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GENETIC BASIS OF SHELL VARIATION IN *THAIS EMARGINATA* (PROSOBRANCHIA, MURICACEA). I. BANDING IN POPULATIONS FROM VANCOUVER ISLAND

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ABSTRACT

Thais (or *Nucella*) *emarginata*, a rocky intertidal gastropod inhabiting the Pacific coast of North America, is polymorphic for shell banding in nearly all habitats in which it is found. Two generations of breeding revealed that this polymorphism has a simple genetic basis. Banding of the outer shell was inherited as if controlled by a single autosomal locus (OB) with two alleles (OB^B = banded and OB^U = unbanded), with banding dominant. Banding also assorted independently of the major shell color locus (OC), thus reinforcing an earlier conclusion that shell traits in this species are not tightly linked in a 'super gene' as in many terrestrial pulmonates. The clarity of banding, however, was found to depend on other genes or alleles influencing pigment intensity; individuals carrying these alleles at the banding or other loci exhibited much less pronounced banding or in some cases a complete loss of pigment in the outer shell. Most commonly, spiral bands appear as regularly spaced lines of pigment set against a largely unpigmented background (= white to pale grey ground color), but bands may also occur against other ground colors. Mechanistically, however, banding appears to result from regularly spaced zones of suppressed pigmentation in the outer shell. Preliminary distributional data revealed that the frequency of banded individuals in field populations increased with increasing wave exposure; however, the adaptive value of this polymorphism is not clear at present.

INTRODUCTION

Many species of marine, shallow-water, prosobranch gastropods exhibit intraspecific variation in shell pigmentation (Kincaid, 1957, 1964; Spight, 1976; Clarke, 1978). The rather conspicuous variation in some species has prompted numerous studies of the correlation between morph-frequencies and environmental conditions (Colton, 1922; Pelseneer, 1935; Moore, 1936; Fischer-Piette *et al.*, 1963; Daguzan, 1968; Safriel, 1969; Berry and Crothers, 1974; Heller, 1975; Pettitt, 1975; Hoagland, 1977; Osborne, 1977; Davis, 1980; Bowman, 1981; Reimchen, 1981; Smith, 1981). Surprisingly few have examined experimentally the adaptive value of variation in shell pigmentation (Giesel, 1970; Reimchen, 1979; see also Mercurio *et al.*, 1985). In addition, and in contrast to the situation for aquatic and terrestrial pulmonates (reviewed by Murray, 1975), little is known about the genetic basis of this variation (but see Cole, 1975; Palmer, 1984a). I report here the results from two generations of breeding, including backcrosses: that variation in shell banding in populations of the rocky intertidal gastropod *Thais* (or *Nucella*) *emarginata* is controlled predominantly by a pair of alleles at a single autosomal locus. Knowledge of the genetic basis of shell banding

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variation now sets the stage for an analysis of the factors which maintain this variation as an apparently stable polymorphism in natural populations.

Thais emarginata is a common and well-studied inhabitant of the middle and upper intertidal of rocky shores (Emlen, 1966; Spight and Emlen, 1976; Kitching, 1976; Davis, 1980; Morris *et al.*, 1980; Palmer, 1983, 1984a, b). It has one of the broadest geographic ranges along the Pacific coast of North America of all intertidal gastropods (Palmer, 1984a), and it is an important predator of barnacles and mussels (Palmer 1983, 1984b). Individuals of *T. emarginata* also exhibit a broad range of variation in shell color, banding, sculpture, thickness, and shape (Kincaid, 1964; Emlen, 1966; Abbott, 1974; Kitching, 1976; Davis, 1980; Morris *et al.*, 1980; Palmer 1984a, 1985). By virtue of their direct development (Lyons and Spight, 1973) and the fact that newly hatched snails will feed readily on very small barnacles, *Thais emarginata* may be bred and raised in the laboratory without difficulty (Palmer, 1984a).

MATERIALS AND METHODS

Immature specimens of *Thais emarginata* (<15 mm in nearly all cases) were collected from Wizard Island (48°51'N, 125°09'W), in Barkley Sound near the Bamfield Marine Station, on the west coast of Vancouver Island, British Columbia (Canada). Individuals were sexed according to relative penis size (see also Hargis, 1957); single pairs were placed in separate cages, made from plastic freezer containers whose sides had been replaced with VEXAR® plastic mesh, and were provided with one of their preferred prey (the barnacle, *Balanus glandula;* Palmer, 1984b). Cages were checked at approximately two-week intervals to monitor growth to maturity and egg-capsule production. When laying was completed, each clutch was transferred to a separate, small envelope of plastic window screening with VELCRO® closures where it was held for approximately 60 days while the embryos developed. These envelopes permitted greater water exchange than cages in which hatchlings were raised and resulted in a higher hatching success. Prior to hatching, the capsules were transferred to one liter freezer containers whose sides had been replaced with 500 μm NITEX® screening and provided with stones covered with very young (<3 mm basal diameter) barnacles (primarily *Balanus glandula* or *Chthamalus dalli*). Stones were checked at two week intervals and were replaced as barnacles were eaten; when the hatchlings had reached >3 mm shell length, they were transferred to larger freezer containers with sides of plastic window screening. Once larger than about 5 mm, juvenile *Thais emarginata* were capable of eating adult *B. glandula* (8–10 mm basal diameter). Beyond this size, stones with fresh barnacles were provided at approximately 3 week intervals.

