

TEMPERATURE-RELATED DIVERSITY OF SHELL COLOUR IN THE INTERTIDAL GASTROPOD *BATILLARIA*

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ABSTRACT

The intertidal snail *Batillaria* exhibits remarkable variation in the shell colour within and among populations. Field study was conducted to determine the factors in maintaining observed shell colour polymorphism. Geographical variations in shell colour polymorphisms in *B. attramentaria* were significantly correlated with the temperature of the locality of the population. Darker morphs were predominant in colder regions, whereas lighter morphs increase their proportion in warmer regions. A consistent association was also found in *B. multiformis* that co-existed with *B. attramentaria*. Strong predatory pressure imposed by digenean trematode parasites was observed in *B. attramentaria*. However, it is unlikely to affect the colour variations, because no correlation exists between colour morphs and trematode parasitism. Although visual selection may also contribute to colour variation in *Batillaria*, no evidence is found for the existence of visual predators that affect colour patterns of these snails. The deficit of variation in cold regions is possibly due to selection against brighter morphs, because bright colours reflect heat. Although dark shells absorb sunlight and may therefore be exposed to the risks of overheating and drying up in a hot habitat, the darkest morph was frequently observed in the warmer regions, suggesting that physical selection on the colour morphs can be relaxed in the warmer environment. Our results suggest that climatic selection is one of the significant factors maintaining shell colour polymorphism in these intertidal snails.

INTRODUCTION

Shell colour polymorphism is common in intertidal and terrestrial gastropods (e.g. Cain & Sheppard, 1954; Berry & Crothers, 1974; Raffaelli, 1982; Etter, 1988; Sokolova & Berger, 2000). These colour polymorphisms are thought to be maintained by natural selection (Heller, 1981; Hughes & Mather, 1986; Etter, 1988). In many cases, shell colours are associated with physical selection, due to climate, salinity and desiccation (Heath, 1975; Cook & Freeman, 1986; Etter, 1988; Sokolova & Berger, 2000); they are also related to predation (Cain & Sheppard, 1954; Heller, 1975; Hughes & Mather, 1986). For example, light shell morphs can be less likely to reach lethal temperatures in warm, sunny habitats, whereas dark morphs may have more of an advantage in a cool habitat since they would warm more rapidly by insolation than light shells (Jones, 1973; Heath, 1975; Stine, 1989). Additionally, shell colours that are similar to the background coloration may not be discovered by predators and are thus probably favoured through visual selection (Hoagland, 1977; Heller, 1981). Therefore, patterns of colour polymorphism are frequently associated with the biotic and physical stresses in the habitat. In addition, genetic processes, including genetic drift, the founder effect and population bottlenecks may affect the colour variation in a population, since these processes could exhaust the genetic variation in a colour polymorphism (Roulin, 2004).

The mud snails, *Batillaria attramentaria* (= *B. cumingi*) and *B. multiformis* are usually the most abundant macroinvertebrates in salt marshes and mudflats on the northeast coast of Asia (Hasegawa, 2000). Based on the phylogenetic study of Kojima *et al.* (2001), these two species are phylogenetically closely related and derived from *B. zonalis*, which is distributed on the southern coast of Japan, Korea and China. Although both *B. attramentaria* and *B. multiformis* exhibit variability in shell

