Biological Journal of the Linnean Society (1990), *40:* 3–10. With 1 figure

Shell colour polymorphism associated with substrate colour in the intertidal snail *Littorina saxatilis* Olivi (Prosobranchia: Littorinidae)

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Received 9 November 1988, accepted for publication 8 June 1989

Littorina saxatilis Olivi (1792), the rough winkle, is highly polymorphic in shell colour. Shell colour frequencies were studied at six locations in south-western Wales, U.K., each at a geological contact between red sandstone and grey limestone or volcanic rock. At each site shell colour frequencies were determined in samples from the contact zone and on red or grey rock on either side. Highly significant associations were found between shell colour frequencies and substrate colour. Grey shells were always more common on grey rock than on red rock, and brown shells were usually more common on red than on grey rock, suggesting selection for cryptic colouration. Shell colour frequency differences were also found between replicate samples taken only 5 m apart from the same kind of rock, and between samples from the same kind of rock at the six study sites. These latter differences suggest that selection for camouflage is not the only factor involved in maintaining shell colour polymorphism in this species.

KEY WORDS:—*Littorina saxatilis* – colour polymorphism – substrate colour matching.

CONTENTS

INTRODUCTION

The rough winkle, *Littorina saxatilis* Olivi (1792) is an abundant intertidal snail found on the shores of the North Atlantic, in the Mediterranean and Baltic Seas, and at least as far east as the White Sea (Snyder & Gooch, 1973). This species is unusually interesting to evolutionary biologists for many reasons, including its high level of shell colour polymorphism. Pettitt (1973a, 1975) and Heller (1975) reported associations between shell colour frequencies in populations and the colour of the rock substrate on which they were found, suggesting selection for cryptic colouration as the cause of the polymorphism. More recently other

0024–4066/90/050003+08 $03.00/0

workers have questioned this explanation, instead speculating that apostatic selection (Atkinson & Warwick, 1983) or selection acting on the pleiotropic effects of shell colour alleles (Raffaelli, 1979) is responsible for maintaining the polymorphism.

This paper reports on a study of associations between shell colour frequencies and rock colour over very short distances at six sites of geological contact between red and grey rock on the Pembroke Peninsula of south-western Wales.

METHODS

The southern Pembroke Peninsula, Dyfed, U.K., was chosen for this study because of its many geological transitions between red Old Red Sandstone and grey Carboniferous limestone or Silurian volcanic rocks (Barrett, 1974). Six zones of contact between red and grey rock were chosen as study sites (Fig. 1). At some of these the rock colour transition occurred cleanly at a single line in a cliff face or rock outcrop; at others the rock was more broken and the transition gradual.

At each site two replicate samples of winkles were collected from 1 m^2 areas 5 m apart on either side of the rock contact and approximately 100 m onto red and grey rock on either side. Locations of the 1 m^2 replicate samples were chosen to match each other as nearly as possible in slope of the rock surface, aspect (direction faced), and especially height in the tide zone, so that they would be ecologically equivalent. Samples were typically taken from steeply sloping or nearly vertical rock surfaces, with the bottom edge of the 1 m^2 at the top of the barnacle line (Lewis, 1966), and from among scattered patches of *Verrucaria*