Egg-capsules produced by crosses initiated with mature females (*e.g.*, backcrosses of offspring to parents) were saved only after 6 months had elapsed from the time of first pairing. Few if any sperm remain from previous matings after this time (Palmer, in prep.).

The labelling convention used below encodes several pieces of information (general form = year—lineage number—generation—cross identifier):

Year—year in which the lineage (a unique collection of genes) was initiated.

Lineage number—the number of the lineage initiated in that year.

Generation—the laboratory generation of the offspring to be produced (when referring to a pair of parents) or of the individuals themselves (when referring to a clutch). The prefix B indicates a backcross to a parent or grandparent. For first-generation crosses, a suffix letter (*e.g.*, A, B, etc.) identifies a particular F1 clutch.

Cross identifier (3 parts; not present for first generation crosses)—
Letter—for bookkeeping (if present).
Digit(s)—identifies the parents of a cross established for a given generation of a given lineage (the prefix M or F indicates a backcross to either a male or female parent).
Letter(s)—identifies a clutch or clutches produced by a given pair of parents (labelled sequentially starting with A for each pair of parents).

The frequency of banded individuals in field populations was determined from samples of snails from four different sites: a site with relatively low wave action (NW side of Wizard Island), a site with intermediate wave action (N end of Prasiola Point—48°48′N, 125°10′W), and two very exposed sites (SW tip of Cape Beale—48°47′N, 125°13′W, and SW tip of Cree Island—48°51′N, 125°20′W). At both Wizard Island and Cape Beale, two or more samples were taken from different microhabitat types: either predominantly from among mussels or predominantly from among barnacles. To ensure an unbiased sample, I collected all snails encountered within a given microhabitat type until approximately 100 individuals had been collected. Snails having badly eroded shells were not counted. Within mussel beds, only visible snails were collected (*i.e.*, the mussel bed was not disturbed to locate snails in the interstices).

Wave exposure indices were obtained from Craik (1980), who used weight lost by swinging cement blocks anchored to the bottom as a relative measure of wave activity.

Except for the field samples, Chi-square values presented below were calculated from a comparison of observed frequencies, corrected for continuity (Sokal and Rohlf, 1981, p. 710), to the expected Mendelian frequencies, assuming banding was controlled by a single, autosomal locus with two alleles.

RESULTS

Although variation did exist in the clarity with which banding was expressed, banded individuals could be distinguished readily from unbanded ones (Fig. 1). The inheritance of banding was determined primarily from the genealogy of two lineages, 80-17 and 80-18 (Table I). The parents of 80-17-1A (banded male × unbanded female) produced approximately equal numbers of banded and unbanded offspring (25:29) whereas the unbanded parents of 80-18-1A produced only unbanded progeny. Crosses between unbanded, F1 individuals from these two lineages yielded only unbanded offspring (80-17-2-A4A, -A5A, -A8A, 80-18-2-pool, and 81-79-1A). A nearly 1:1 ratio of phenotypes resulted from crosses between banded and unbanded individuals, regardless of which parent was banded: 260:288 banded:unbanded progeny when the male was banded ($\chi^2 = 1.33$, $P = 0.25$; pooled from clutches 80-17-2-A1A, -A2A,B, -A3A, -A10A, -A12A, -A13A, -A16A, 81-77-1A, and 81-78-1A) and 123:121 banded: unbanded offspring when the female was banded ($\chi^2 = 0.004$, $P = 0.95$; pooled from clutches 80-17-2-A9A,B, -A11A,B, and -A15A). In addition, crosses between two banded F1 individuals yielded close to the predicted 3:1 ratio of banded to unbanded offspring (141:42, $\chi^2 = 0.31$, $P = 0.58$; pooled from clutches 80-17-2-A6A and -A7A). Backcrosses with unbanded (homozygous recessive) F1 offspring confirmed that the banded, male parent of 80-17-1A was heterozygous (80-17-B2-MA), and that the remaining three unbanded parents in both lineages were homozygous recessive (80-17-B2-FD; 80-18-B2-MA, and -FE). Finally, backcrosses between unbanded F1 individuals and unbanded, field-collected individuals yielded only unbanded offspring (81-80-1A, 81-81-1A, 81-82-1B). These patterns were consistent with a genetic model incorporating a single, autosomal locus (OB for outer shell banding) having two alleles: OB^B, a