colour pattern, the factors that determine and maintain the colour variations have scarcely been studied for either snail. Intertidal snails are usually preyed upon by several types of predators, such as crabs, fish, birds and mammals (e.g. Etter, 1988), and such predatory selection can markedly affect the shell colour polymorphisms in the snails (Hughes & Mather, 1986). However, no shell fragments of *Batillaria* have been found in bird faecal material, suggesting that birds do not prey on these species (Whitlatch, 1974). Furthermore, other predators, including crabs and fish, were never observed preying upon these snails (Whitlatch, 1974; O. Miura, unpubl.). These observations suggest that visual predators do not impose a severe selective pressure on the *Batillaria* species. The main predators of *Batillaria* are likely to be parasites (Whitlatch, 1974). *Batillaria attramentaria* is infected, as a first intermediate host, by a suite of eight morphologically distinct digenean trematode species (Ito, 1957; Shimura & Ito, 1980; Harada, 1989; Harada & Suguri, 1989; R.F. Hechinger, unpubl.), and *B. multiformis* is infected by at least three species of trematode parasites (O. Miura, unpubl.). All of these trematodes induce complete castration of the host snail. Since the prevalence of these trematodes can sometimes reach more than 50–90% on the northern coast of Japan (Miura *et al.*, 2005, 2006), the predatory pressure derived from parasitism could affect colour polymorphisms of *Batillaria* species if resistance genes against trematode infection are linked to the genes for the colour polymorphisms.

In this study, we test several hypotheses addressing how colour variation is maintained in *Batillaria*. We collected *Batillaria* snails from the north to south coasts of Japan to investigate the effect of climatic selection on shell colour polymorphism. Additionally, we investigated trematode parasitism to examine the relation between predatory pressure and colour variation. Further, the effects of population bottlenecks on shell colour variation are discussed using a published genetic study of *Batillaria* snails. Finally, we compare the colour polymorphism patterns of two related species to determine the generality of our findings.

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MATERIAL AND METHODS

Samples were collected from 13 muddy shores and estuarine sites for *Batillaria atramentaria* and eight sites for *B. multiformis* at daytime low tides (Fig. 1). Because most *Batillaria* are found on the surface of the mud, spending a long time out of water, they are exposed directly to solar radiation for a long period. Snails were identified following Adachi & Wada (1998). To collect *B. atramentaria* snails randomly, we threw a 15 cm × 30 cm quadrat in several directions and collected all snails within the quadrat. This operation was repeated several times to collect more than 100 snails from each site in order to examine the colour variation of the shell, and to investigate parasitism. Since the density of *B. multiformis* in the field was not high at most of the study sites, we collected this species without a quadrat. More than 50 *B. multiformis* snails were collected from the mud flat at each site. The shell colour patterns were recorded individually for both species. The temperature records of the nearest city were obtained at the website of the Japan Meteorological Agency (<http://www.jma.go.jp/jma/indexe.html>), and were used to investigate the correlation between climate and colour polymorphism. The colour diversity (H) was measured

using Shannon's diversity index, calculated as

$$H = - \sum P_i \ln P_i$$

where P_i is the frequency of a given colour pattern. Averages of the extreme low temperatures for the past 20-year period (1986–2005) were compared with the shell colour diversity. A linear regression model was then used to test whether climatic selection (temperature) was correlated with shell colour polymorphism, and also to test whether variation in shell patterns of two *Batillaria* snails were correlated with each other.

After morphological observations, the snails were dissected under a stereomicroscope and trematode species were identified based on previous work on cercariae from *B. atramentaria* (Ito, 1957; Shimura & Ito, 1980; Harada, 1989; Harada & Suguri, 1989; R.F. Hechinger, unpubl.). Since no description of cercariae from *B. multiformis* was available, we classified the trematodes from *B. multiformis* at the family level. We examined the prevalence of trematode infection in each shell colour pattern. Independence between shell colour patterns and parasitism was tested using the chi-square statistics.

RESULTS

We found six shell colour patterns for *Batillaria atramentaria*, and two patterns for *B. multiformis* (see Fig. 1). A dark unbanded shell (D type) and a shell with a white line on the upper side of each whorl (UL type) were common patterns in both species. Four colour patterns were found only in *B. atramentaria*: shells with a white line on the lower side of each whorl (LL type), shells with white lines on both the upper and lower sides of each whorl (DL type), shells with multiple white lines (ML type) and white unbanded shells (W type). These colour patterns are shown in Figure 2A for *B. atramentaria* and Figure 2B for *B. multiformis*.