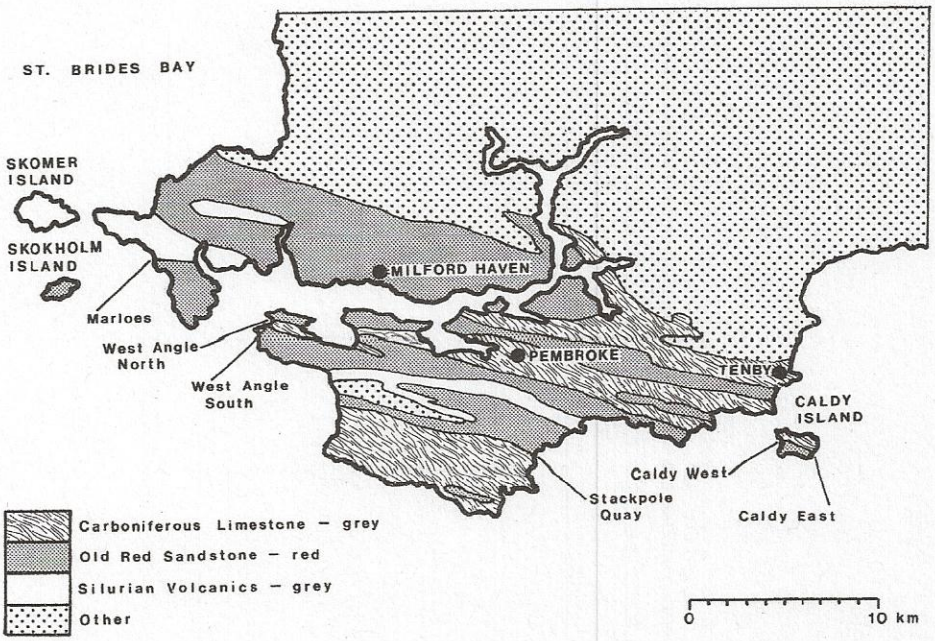

Figure 1. Geological map of southern Pembroke Peninsula showing locations of the six study sites at contacts between red and grey rock.

maura, clumps of *Pelvetia canaliculata,* and scattered *Chthamalus montagui.* Sample sizes from these 1 m^2 areas ranged from 86–208, with a mean of 136 (Table 1). Samples were collected from 18 to 29 June, 1984.

Shell colours of each sample were scored using the system devised by Pettitt (1973b). Ground colour was separated into six categories—brown (B), grey (G), white (W), orange (O), fawn (F), purple (P), and black (BL)—and the presence of bands (B^+), tesselations (T^+), or lines in grooves (LG) were noted. All individuals were scored while alive, and shells were wetted before scoring.

Differences in shell colour frequencies between replicate samples, rock substrate colours, and study sites were tested using G-tests of independence (Sokal & Rohlf, 1987). In these analyses brown and grey shells, which were generally common, were treated as separate categories; all other shell colours, which were generally much less common, were pooled in order to make the expected frequency in each cell greater than 5.

RESULTS

Shell colour differences between replicate samples

Raw shell colour data from all samples are given in Table 1. Thirteen out of eighteen 2×3 G-tests of independence between replicate samples collected 5 m apart showed statistically significant differences in shell colour frequencies. Replicate samples that did not differ significantly occurred at Stackpole Quay on red rock and at the contact zone, at Caldy West at the contact zone, and at Caldy East on both red and grey rock.

Shell colour differences and substrate colour

Shell colour frequencies from snails from red rock, grey rock, and the contact zone were compared using 3×3 G-tests of independence after pooling the replicate samples from each substrate. Because replicates usually differed in shell colour frequency, pooling replicates is a conservatism that should reduce the possibility of spurious associations between shell colour and rock colour. Highly significant associations between shell colour and rock colour were found at all six of the study sites: Marloes—$G=66.7$, $P<0.001$ (all degrees of freedom $=4$); West Angle North—$G=53.8$, $P<0.001$; West Angle South—$G=229.5$, $P<0.001$; Stackpole Quay—$G=79.7$, $P<0.001$; Caldy West—$G=297.4$, $P<0.001$; Caldy East—$G=28.1$, $P<0.001$.

These substrate-shell colour associations are presented in Tables 2 and 3. Brown shells were more common on red rock than on grey rock, with intermediate frequencies at the contact zone, at each site except Stackpole Quay (Table 2). Shell colours at Stackpole Quay were unusual in having a very high frequency of the orange banded morph (Table 1). The ground colour of this morph is orange, with bands of black, brown, purple, purplish grey or grey. At this site the orange banded morph made up 68% of the pooled replicate samples from red sandstone, 57% of the samples from the contact zone, and 30% of the samples from grey limestone. Grey shells were always more common on grey rock than on red rock, with intermediate frequencies at the contact zone (Table 3). Frequencies of shells of colours other than brown or grey generally did