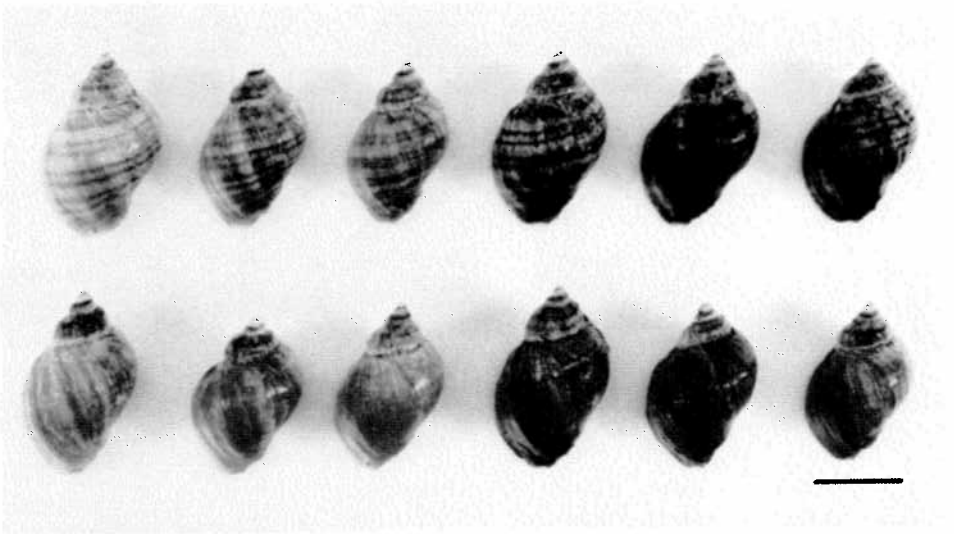

FIGURE 1. Shell banding dimorphism in *Thais* (or *Nucella*) *emarginata* (Deshayes, 1839). Banding phenotypes from clutch 80-17-B2-MA (backcross of unbanded F1 female to original banded male parent; actual phenotype frequencies in Table I). The three left-most individuals in each row are predominantly orange, the three right-most, black. All snails in these clutches were raised from egg capsules deposited in the lab; only the three largest individuals of each phenotype combination were photographed. Approximate age of snails = 9 mos. Scale bar = 10 mm.

dominant allele causing outer shell pigment to be localized in spiral bands, and OB^U, a recessive allele resulting in uniformly pigmented shells.

Crosses in which one parent was heterozygous at both the banding (OB^B/OB^U) and the primary outer shell color locus (OC^{OR}/OC^{BL}), while the other parent was homozygous recessive at both loci (OB^U/OB^U, OC^{BL}/OC^{BL}) revealed that alleles at the banding locus assorted independently from those at the color locus [Group I, Table II; in populations from Barkley Sound the orange, outer shell color allele (OC^{OR}) is usually, but not always dominant to the black allele (OC^{BL}) (Palmer, unpub.)]. Differential survival of phenotypes was suggested by the results of Table II since unbanded, black offspring (OB^U/OB^U, OC^{BL}/OC^{BL}) were not only the most frequent in crosses of Group I, where linkage was tested, but also in the crosses of Group II, where no linkage would be detectable since one parent was heterozygous for banding (OB^B/OB^U), while the other was heterozygous for color (OC^{OR}/OC^{BL}). However, the overall deviation from expected of both groups pooled was not significant ($P = 0.27$, 'Pooled', Table II).

The F1 phenotypes in clutch 82-52-1A (Table I), although consistent with the proposed Mendelian model, reveal the further complexity that the intensity of banding was influenced by other alleles or loci. In this cross, a predominantly unpigmented, essentially unbanded male (light grey shell with pale tan mottling and a white lip), was crossed with a yellow-orange, strongly banded female, yielding six fairly discrete phenotypes (Fig. 2a, b; Table III). Two strongly pigmented morphs (orange and black) exhibited strong banding [two leftmost individuals of rows 3 and 4 respectively, Fig. 2a, b; except for one unbanded black individual (Table III) which, because of its shell shape, was probably a contaminant from another cross or was introduced accidentally with barnacles from the field, a rare but nearly unavoidable problem given the large

TABLE I

Sex, origin, phenotypes, and inferred genotypes of parents and offspring of crosses with Thais emarginata[1]

	Parents				Progeny				
Clutch label	Sex	Source	Pheno.	Genotype	Sex	Banded N	Unband N	χ^2	*P*
F1									
80-17-1A	m	Wizard Is.	banded	OB^B/OB^U	m	15	12	1.48	0.69
	f	Wizard Is.	unband	OB^U/OB^U	f	10	17		
80-18-1A	m	Wizard Is.	unband	OB^U/OB^U	m	—	10	1.33	0.25
	f	Wizard Is.	unband	OB^U/OB^U	f	—	17		
82-52-1A	m	Wizard Is.	unband[2]	?[2]	—	44	39	0.19	0.66
	f	Wizard Is.	banded	OB^B/OB^U, OB^B/OB^B?					
F2									
80-17-2-A1A	m	prog 80-17-1	banded	OB^B/OB^U	—	36	37	0.00	1.00
	f	prog 80-17-1	unband	OB^U/OB^U					
80-17-2-A2A,B	m	prog 80-17-1	banded	OB^B/OB^U	—	58	45	1.40	0.24
	f	prog 80-17-1	unband	OB^U/OB^U					
80-17-2-A3A	m	prog 80-17-1	banded	OB^B/OB^U	—	28	34	0.40	0.53
	f	prog 80-17-1	unband	OB^U/OB^U					
80-17-2-A4A	m	prog 80-17-1	unband	OB^U/OB^U	—	—	32	—	—
	f	prog 80-17-1	unband	OB^U/OB^U					
80-17-2-A5A	m	prog 80-17-1	unband	OB^U/OB^U	—	—	43	—	—
	f	prog 80-17-1	unband	OB^U/OB^U					
80-17-2-A6A	m	prog 80-17-1	banded	OB^B/OB^U	—	67	20	0.10	0.75
	f	prog 80-17-1	banded	OB^B/OB^U					
80-17-2-A7A	m	prog 80-17-1	banded	OB^B/OB^U	—	74	22	0.13	0.72
	f	prog 80-17-1	banded	OB^B/OB^U					
80-17-2-A8A	m	prog 80-17-1	unband	OB^U/OB^U	m	—	17	0.13	0.72
	f	prog 80-17-1	unband	OB^U/OB^U	f	—	14		
80-17-2-A9A, B	m	prog 80-17-1	unband	OB^U/OB^U	—	77	76	0.00	1.00
	f	prog 80-17-1	banded	OB^B/OB^U					
80-17-2-A10A	m	prog 80-17-1	banded	OB^B/OB^U	—	19	30	2.04	0.15
	f	prog 80-17-1	unband	OB^U/OB^U					
80-17-2-A11A, B	m	prog 80-17-1	unband	OB^U/OB^U	—	20	21	0.00	1.00
	f	prog 80-17-1	banded	OB^B/OB^U					
80-17-2-A12A	m	prog 80-17-1	banded	OB^B/OB^U	—	14	26	3.03	0.08
	f	prog 80-17-1	unband	OB^U/OB^U					
80-17-2-A13A	m	prog 80-17-1	banded	OB^B/OB^U	—	40	41	0.00	1.00
	f	prog 80-17-1	unband	OB^U/OB^U					
80-17-2-A15A	m	prog 80-17-1	unband	OB^U/OB^U	—	26	25	0.00	1.00
	f	prog 80-17-1	banded	OB^B/OB^U					
80-17-2-A16A	m	prog 80-17-1	banded	OB^B/OB^U	—	6	9	0.27	0.61
	f	prog 80-17-1	unband	OB^U/OB^U					
80-18-2-pool[3]	m	prog 80-18-1	unband	OB^U/OB^U	—	—	406[3]	—	—
	f	prog 80-18-1	unband	OB^U/OB^U					