For *B. atramentaria*, the sites on the north coast of Japan (Toga Bay, Yamada Bay, Mangoku Bay and Matsushima Bay) were mostly occupied by the D type, whereas several colour patterns were observed at Kasuga River, Hiroshima Bay and Ariake Bay, sites in the southern part of Japan (Fig. 1A). The diversity index was significantly correlated with the averages of the extremely low temperatures (Fig. 3A, $R^2 = 0.60$, $F_{1,11} = 6.31$, $P < 0.05$). A consistent association was found in *B. multiformis*; D type shells were frequently observed on the north coast of Japan,

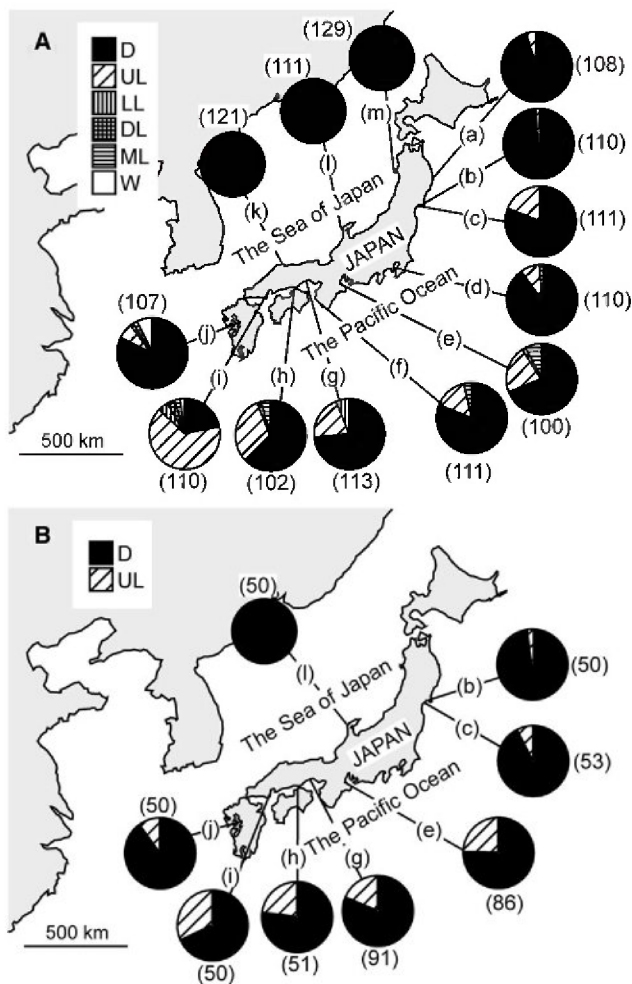


Figure 1. Distribution of colour patterns for *Batillaria atramentaria* (A), and for *B. multiformis* (B). Sample size is shown in parentheses. Letters indicate sampling sites: (a) Yamada Bay, (b) Mangoku Bay, (c) Matsushima Bay, (d) Obitsu River, (e) Kumode River, (f) Tanabe Bay, (g) Ibo River, (h) Kasuga River, (i) Hiroshima Bay, (j) Ariakekai, (k) Nanarui Bay, (l) Minatsuki Bay, (m) Toga Bay.

