

Shell Color Polymorphism in Populations of the Intertidal Gastropod *Nerita plicata* (L., 1758) from the Cook Islands

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Abstract

The gastropod *Nerita plicata* is widely distributed across the Indo-Pacific Ocean's upper intertidal zone and exhibits shell color polymorphism. This study examined *Nerita plicata*'s shell color diversity according to substrate type (i.e., basalt or beach rock). This study was performed on the Rarotonga (in August 2008 and August 2018) and Aitutaki (September 2009) Islands, which belong to the Cook Islands. Three different shell color morphs (fully white, partially black-spotted, and fully black-spotted) were recorded on basalt and beach rocks with a different frequency of distribution in the two habitats. The frequency of shell color distribution in *N. plicata* is closely related to the type of habitat, with white shells occurring more frequently in the light-colored beach rocks than in the dark-colored basalt rocks. The relationship between shell color polymorphism and environmental and genetic factors is discussed in this study, as well as the possible role of humans in habitat changes that drive shell color polymorphisms.

Key words: basalt, beach rock, color polymorphism, Indo-Pacific, *Nerita plicata*, substrate

Introduction

Polymorphisms can be driven both by environmental fluctuations and genetic diversity (FALCONER and MACKAY 1996). In marine gastropods from intertidal regions, shell shape and color pattern polymorphisms are common (LITTLE and KITCHING 1996, JOHANNESSON 2016). Intertidal gastropods live in a variable habitat, influenced by many environmental factors, such as temperature, salinity, wave action, prey type, desiccation, and predation risk (HUGHES 1986, LITTLE and KITCHING 1996). The juvenile muricid snail *Concholepas concholepas*, for example, exhibits shell color plasticity according to the presence of different prey species (MANRÍQUEZ *et al.* 2009). Visual selection by predators has been suggested as a driving force of shell color polymorphism in the intertidal snails *Littorina saxatilis* (BYERS 1990) and *Littorina filose* (MCKILLUP and MCKILLUP 2002). Distinct shell colors and morphologies across populations are a result of various genetic factors. (e.g. JOHANNESSON and JOHANNESSON 1996).

The gastropod *Nerita plicata*, which dwells on the rocky shores of the Indo-Pacific Ocean's upper intertidal zone, exhibits shell color polymorphism (ABBOTT and DANCE 1985, NEVILLE 2003). This species inhabits substrates with different background colors, such as beach rocks, limestone, and basalt rocks (VERMEIJ 1971a).

The Cook Islands, located in the tropical Pacific Ocean, comprise volcanic islands and atolls. The islands contain several tropical intertidal substrates of different colors, such as basalt rock (black), limestone (white/gray), and beach rock (white/gray). The present study aimed to investigate the shell color polymorphism of *N. plicata* on different substrate types in two of the Cook Islands. Also discussed is the underlying mechanism of shell color polymorphism in this species.

Materials and Methods

Study Sites

This study was performed in two of the Cook Islands: Rarotonga Island (21°14'54" S/159°47'7" W), during August 2008 and August 2018; and Aitutaki Island (18°51'00" S/159°45'0" W), during September 2009 (Fig.1). Rarotonga is a volcanic island with both beach and basalt rocks scattered throughout its sandy beaches, and Aitutaki is a lagoon island located approximately 260 km north of Rarotonga Island. Aitutaki presents basalt rocks scattered throughout its beaches, whereas its north shore has many beach rocks. In Rarotonga Island, research stations were located at four sites containing beach rocks (stations a, c, e, and f) and at three sites containing basalt rocks (stations b, d, and g). In Aitutaki Island, research stations were located at four sites containing basalt rocks (stations h, i, j, and l) and one site containing beach rocks (station k) (Fig.1). *N.*

plicata's shell color polymorphism was studied on seven stations (stations a, b, c, d, e, f, and g) in Rarotonga Island in 2008, five stations (stations h, i, j, k and l) in Aitutaki Island in 2009, and on two stations (stations e and d) again in Rarotonga Island in 2018.

In *N. plicata*'s existing sites, salinity and air and water temperature were measured using an environmental measurement device (YSI 63/ Ohio 45387 USA) during daylight hours from 10:00 to 15:00, on 25 August 2008 on Rarotonga Island (station b) and on 21-24 September 2009 on Aitutaki Island (stations h, k, and l) (Fig. 1). In both islands the air and water temperatures ranged from 24°C-30°C. Salinity was approximately 36‰ in stations b, h, and k, which faced open sea, and 25‰ in station l, located in Aitutaki Island's interior lagoon.