TABLE I (*Continued*)

Clutch label	Parents				Progeny				
	Sex	Source	Pheno.	Genotype	Sex	Banded N	Unband N	χ^2	*P*
F2									
81-77-1A	m	prog 80-17-1	banded	OB^B/OB^U	—	28	35	0.57	0.45
	f	prog 80-18-1	unband	OB^U/OB^U					
81-78-1A	m	prog 80-17-1	banded	OB^B/OB^U	—	31	35	0.14	0.71
	f	prog 80-18-1	unband	OB^U/OB^U					
81-79-1A	m	prog 80-17-1	unband	OB^U/OB^U	—	—	50	—	—
	f	prog 80-18-1	unband	OB^U/OB^U					
Backcrosses									
80-17-B2-MA	m	par 80-17-1	banded	OB^B/OB^U	—	32	28	0.15	0.70
	f	prog 80-17-1	unband	OB^U/OB^U					
80-17-B2-FD	m	prog 80-17-1	unband	OB^U/OB^U	—	1[4]	59	—	—
	f	par 80-17-1	unband	OB^U/OB^U					
80-18-B2-MA	m	par 80-18-1	unband	OB^U/OB^U	—	—	53	—	—
	f	prog 80-18-1	unband	OB^U/OB^U					
80-18-B2-FE	m	prog 80-18-1	unband	OB^U/OB^U	—	—	47	—	—
	f	par 80-18-1	unband	OB^U/OB^U					
81-80-1A	m	Wizard Is.	unband[2]	OB^U/OB^{U2}	—	—	125	—	—
	f	prog 80-18-1	unband	OB^U/OB^U					
81-81-1A	m	Wizard Is.	unband[2]	OB^U/OB^{U2}	—	—	38	—	—
	f	prog 80-17-1	unband	OB^U/OB^U					
81-82-1B	m	Wizard Is.	unband[5]	OB^U/OB^{U5}	—	—	28	—	—
	f	prog 80-17-1	unband	OB^U/OB^U					

[1] Entries under Source: Wizard Is. = collected as immature animals from the field, prog = progeny of specified cross, par = original parent of specified cross. Pheno. = observed phenotype of parents or offspring. Genotype = inferred genotype(s) of parents (OB^B = dominant allele for 'banded', OB^U = recessive allele for 'unbanded'). χ^2 = Chi-square value from comparing observed phenotype frequencies with those expected from a two allele polymorphism at a single autosomal locus. *P* = exact probability.

[2] Parent was unpigminted, thus the parental genotype could only be inferred from the offspring phenotype frequencies.

[3] Pooled offspring from 12 separate crosses.

[4] Probably an accidental contaminant (see text).

[5] Parent was largely unpigmented (with very faint tan mottling), see footnote 2.

number of crosses and their frequency of handling]. Two morphs with pale pigmentation (pale orange and pale brown) exhibited faint banding that increased in intensity towards the more anterior and more posterior edges of the whorl (two rightmost individuals of rows 1 and 2 respectively, Fig. 2a, b). Finally, two morphs were unbanded and their outer shell coloration intergraded to some extent; both were predominantly unpigmented (very pale grey, although some exhibited very pale yellow mottling), but one morph had a white columella and the other clearly had a purple columella (two rightmost individuals of rows 3 and 4 respectively, Fig. 2a, b).