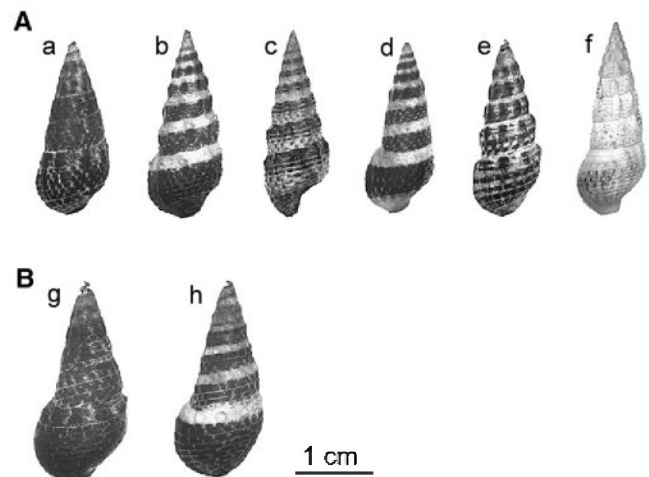


Figure 2. A. Colour patterns for *Batillaria atramentaria*: (a) D type, (b) UL type, (c) LL type, (d) DL type, (e) ML type, (f) W type. B. Colour patterns for *B. multiformis*: (g) D type, (h) UL type.

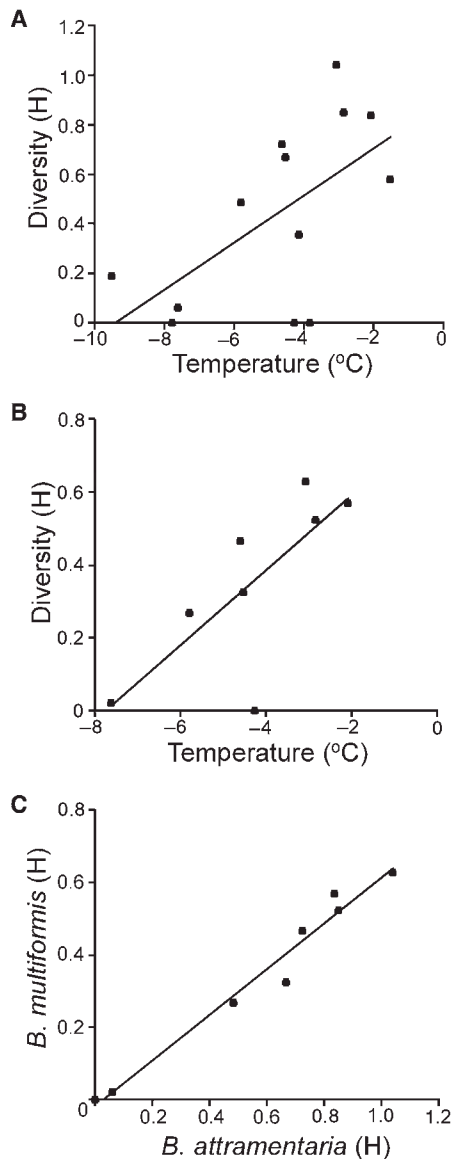


Figure 3. Positive correlations between colour diversity (H) and the averages of the extreme low temperature for 20 years for *Batillaria attramentaria* (A) and for *B. multiformis* (B), and between colour diversities for the two species (C).

while the UL type shells frequently appeared on the south coast (Fig. 1B). Frequencies of shell colour patterns are shown in Table 1. The colour diversity index of *B. multiformis* was also significantly correlated with the extreme low temperatures (Fig. 3B; $R^2 = 0.76$, $F_{1,6} = 7.98$, $P < 0.05$). Importantly, there were strong correlations between colour diversity of *B. attramentaria* and that of *B. multiformis* (Fig. 3C, $R^2 = 0.99$, $F_{1,6} = 214.55$, $P < 0.001$). The colour diversity is clearly different between the Pacific Ocean and the Sea of Japan coasts. While several colour variations were observed on the Pacific coast of Japan, only D type shells appeared on the Japanese coast of the Sea of Japan. This pattern was observed for both *B. attramentaria* and *B. multiformis* (Fig. 1A, B).

Of the eight trematode species reportedly from *B. attramentaria* (Ito, 1957; Shimura & Ito, 1980; Harada, 1989; Harada & Suguri, 1989; R.F. Hechinger, unpubl.), we found *Cercaria batillariae* (60.5% of all infections) and *C. hosoumininae* (27.5%) at high frequency. We found a high level of trematode prevalence

Table 1. Frequencies of shell colour patterns in each population of *Batillaria*. Sampling sites refer to characters in Figure 1. Abbreviations of each colour patterns are explained in the text.