TABLE 1. Shell colour frequencies* from six sites and three different substrate colours at each site

Location	N	B	G	W	O	F	P	BL	B+	T+	LG
	Frequency (%)										
Marloes											
Red:											
Sample 1	108	40.7	1.9	0	0	2.8	2.8	0	1.9	0.9	49.1†
Sample 2	122	73.8	0.8	0	0	0.8	4.1	0	0.8	1.6	18.0
Contact:											
Sample 1	136	53.7	3.7	0	0	0.7	15.4	0	1.5	0	25.0
Sample 2	119	60.5	9.2	0.8	0	4.2	15.1	0	0.8	0.8	8.4
Grey:											
Sample 1	117	54.7	15.4	0	0	0	6.0	8.6	0.9	9.4	5.1
Sample 2	119	45.4	29.4	0	0	0	17.7	5.0	0	0	2.5
W. Angle North											
Red:											
Sample 1	137	61.3	16.1	0	13.9	8.0	0	0	0.7	0	0
Sample 2	140	77.1	10.7	0	5.7	5.0	1.4	0	0	0	0
Contact:											
Sample 1	101	25.7	46.5	0	5.0	20.8	0	2.0	0	0	0
Sample 2	124	75.0	16.1	0	1.6	5.7	0	1.6	0	0	0
Grey:											
Sample 1	155	26.5	48.4	0	0	12.9	0	11.6	0.7	0	0
Sample 2	165	61.2	28.5	0	0.6	7.3	0.6	1.2	0.6	0	0
W. Angle South											
Red:											
Sample 1	156	87.2	3.9	0	1.3	6.4	0	0.6	0.6	0	0
Sample 2	149	94.0	2.0	0	0.7	0.7	0.7	0.7	1.3	0	0
Contact:											
Sample 1	97	21.7	53.6	0	0	20.6	0	4.1	0	0	0
Sample 2	129	68.2	10.1	0	0.8	18.6	0.8	1.6	0	0	0
Grey:											
Sample 1	86	5.8	70.9	0	0	15.1	0	8.1	0	0	0
Sample 2	132	50.0	16.7	0	0.8	30.3	1.5	0.8	0	0	0
Stackpole Quay											
Red:											
Sample 1	196	8.7	13.8	0	0.5	2.0	1.5	0.5	72.5‡	0.5	0
Sample 2	149	7.4	16.1	0.7	1.3	2.0	1.3	0	71.1	0	0
Contact:											
Sample 1	133	12.8	21.8	0	0	1.5	0.8	0	63.2	0	0
Sample 2	148	8.1	31.8	0	0	0.7	1.4	0	58.1	0	0
Grey:											
Sample 1	153	5.2	36.0	0	1.3	2.6	1.3	5.2	48.4	0	0
Sample 2	114	11.4	61.4	0	0.9	4.4	6.1	0.9	14.9	0	0
Caldy West											
Red:											
Sample 1	170	57.6	22.4	1.2	0	4.7	1.8	4.7	5.9	1.8	0
Sample 2	166	39.8	27.7	0.6	0	9.0	4.2	6.0	9.0	3.0	0.6
Contact:											
Sample 1	105	10.5	63.8	7.6	0	8.6	2.9	0	4.8	1.9	0
Sample 2	85	12.9	54.1	14.1	0	3.5	4.7	0	7.1	3.5	0
Grey:											
Sample 1	148	3.4	91.2	0	0	2.0	0.7	1.4	1.4	0	0
Sample 2	188	11.2	79.8	0	0	3.7	0.5	2.7	1.6	0.5	0
Caldy East											
Red:											
Sample 1	121	58.7	12.4	0	1.7	17.4	0.8	0	9.1§	0	0
Sample 2	99	48.5	13.1	0	1.0	32.3	0	0	5.1	0	0
Contact:											
Sample 1	117	43.6	7.7	0	18.8	6.0	0	0	23.9	0	0
Sample 2	140	37.9	30.0	0	8.6	14.3	0	0	9.3	0	0

