in the field, exhibited a depressed activity level. The Bermudan urchins all righted within 74-200 seconds. Kleitman (1941) found that the righting-reaction time for Lytechinus from Bermuda varied with temperature and that the optimum

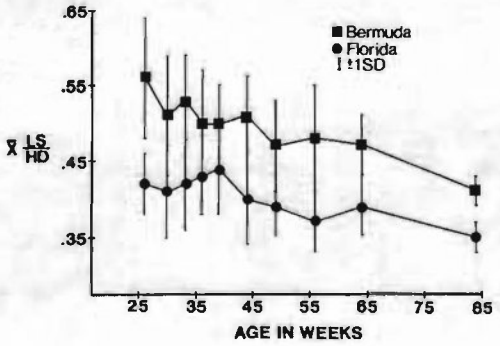

Figure 5. The relation between mean ratios of longest spine length (LS)/horizontal test diameter (HD) and age in Lytechinus variegatus. Bermuda, N = 20; Florida, N = 22.

temperature for righting was 26°C. Below this temperature, the righting time increased progressively.

5 DISCUSSION

We conclude that the consistent morphological differences noted for the Bermudan and Floridan Lytechinus variegatus have a genetic basis unrelated to influence by environmental factors. It follows that Bermudan L. variegatus are genetically isolated from populations to the south and southwest. Some extrinsic or intrinsic barriers are preventing the incorporation of the Florida-Caribbean genome into the Bermudan populations. We suggest that larval stages of L. variegatus from the south and southwest are not reaching Bermuda or are not surviving there. Some larvae may arrive, settle and metamorphose, but the resulting infusion of genetic material is being swamped by the existing Bermuda genome. Alternatively, because of some pre-mating behavioral idiosyncrasy, the Floridan urchins that reach Bermuda and survive there do not spawn in synchrony with the Bermudan urchins and therefore have no influence on the Bermuda genome. These last two possibilities seem remote; during a study of apical systems in L. variegatus at Bermuda in the early 1970's, one of us (DLP) examined in excess of 2,000 urchins from several localities, and not a single "Florida type" urchin was found.

If our suggestions are correct, recruitment to the Bermudan populations of L. variegatus is entirely internal. Apparently local favorable current patterns retain some larvae in the Bermuda area until they

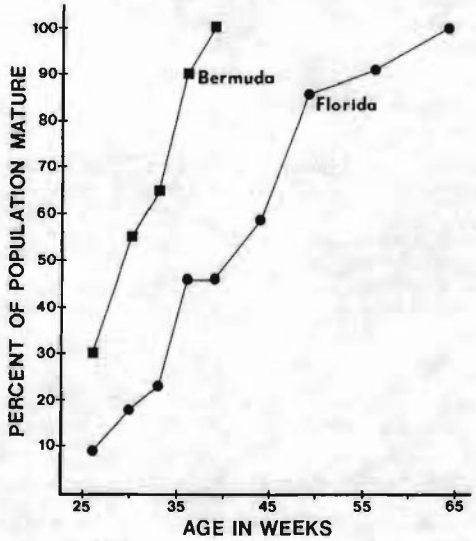

Figure 6. Rate of maturation of laboratory-reared Lytechinus variegatus.

are competent to settle and metamorphose.

It has already been noted that the other shallow-water Bermudan echinoderms appear to be morphologically identical with conspecifics to the south and southwest. For these species, it appears that a genetic continuity exists through larval dispersal. Unfortunately there is no empirical evidence available to support or refute this contention. Information on the composition of oceanic plankton and on surface and near-surface water movements in the vicinity of Bermuda is lacking. The Gulf Stream usually passes well to the west of Bermuda, but the influence of its eddies and rings (Richardson 1976, Wiebe 1976) is probably reflected in the Bermudan echinoderm fauna.

At present, the Bermudan Lytechinus is best regarded as a subspecies of L. variegatus in the sense of Clark (1912) and Serafy (1973). However this designation might not adequately describe the systematic position of the Bermudan populations.

The theory of allopatric speciation proposed by Mayr (1963, 1970), requires that reproductive isolation and morphological change result from reduced genetic variability due to founder effects or genetic drift. Lande (1980) notes that in a population geographically isolated from the main range of a species, reproductive isolation can evolve only incidentally as a byproduct of genetic divergence occurring for other reasons. Whatever these reasons might be, the Bermudan L. variegatus, is apparently genetically distinct from the

Floridan L. variegatus. We believe that the Bermudan L. variegatus is undergoing incipient speciation or, in the sense of White (1978), a species in statu nacendi.

6 LITERATURE CITED

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