Site	<i>B. attramentaria</i>						<i>B. multiformis</i>	
	D	UL	LL	DL	ML	W	D	UL
a	103	5						
b	109	1					49	1
c	90	21					49	4
d	99	10				1		
e	69	22	1		8		64	22
f	90	17			4			
g	83	23			7		75	16
h	64	32	1		5		40	11
i	24	72	5	4	3	2	34	16
j	88	8		1	2	8	45	5
k	121							
l	111						50	
m	129							

Table 2. Trematode prevalence for each colour pattern in *B. attramentaria* and *B. multiformis*. Number of individuals for each colour patterns is also shown.

Species	Colour	N	Prevalence (%)
<i>B. attramentaria</i>	D	1180	22.7
	UL	211	21.8
	LL	7	28.6
	DL	5	0.0
	ML	30	26.7
	W	10	0.0
<i>B. multiformis</i>	D	404	3.7
	UL	7	7.9

in *B. attramentaria* (22.5% of all individuals). These trematodes randomly infect snails of each shell pattern, since independence between the colour patterns and parasitism was not rejected ($n = 1443$, $\chi^2 = 4.90$, $P = 0.43$ Table 2). Three undescribed cercariae were found in *B. multiformis* (the heterophyid cercaria, microphallid cercaria and philophthalmid cercaria). The trematode prevalence in *B. multiformis* was relatively low at all sites (4.4% of all individuals). Further, independence between the colour variation and parasitism was not rejected for *B. multiformis* ($n = 481$, $\chi^2 = 1.69$, $P = 0.19$, Table 2).

DISCUSSION

The intertidal zone is a habitat with strong physical stresses. During the period of tidal emersion, fluctuations of temperature, desiccation and osmotic stress are routinely imposed on the intertidal area; such stresses may profoundly affect the habitat use and mortality of intertidal organisms (Newell, 1979). Globally, the latitudinal pattern is related to the physical stress due to climatic changes along the latitudinal gradient. The average temperature, as well as the extreme high and low temperatures, should change with latitude. High temperature induces high desiccation stress and mortality caused by overheating in regions of lower latitude, whereas high mortality can be caused by freezing in areas at higher latitude (Murphy & McCausland, 1980; Hylleberg & Siegismund, 1987; Etter,

1988; Sokolova & Berger, 2000). The combined effects of tidal emersion and climatic factors can strongly affect the colour polymorphisms of intertidal organisms.

Several authors have pointed out that differences exist in the ecology of *B. atramentaria*, *B. multififormis* and their related species (Adachi & Wada, 1998; Yamamoto & Wada, 1999; Wakamatsu & Tomiyama, 2000). *Batillaria atramentaria* generally prefers to live slightly lower on the shore than *B. multififormis* (Adachi & Wada, 1998). Another cerithioidean species, *Cerithiidea cingulata*, was observed lower than the *Batillaria* species, whereas *C. rhizophorarum* was frequently found in the upper tidal zone (Yamamoto & Wada, 1999). These habitat preferences may reflect tolerance to physical stresses such as low salinity and desiccation (Yamamoto & Wada, 1999; Wakamatsu & Tomiyama, 2000). Despite its potential importance, the effects of the latitudinal gradient on the distribution of intertidal organisms have been little studied. The Japanese islands span a wide range of latitude and have long coastlines from north to south; they include sub-arctic, temperate and subtropical climates. The distributions of the four *Batillaria* species reported from Japan are likely to be affected by climatic factors. *Batillaria zonalis* and *B. flectosiphonata* are mainly distributed in the subtropical areas of Japan (Kojima *et al.*, 2003, 2005). *Batillaria multififormis* is distributed in the temperate areas of Japan, whereas *B. atramentaria* primarily ranges from temperate to sub-arctic areas of Japan (Kojima *et al.*, 2004, 2005). These differences in distribution likely reflect tolerances to extreme temperatures. On the northern coast of Japan, the salt marshes are frequently covered by snow in the winter, and temperatures fall below freezing. In contrast, the temperature on the south coast sometimes exceeds 40°C in summer. Therefore, tolerance to extreme temperature can profoundly impact with the distribution and thus the mortality of these intertidal snails.