TABLE 1. *continued*

Location	N	B	G	W	O	F	P	BL	B+	T+	LG
	Frequency (%)										
Caldy East											
Grey:											
Sample 1	150	28.7	30.7	0	10.7	11.3	2.0	0	13.3	3.3	0
Sample 2	208	35.6	19.2	0	12.0	11.5	1.9	0.5	17.3	1.9	0
Total	4882	39.7	27.7	0.5	2.5	7.8	2.6	1.9	13.8	0.8	2.6

*Abbreviations for shell colours: B=brown; G=grey; W=white; O=orange; F=fawn; P=purple; BL=black; B+=banded, all ground colours; T+=tesselated, all ground colours; LG=lines in grooves, all ground colours. Frequencies sum to 100% except for minor rounding differences.

†Lines-in-grooves (LG) morph at Marloes mostly very light grey ground colour with purple lines.

‡Banded morph at Stackpole Quay mostly orange ground colour with black, brown, purple, purplish grey, or grey bands.

§Banded morph at Caldy East mostly orange ground colour with black, brown, purple, purplish grey or grey bands.

not show consistent associations with substrate colour. For example, fawn shells were more common on red rock than on grey at Caldy Island, more common on grey rock than on red at West Angle South and Stackpole Quay, and more common at the contact zone than on either side at Marloes and West Angle North (Table 1). Snails with lines in grooves were common at Marloes and virtually absent elsewhere (Table 1).

TABLE 2. Frequencies of brown shells on three substrates (replicate samples pooled)

Location	Red rock	Contact zone	Grey rock
	Frequency of brown shells (%)		
Marloes	58.3	56.9	50.0
West Angle North	69.3	52.9	44.4
West Angle South	90.5	48.2	32.6
Stackpole Quay	8.4	10.3	7.9
Caldy West	48.8	10.5	7.7
Caldy East	54.1	40.5	32.7
Pooled	53.4	36.7	28.5

TABLE 3. Frequencies of grey shells on three substrates (replicate samples pooled)

Location	Red rock	Contact zone	Grey rock
	Frequency of grey shells (%)		
Marloes	1.3	6.3	22.5
West Angle North	13.4	29.8	38.1
West Angle South	3.0	28.8	38.1
Stackpole Quay	14.8	27.0	46.8
Caldy West	24.7	59.5	84.8
Caldy East	12.7	19.8	24.3
Pooled	12.3	27.1	43.5

Shell colour differences between sites

Shell colour frequencies in samples from rock of the same colour at the six study sites were compared using 3×6 G-tests of independence. There were highly significant differences in colour frequencies on each of the three substrates: on red rock ($G=659.7$, d.f.$=10$, $P<0.001$), at the contact zone ($G=374.6$, d.f.$=10$, $P<0.001$), and on grey rock ($G=494.6$, d.f.$=10$, $P<0.001$).

DISCUSSION

Strong and consistent matching of shell and substrate colour was found in this study, supporting the hypothesis that visually-hunting predators are in part responsible for maintaining shell colour polymorphism. Grey shells were always more common on grey than on red rock, brown shells were more common on red than on grey rock at five of the six study sites, and colour frequencies at the contact zone were intermediate.

The one exception to the general observation that brown shells were more common on red than on grey rock, found at Stackpole Quay, can also be explained as the result of selection for substrate colour matching. At this site the most common shell colour was orange with bands of black, brown, purple, purplish grey or grey. These shells gave an overall impression of reddish brown colour when either wet or dry, and were most camouflaged on red sandstone. Frequencies of the orange banded morph were 68% on red rock, 57% at the contact zone, and 30% on grey rock.

Both Heller (1975) and Pettitt (1973a) found associations between shell colour frequencies and substrate colour, and proposed visual selection as a cause of the polymorphism. The results of this study support the hypothesis of these authors, and extend the findings to a much smaller spatial scale.

A number of studies of intertidal gastropods other than *L. saxatalis* have found evidence that visually-hunting predators help maintain shell colour polymorphisms (Byers, 1989; Hoagland, 1977; Hughes & Jones, 1985; Hughes & Mather, 1986; Reimchen, 1979).