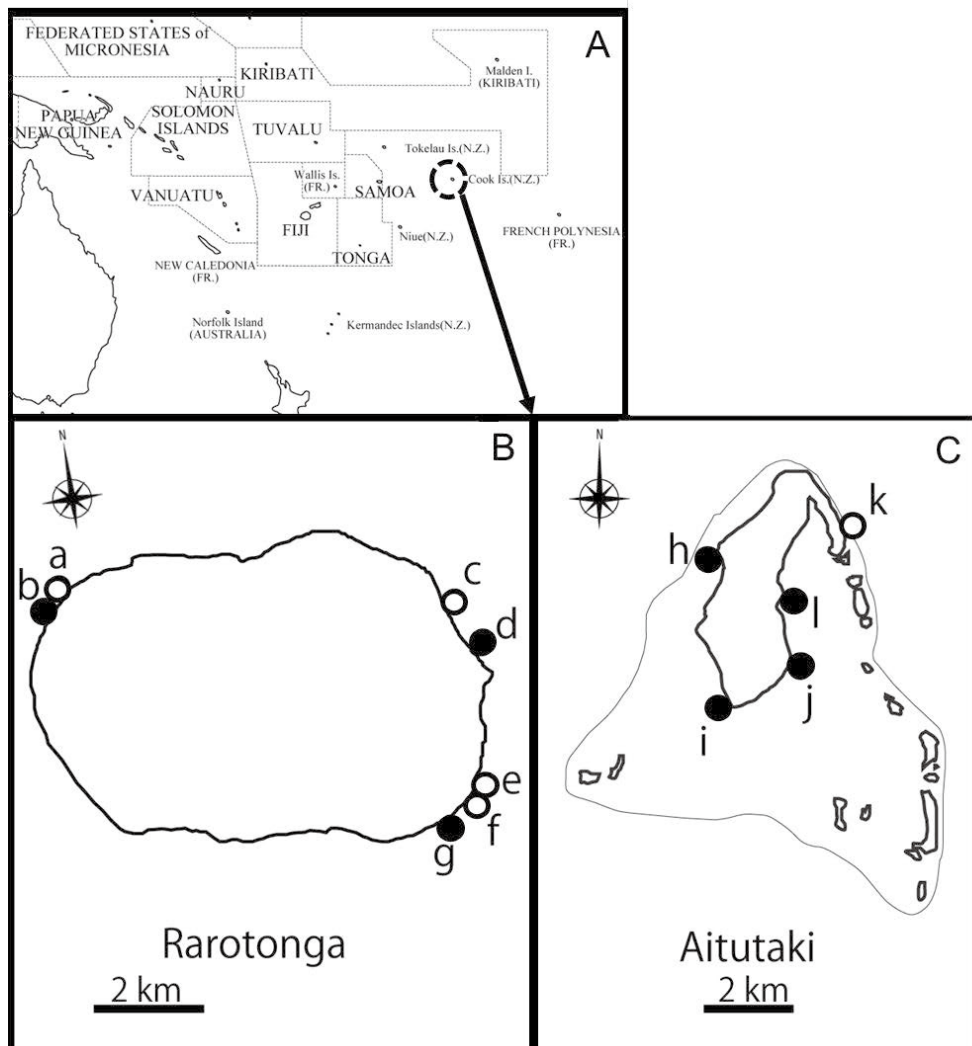


Fig.1. Research stations in (A) the Cook Islands, (B) Rarotonga Island, and (C) Aitutaki Islands. Substrates were basalt rock (black circles: stations b, d, g, h, i, j, and l) and beach rock (white circles: stations a, c, e, f, and k). The narrow line in C indicates the reef edge.

Shell color polymorphism

Over 30 individuals of *N. plicata* (shell length > 15 mm) were collected from each station. The specimens were fixed with 70% ethyl alcohol, and transported to the laboratory for further examination of shell color patterns. Shell surfaces were carefully observed, and the features of these surfaces were recorded.

Shell pattern distribution was compared between different islands with the same substrate, between different substrates on the same island, and between 2008 and 2018 on the same substrate (stations e or d). Statistical analysis was performed using logistic regression. All statistical tests were performed using SPSS version 25.