Among the physical stresses in the intertidal zone (e.g. temperature, salinity and hypoxia), temperature is likely to be the most significant factor for shell colour variations, because shell colour patterns are frequently correlated with tissue temperatures of intertidal gastropods (Heath, 1975; Cook & Freeman, 1986; Etter, 1988). We found a strong correlation between the colour patterns and low temperatures for *B. atramentaria* (Fig. 3A). The D type (blackish shell) is dominant on the north coast (and hence cold region) of Japan (Fig. 1A). Although the relationship between shell colour patterns and body temperature of *Batillaria* snails has not yet been examined, other studies of intertidal gastropods have demonstrated that dark shells could be adapted to cold temperature, since dark shells are more rapidly heated by solar radiation, whereas light shells could be adapted to hot temperature, because they may be less likely to reach lethal temperatures in sunlight (Jones, 1973; Heath, 1975; Cook & Freeman, 1986; Stine, 1989). Thus, the D type shell (the darkest shell in *Batillaria*) may have a selective advantage on the north coast of Japan, and is the dominant morph in this region. Strong climatic selection is probably imposed on the whitish shells (UL, LL, DL, ML and W type) in the north (Fig. 1A). This climatic-selection hypothesis is supported by the parallel variation in the two *Batillaria* species; the colour diversity index was decreased with extreme low temperatures for both *B. atramentaria* and *B. multififormis* (Fig. 3A, B). This consistency suggests that physical stress (low temperature) restricts the colour diversity of both species on the north coast. Importantly, the colour diversity of each of these related snails was strongly correlated with each other (Fig. 3C), suggesting the climatic selection could simultaneously affect the distribution and frequency of the colour morphs, and synchronize the shell colour diversity of these snails. Although *B. atramentaria* occurs lower on the shore than *B. multififormis*, there is high habitat overlap, and there seems to be no difference in the influence of solar radiation on these species. Our work is in

agreement with several earlier studies that have demonstrated a correlation between the geographic distribution of colour patterns and climate (Jones, 1973; Bantock, 1980; Heller, 1981). As in our findings, they demonstrated that dark shells are more prevalent in cool habitats, whereas whitish shells are frequent in warmer regions.

Studies on intertidal snails have suggested that dark morphs have a disadvantage in hot habitats, since they more quickly become hot and dried in sunlight (Etter, 1988). Additionally, light shells can be exposed to risks in cold habitats since they cannot be warmed too quickly (Sokolova & Berger, 2000). One might expect high colour diversity in the mid-temperature regions, such as the central coast of Japan, because the physical selection on particular shell colours would be relaxed, and hence, shell colours would be free to vary. However, our observations were inconsistent with this expectation. High colour diversity was observed on the south coast of Japan for both species (Fig. 1). This might be because physical selection on *B. atramentaria* was high in the north but only moderate on the south coast. The shell morphs with a white background or white lines (UL, LL, DL, ML and W type) were mostly absent from the north coast (contributing only 5.9% of total colour morphs in the northern population), suggesting that a high level of physical selection (low temperature) was imposed on the light morphs on the north coast. However, the dark shell morph (D type) frequently appeared on the south coast (contributing 55.2% of total colour morphs in the southern population), suggesting that the temperature did not rise to a lethal level for the dark colour morph on the south coast. Thus, physical selection on the shell colour morphs may be relaxed on the south coast, which is likely to increase the selectively neutral colour morphs in the warmer habitat, and induce a high colour diversity on the south coast of Japan. Although there is very little evidence of predation based on appearance (see below), apostatic selection by visual predation may also increase polymorphisms and frequency of bright colour, because if climatic selection is relaxed, selection for being different (i.e. apostatic selection) has a chance to operate. Further studies of visual predation on *Batillaria* are needed to evaluate this possibility.