Interpretation of both the study reported here and earlier work on shell colour polymorphism is complicated by the fact that the ovoviviparous *L. saxatilis* has an oviparous sibling species, *L. arcana* (Hannaford Ellis, 1978), which is generally indistinguishable in the field. In some locations shell colour frequencies differ between these species (Atkinson & Warwick, 1983; Hannaford Ellis, 1985).

Littorina saxatilis and *L. arcana* were not distinguished in this study. However, if the strong and consistent matching of shell colour and substrate colour reported here was an artifact of failure to separate *L. arcana* and *L. saxatilis* and not the result of selection for crypsis, one of the species would consistently have to occur at higher frequencies on one of the substrates, and strong and consistent differences in shell colour would have to exist between the species. The first requirement is unlikely to have been met because of the ecological similarity of the sampling sites on the different substrates (see Methods), although subtle ecological differences can not be ruled out. The second requirement is unlikely to have been met because Atkinson & Warwick (1983) found that shell colour differences between the species were not consistent—higher frequencies of brown

shells can occur in *L. arcana* at one site, but in *L. saxatilis* at another, for example. Nor were the shell colour differences between the two species reported by Atkinson & Warwick (1983) as large as the differences between winkles from red or grey rock found in this study.

Most of the work on shell colour polymorphism in *L. saxatilis* assumes that this character is under genetic control. This assumption is probably correct given the unequivocal demonstration of a genetic basis for shell colour in *L. obtusata* and *L. mariae* (Reimchen, 1979), and unpublished evidence from studies by T. Warwick, A. J. Knight and R. D. Ward that at least some of the colours and banding patterns of *L. saxatilis* are genetically determined (R. D. Ward, personal communication).

Shell colour frequencies often differed over a distance of 5 m. Such microgeographic differentiation is undoubtedly made possible by the reproductive biology of this species—*L. saxatilis* has internal fertilization and lacks a planktonic larval stage. It is also undoubtedly due to the low mobility of adults, in the range of 1–4 m in 3 months (Janson, 1983). These microgeographic differences are not due to selection for substrate colour matching, because they occur between replicate samples taken from rock of the same colour. Because replicate samples were collected in such a way as to minimize ecological differences (see Methods), these differences are not likely to be due to other kinds of selective forces proposed to influence shell colour in this species—apostatic selection (Atkinson & Warwick, 1983) or selection on pleiotropic effects of shell colour alleles or closely-linked loci (Raffaelli, 1979)—or selection based on the thermoregulatory effects of shell colour as proposed by Etter (1988) for *Nucella lapillus*. The most likely explanation of these differences is that they are the result of stochastic processes operating in a species with very restricted gene flow. Janson & Ward (1984) found differences in allozyme frequencies over distances as short as 4 m in this species.

The fact that there were significant differences in shell colour frequency in populations from rocks of the same colour at the six sites indicates that selection for crypsis is not the only factor controlling shell colour polymorphism, especially on a macrogeographic scale. Selection for crypsis does not appear to explain the high frequency of snails with lines in grooves at Marloes, of banded snails at Stackpole Quay, and of fawn snails at the Angle and Caldy sites, for example. At a larger spatial scale, apostatic selection, as proposed by Atkinson & Warwick (1983), or selection acting on the pleiotropic effects of shell colour alleles or on closely-linked loci, as suggested by Raffaelli (1979), could be important factors as well. Selection of shell colour based on its influence on heat stress has been demonstrated in the intertidal snail *Nucella lapillus* (Etter, 1988), and such selection might also be a factor in *L. saxatilis*. As in *Cepaea* (Jones, Leith & Rawlings, 1977), shell colour polymorphism in *Littorina* is undoubtedly the result of complex interactions between many selective forces.

ACKNOWLEDGEMENTS

My warmest thanks go to J. Butler and A. Bryn for assistance in the field; to J. A. Beardmore, B. L. James, K. Johannesson, A. J. Knight, D. Raffaelli, R. D. Ward and T. Warwick for helpful discussions while this work was in progress; and to the winkles!

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