The results of the field surveys for banding phenotype frequencies revealed a pronounced increase in the frequency of banded individuals with increasing wave exposure

TABLE II

Evidence for independent assortment of outer shell color and outer shell banding in Thais emarginata[1]

				Offspring phenotype[2]					
	Parent			'Orange'		Black			
Clutch label	Sex	Phenotype[2]	Inferred genotype[3]	Band	Unb	Band	Unb	χ^2	P
80-17-1A	m	Orange, banded	OC^{OR}/OC^{BL}, OB^{B}/OB^{U}	11	17	14	12	1.04	0.79
	f	Black, unband	OC^{BL}/OC^{BL}, OB^{U}/OB^{U}						
81-77-1A	m	Orange, banded	OC^{OR}/OC^{BL}, OB^{B}/OB^{U}	19	12	9	23	6.52	0.09
	f	Black, unband	OC^{BL}/OC^{BL}, OB^{U}/OB^{U}						
80-17-2-A1A	m	Black, banded	OC^{BL}/OC^{BL}, OB^{B}/OB^{U}	16	16	20	21	0.70	0.87
	f	Orange, unband	OC^{OR}/OC^{BL}, OB^{U}/OB^{U}						
80-17-2-A2A, B	m	Orange, banded	OC^{OR}/OC^{BL}, OB^{B}/OB^{U}	28	18	30	27	2.73	0.44
	f	Black, unband	OC^{BL}/OC^{BL}, OB^{U}/OB^{U}						
80-17-2-A6A	m	Orange, banded	OC^{OR}/OC^{BL}, OB^{B}/OB^{U}	39	17	23	7	4.39	0.22
	f	Orange, banded	OC^{OR}/OC^{BL}, OB^{B}/OB^{U}						
80-17-2-A12A	m	Black, banded	OC^{BL}/OC^{BL}, OB^{B}/OB^{U}	10	11	4	15	5.08	0.17
	f	Orange, unband	OC^{OR}/OC^{BL}, OB^{U}/OB^{U}						
80-17-2-A13A	m	Black, banded	OC^{BL}/OC^{BL}, OB^{B}/OB^{U}	24	24	16	17	2.11	0.55
	f	Orange, unband	OC^{OR}/OC^{BL}, OB^{U}/OB^{U}						
80-17-2-A15A	m	Black, unband	OC^{BL}/OC^{BL}, OB^{U}/OB^{U}	10	8	16	17	3.51	0.32
	f	Orange, banded	OC^{OR}/OC^{BL}, OB^{B},OB^{U}						
80-17-2-A16A	m	Black, banded	OC^{BL}/OC^{BL}, OB^{B},OB^{U}	3	3	3	6	0.87	0.83
	f	Orange, unband	OC^{OR}/OC^{BL}, OB^{U}/OB^{U}						
Group I[4]	—	Orange, banded	OC^{OR}/OC^{BL}, OB^{B}/OB^{U}	68	55	69	79	3.93	0.27
	—	Black, unband	OC^{BL}/OC^{BL}, OB^{U}/OB^{U}						
Group II[5]	—	Orange, unband	OC^{OR}/OC^{BL}, OB^{U}/OB^{U}	53	54	43	59	2.24	0.52
	—	Black, banded	OC^{BL}/OC^{BL}, OB^{B}/OB^{U}						
Pooled[6]	—	—	—	121	109	112	138	3.94	0.27

[1] Data only for crosses in which one or both parents were banded (OB^{B}/OB^{U}) and orange (OC^{OR}/OC^{BL}).

[2] 'Black' is a fairly discrete phenotype; 'Orange' individuals range from bright yellow-orange to orange-brown or dull orange with black mottling. 'Orange' individuals have been pooled because evidence from these and other crosses (Palmer, unpub.) strongly suggest this is a single allele whose phenotypic expression is influenced epistatically.

[3] See text for allele designations.

[4] 80-17-1A, 81-77-1A, 80-17-2-A2A, B, and -A15A pooled.

[5] 80-17-2-A1A, -A12A, -A13A, and -A16A, pooled.

[6] Group I and Group II, pooled.

(Table IV; Fig. 3). Rather curiously, banding frequency was not correlated with microhabitat type; at both Wizard Island and Cape Beale, the banding frequencies were not statistically different between samples taken predominantly or exclusively from within mussel beds (*Mytilus californianus*) or from among barnacles (Table V). At Cape Beale, unbanded individuals were statistically less common in 1984 than in 1983, but different areas were sampled in the two years, and the differences were small compared to those observed over the wave exposure gradient.

TABLE III

Color and banding phenotypes in F1 progeny from cross 82-52-1A (see also Fig. 2)[1]

Pigmentation		Number of offspring			Inferred genotype[2]		
Outer shell	Columella[3]	Strong banding	Weak banding	Un-banded	Shell color	Banding	Pigment reduction
Intense orange	or-br	9	—	—	OC^{OR}/OC^{BL}	OB^{B}/OB^{B}	OI^{-}/OI^{-}
Intense black	purp	10	—	1[4]	OC^{BL}/OC^{BL}	OB^{B}/OB^{B}	OI^{-}/OI^{-}
Pale orange	or-br	—	13	—	OC^{OR}/OC^{BL}	OB^{B}/OB^{B}	OI^{-}/OI^{R}
Pale brown	purp	—	12	—	OC^{BL}/OC^{BL}	OB^{B}/OB^{B}	OI^{-}/OI^{R}
Mostly unpigmented[5]	white	—	—	18	OC^{WH}/OC^{OR}	OB^{B}/OB^{B}	OI^{-}/OI^{-}, OI^{-}/OI^{R}
Mostly unpigmented[5]	purp	—	—	21	OC^{WH}/OC^{BL}	OB^{B}/OB^{B}	OI^{-}/OI^{-}, OI^{-}/OI^{R}

[1] Male = light grey with tan mottling and white lip, hint of banding (inferred genotype: OC^{WH}/OC^{BL}, OB^{B}/OB^{B}, OI^{-}/OI^{R}) Female = yellow-orange, banded (inferred genotype: OC^{OR}/OC^{BL}, OB^{B}/OB^{B}, OI^{-}/OI^{-}); see text for allele designations and alternative inferred genotypes.