Predatory selection by visual predators can markedly affect shell colour polymorphisms in snails (Hughes & Mather, 1986). *Batillaria* snails possibly are prey items for shore birds, intertidal crabs and fish, because *Batillaria* are the most abundant snail species in the intertidal zone. However, no shell fragments of *Batillaria* were observed in bird faecal material (Whitlatch, 1974), and there are no observations of intertidal crabs and fish preying upon these snails (Whitlatch, 1974; O. Miura, unpubl.). Although the available records and our observations suggest that visual predators are unlikely to impose a severe selective pressure on the *Batillaria* species, it cannot be concluded that visual predators have no effect, because evidence of predation is not always easy to detect, and low levels of selective predation over long periods of time could be effective in modifying colour patterns. Thus, careful research on the predators of *Batillaria* is needed to investigate the relation between selection by visual predators and shell colour variation. In contrast, parasitism clearly imposes severe predatory pressure on the snails because of its high prevalence (Whitlatch, 1974), especially in northern Japan (Miura *et al.*, 2005). It is possible that trematode infection affects the colour polymorphism if resistance genes against infection are directly linked with the genes controlling colour. We found a high level of trematode infection in *B. atramentaria* but a lower rate in *B. multififormis* (Table 2). This difference could be explained by different adaptation patterns of parasites to these two host snails or different host specificities. If some colour patterns had a stronger resistance than other against trematode infections,

the predatory pressure imposed by parasitism could contribute to the pattern of the colour polymorphism. However, we found no significant correlation between parasitism and colour morphs for either *B. attramentaria* or *B. multiformis*. Thus, it is unlikely that parasitism affects colour variation, and it may not contribute to the maintenance of colour polymorphisms in these *Batillaria* species.

As another mechanism, population bottlenecks can effectively reduce colour variation (Roulin, 2004). Using the population genetics approach, Kojima *et al.* (2004) suggested that *B. attramentaria* in the Sea of Japan was probably isolated during the last glacial period, and physical stresses, such as low salinity, anoxia and decreased water temperature imposed a strong population bottleneck on the snails. Due to this bottleneck event, the genetic variation of the *B. attramentaria* populations in the Sea of Japan was substantially lower than on the Pacific side (Kojima *et al.*, 2004). We found a high level of colour variation in *B. attramentaria* on the Pacific side, while only the D type appeared on the northern and southern coasts of the Sea of Japan (Fig. 1). This reduced colour variation corresponds to the bottleneck patterns and, thus, the population bottlenecks may have results in the loss of genetic variation in the colour polymorphism. A similar pattern was observed in *B. multiformis*, but the role of population bottleneck is unclear, because there are as yet no genetic studies in this species.

In conclusion, we found that climatic selection profoundly contributes to maintaining the shell colour polymorphisms in the *Batillaria* snails. In addition, the population histories, including past population fluctuation and population bottlenecks, could have reduced the shell colour diversity. Although predatory selection can have a remarkable impact on colour variation, absence of visual predators, as well as the lack of a significant association between colour morphs and parasitism suggests that predatory pressure contributes little to the colour polymorphism of the two *Batillaria* species. Importantly, climatic selection appears to affect the colour polymorphism of two *Batillaria* species, and is consistent with geographical patterns in other colour polymorphisms. We expect that other snails may exhibit consistent associations as a result of climatic selection, dark morphs dominating in cold regions, while several selectively neutral morphs appear in warmer regions.

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