[2] See text for allele designations.

[3] Abbreviations: purp = purple, or-br = orange-brown.

[4] Most likely an accidental contaminant (see text).

[5] Most individuals unpigmented, some with very pale yellow mottling.

DISCUSSION

The results presented above are all consistent with the hypothesis that spiral shell banding in *Thais emarginata* is controlled predominantly by a single autosomal locus (OB for outer shell banding) with two alleles, banded (OB^{B}) and unbanded (OB^{U}), and with the banded allele dominant (Table I). That banding should be controlled by a single locus is not surprising given what is known for the genetic control of shell banding in pulmonates (reviewed by Murray, 1975); in most pulmonates, the presence or absence of banding is controlled by a single locus, although the lack of bands may be either dominant [some helicids (2 spp.), Achatinidae (2 spp.)] or recessive [some helicids (2 spp.), Partulidae (1 sp.), Arionidae (1 sp.), Fructicicolidae (1 sp.)] depending on the species.

In contrast to pulmonates, however, the locus for shell banding appears to be unlinked to shell color (Table II; Palmer, 1984a) in *Thais emarginata.* Thus, although the occurrence of blocks of tightly linked genes for shell traits ('super genes') appears to be the rule among pulmonates (Murray, 1975; Murray and Clarke, 1976a, b), such tight linkage may not be as common among prosobranchs. Obviously, the generality of this conclusion must await additional genetic studies of prosobranch shell variation.

An additional difference between the results reported for pulmonates and those reported here is that, while the banding allele in pulmonates (B^{B} for *Cepaea nemoralis, C. hortensis,* and *Arianta arbustorum*) causes bands of a different pigment to be superimposed upon various ground colors of the shell (Murray, 1975), the banded allele in *Thais emarginata* (OB^{B}) causes a regularly spaced suppression of outer shell pigment. In *T. emarginata* from Barkley Sound, then, these 'bands' where pigment has been suppressed appear nearly white.

The results of cross 82-52-1A (Table III) were particularly interesting on two accounts. First, except for some variation within the two predominantly unpigmented forms, phenotypes were very discrete (Fig. 2a, b), suggesting that few loci were involved.

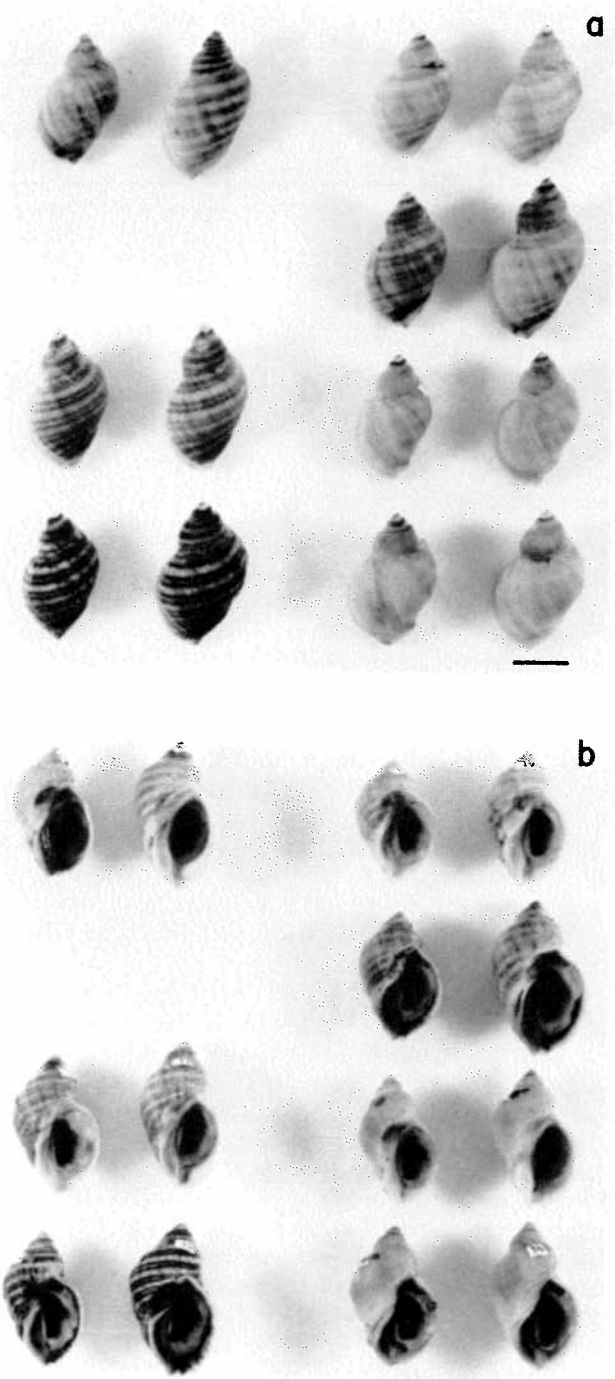

FIGURE 2. Possible evidence for pleiotropy; pigmentation and banding phenotypes from clutch 82-52-1A in *Thais emarginata*. (a) abapertural view, (b) apertural view of the same individuals. Columns 1 and 2: row 1—male parent = light grey shell with tan mottling and white lip, hint of banding, purple columella, female parent = yellow-orange shell and lip, banded, orange columella; row 3—both individuals intense

TABLE IV

Proportions of banding phenotypes in field populations of Thais emarginata *as a function of wave exposure, microhabitat type, and year*

Site	Date[1]	Wave exposure[2]	Micro-habitat[3]	Banded	Weakly banded	Unbanded[4]	N
Wizard Is. 1	9/83	38.8	Mostly M	0.09	0.18	0.73	147
Wizard Is. 2	9/83	38.8	Mixed M & B	0.08	0.13	0.79	132
Prasiola Pt.	6/84	63.4	B only	0.30	0.21	0.49	138
Cree Is.	6/84	75.1	B only	0.66	0.14	0.20	163
Cape Beale 1	7/83	80.3	Mostly B	0.62	0.08	0.31	131
Cape Beale 2	7/83	80.3	Mostly B	0.59	0.13	0.28	143
Cape Beale 3	7/83	80.3	Mixed M & B	0.63	0.13	0.24	134
Cape Beale 4	6/84	80.3	M only	0.61	0.22	0.17	87
Cape Beale 5	6/84	80.3	B only	0.61	0.22	0.17	128

[1] Month/year.

[2] Wave exposure index = entries are from the nearest station measured by Craik (1980): for Cree Is.—Benson Is., for Praisola Pt.—First Beach, for Wizard Is.—Helby Is.

[3] M—bed of *Mytilus californianus*, B—rock covered predominantly with *Balanus glandula*.

[4] Individuals with unpigmented shells pooled with pigmented, unbanded individuals.

Second, the offspring from this cross suggest very strongly that other alleles or loci influence the intensity of banding. The male parent was very pale grey with pale tan mottling and a white lip (= unpigmented) and at best only a hint of banding, and the female parent had a bright orange to yellow-orange, banded shell (leftmost two individuals respectively of row 1, Fig. 2a, b). One genetic hypothesis that accounts for the observed phenotype frequencies of the F1 assumes three, unlinked loci: (1) banding (male = OB^B/OB^B if female = OB^B/OB^U, or male = OB^B/OB^B, OB^B/OB^U or OB^U/OB^U if female = OB^B/OB^B), (2) outer shell color [male = OC^{WH}/OC^{BL}, female = OC^{OR}/OC^{BL} (OC^{WH} = 'white' or unpigmented, OC^{OR} = orange, OC^{BL} = black; in Vancouver Island populations $OC^{OR} > OC^{BL}$ in general, and at least one 'white' allele appears to exist with $OC^{WH} > OC^{OR} > OC^{BL}$ (Palmer, unpub.)], and (3) outer shell pigment intensity [one parent heterozygous OI^-/OI^R, one homozygous recessive (OI^R = partial reduction in pigment intensity in heterozygotes, complete reduction in homozygotes, OI^- = no reduction in pigment intensity; dominance is not known at present, so genotypes cannot be assigned to the parents)]. According to this model, the differences in columella color in the predominantly unpigmented individuals (two rightmost individuals of rows 3 and 4, Fig. 2a, b) would be determined by the allele carried in heterozygous condition with the proposed dominant white allele (OC^{WH}/OC^{OR} results in a white columella, OC^{WH}/OC^{BL} results in a purple columella). However, alternative interpretations of columella color variation are possible.

The above hypothesis derives from three aspects of the F1 phenotype frequencies. First, approximately half the offspring exhibited little or no outer shell pigmentation (bottom two rows, Table III), presumably because the dominant OC^{WH} allele from

orange banded shell and lip, orange-brown columella; row 4—both individuals intense black banded shell and lip, purple columella. Columns 3 and 4: row 1—both individuals very faint orange banded shell and lip, orange-brown columella; row 2—both individuals very faint brown banded shell and lip, purple columella; row 3—both individuals unpigmented, white columella; row 4—both individuals unpigmented, purple columella. Only the largest two individuals from each phenotype were photographed. Actual phenotype frequencies in Table III. Scale bar = 10 mm.

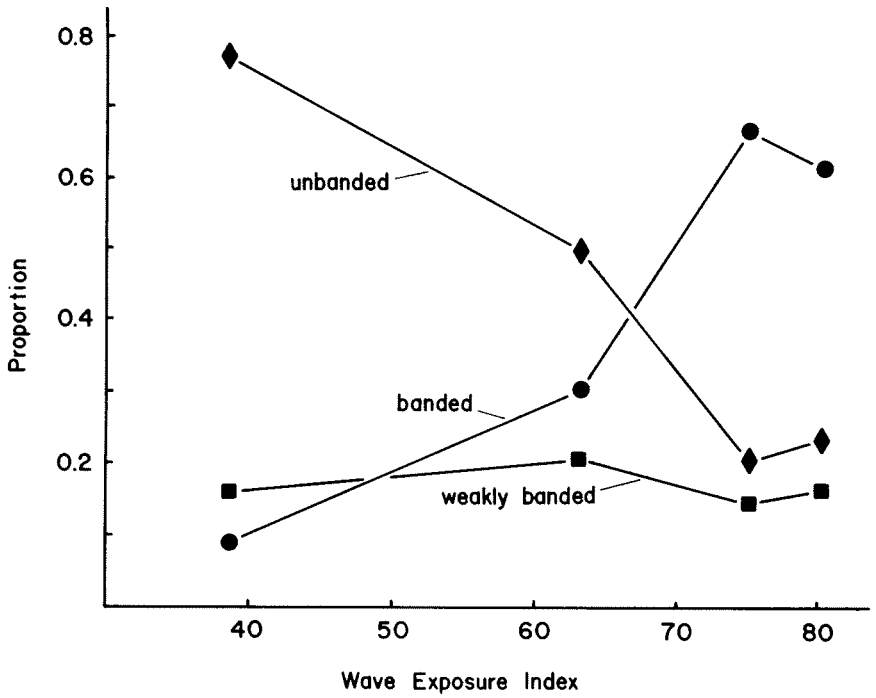

FIGURE 3. Change in proportion of banding phenotypes over a wave exposure gradient (data from Table IV).

the male suppressed expression of the OC^{BL} or OC^{OR} allele from the female parent, and the recessive OC^{BL} allele from the male allowed both female alleles to be expressed. Second, of the half of the clutch exhibiting distinct pigmentation, approximately half were strongly pigmented (upper two rows Table III) while the remaining half (rows 3 and 4) exhibited a similar color, but of much weaker intensity, presumably because half were homozygous recessive and half heterozygous at the pigment intensity locus (OI; until the dominance relationship is established at this locus, genotypes cannot be assigned). It is also possible that the presence of the mottled, pale yellow pigmentation in some of the 'unpigmented' individuals (rows 5 and 6, Table III) resulted from one of the alleles at this proposed pigment intensity locus. Third, all those offspring exhibiting distinct pigmentation showed some evidence of banding (upper four rows);

TABLE V

Results of contingency table analyses of banding frequency variation in Thais emarginata *(data in Table IV)*[1]

Comparison	χ^2	df	*P*	Sig
Between microhabitats, Wizard Is.	1.32	2	0.52	
Among microhabitats, Cape Beale, 1983	3.64	4	0.46	
Between microhabitats, Cape Beale, 1984	<0.01	2	>0.99	
Between years, Cape Beale	16.8	2	<0.001	***
Among sites	295.6	6	<0.001	***

[1] χ^2 = Chi-square value, df = degrees of freedom, *P* = exact probability, Sig = significance.

since outer shell pigmentation assorts independently from the banding locus (Table II; see also Palmer, 1984a), at least one parent had to have been homozygous dominant for banding. This last observation is significant, since it implies that all the weak or unpigmented individuals (bottom two rows) were also either heterozygous (OB^B/OB^U) or homozygous dominant (OB^B/OB^B) for banding even though they were phenotypically unbanded. Thus, banding may not be expressed in individuals carrying the 'unpigmented' allele for outer shell color (OI^R). If this interpretation is correct, these data contrast with those for *Cepaea nemoralis* (Cain *et al.*, 1968, cited in Murray, 1975) and *C. hortensis* (Cook and Murray, 1966) where individuals which are genetically 'banded' (*i.e.*, homozygous recessive) but do not produce any banding pigment (*i.e.*, 'transparent bands' = hyalozonate) nonetheless exhibit at least microstructural evidence of banding. No such 'transparent' bands appeared to be present in unpigmented, but presumably genetically banded individuals of *Thais emarginata.*

I should emphasize that alternative Mendelian models are also consistent with the phenotype frequencies in clutch 82-52-1A. Similar frequencies would obtain if (a) the proposed OC^{WH} allele was not dominant, but resulted in a dimunition of pigment intensity in heterozygotes (*e.g.*, rightmost two individuals of rows 1 and 2, Fig. 2a, b), and (b) the proposed pigment reducing allele (OI^R) was completely dominant to the normal allele (OI^-) instead of exhibiting partial reduction in pigment intensity in heterozygotes. This hypothesis requires that the parental genotypes be: male = OC^{BL}/OC^{WH}, OB^B/OB^B, OI^-/OI^R, female = OC^{OR}/OC^{BL}, OB^B/OB^B, OI^-/OI^-. Thus, with the present data, it is not possible to establish whether the predominantly unpigmented phenotype results (a) from a 'white' (= unpigmented) allele at the outer shell color locus (OC^{WH}), (b) from an independent allele at the proposed pigment reduction locus (OI^R), or (c) possibly from an allele at the banding locus (OB^S) that suppresses outer shell pigment uniformly about the mantle margin rather than in a regularly spaced manner as OB^B appears to do. Additional crosses are required to distinguish among these alternative hypotheses.

The evolutionary significance of the shell banding polymorphism in *Thais emarginata* is, unfortunately, uncertain at present. Although I have no direct evidence, the pronounced correlation of banding frequency with a gradient in wave exposure (Fig. 3), and the similarity of frequencies between samples taken one year apart at the same site compared to the frequencies at different sites (Tables IV and V), suggests rather strongly that the banding polymorphism is maintained by some form of natural selection. Presumably, selection is exerted by one or more species of visual predators, since visual predators are important sources of differential mortality in both marine gastropods [fish (Vermeij, 1978; Palmer, 1979; Reimchen, 1979; Mercurio *et al.*, 1985) and birds (Mercurio *et al.*, 1985, and references therein)] and terrestrial gastropods [birds (Cain and Sheppard, 1954; Jones, 1973)]. However, the lack of any correlation between banding frequency and microhabitat type at two sites (Tables IV, V) suggests such selection is not responsible for maintaining this polymorphism within a particular site. While several potential visual predators forage in habitats occupied by *Thais emarginata* in Barkley Sound [surfperch (*Damalichthys vacca, Embiotoca lateralis*), gulls (primarily *Larus californicus*), oystercatchers (*Haematopus bachmani*), turnstones (*Arenaria melanocephala*), and crows (*Corvus daurinus*); Mercurio *et al.*, 1985], the ones most responsible for maintaining the banding polymorphism in *T. emarginata* are unknown.

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