Results

Three shell color morphs were observed within the study areas: fully white (Fig. 2A and D); partially black-spotted (Fig. 2B and E); and fully black-spotted (Fig. 2C and F). The fully white morph was defined as a completely white from the outer lip to the apex. The partially black-spotted morph was defined as having discontinuous or incomplete small black spots pattern on the shell surface and the fully black-spotted

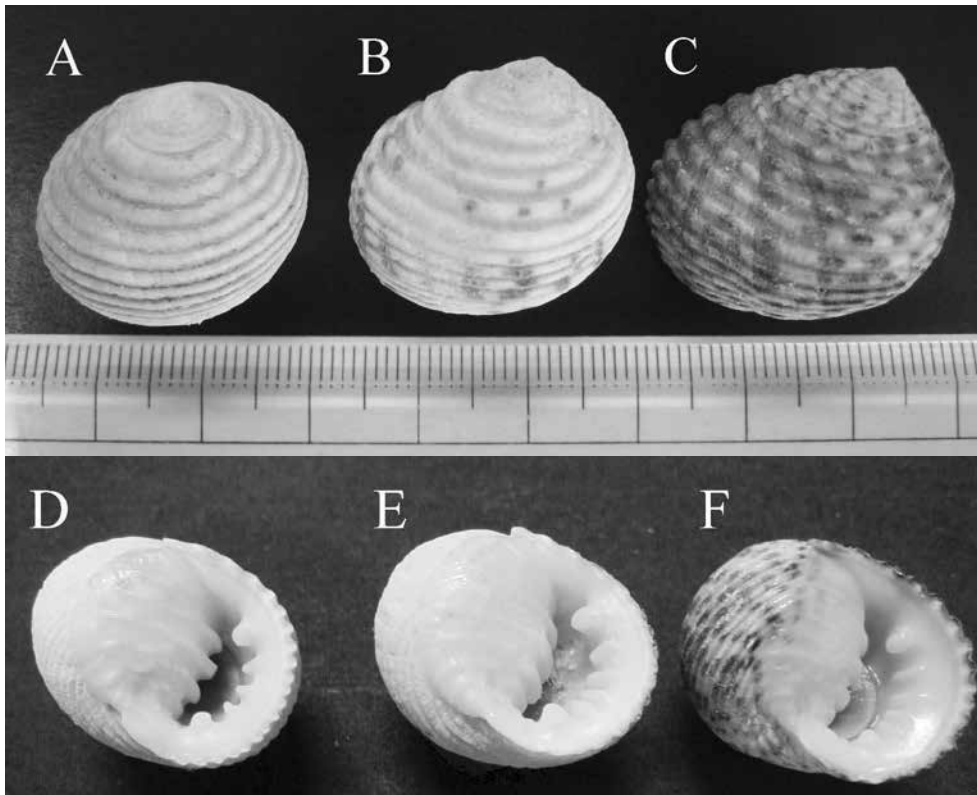


Fig.2. Shell color morphs: (A, D) fully white; (B, E) partially black-spotted; and (C, F) fully black-spotted.

morph was defined as having the continuous and complete small black spots pattern on the shell surface from the outer lip to the apex.

In 2008, in Rarotonga Island, the rocks at stations a, c, e, and f were white-gray and the fully white morph comprised approximately 60% of the observed individuals. Partially and fully black-spotted morphs, on the other hand, comprised approximately 20% of observed individuals each (Fig. 3A). In contrast, on the basalt rocks of stations b, d, and g, the three morphs were distributed at nearly equivalent frequencies.

In 2009, in Aitutaki Island's basalt rocks of stations h, i, j, and l, approximately 70% of the individuals were fully black-spotted, whereas almost all other individuals were partially black-spotted. Fully white morphs were rare (Fig. 3B). In contrast, on the beach rocks of station k, the three morphs were observed at almost equivalent frequencies.

In both Rarotonga Island (2008) and in Aitutaki Island (2009) significant differences were found between the morph frequencies of beach and basalt rocks (Rarotonga Island: $\chi^2 = 52.81$, $df = 1$, $p < 0.001$, Fig. 3A; Aitutaki Island: $\chi^2 = 548.92$, $df = 1$, $p < 0.001$, Fig.3B). Significant differences were also found between the morph

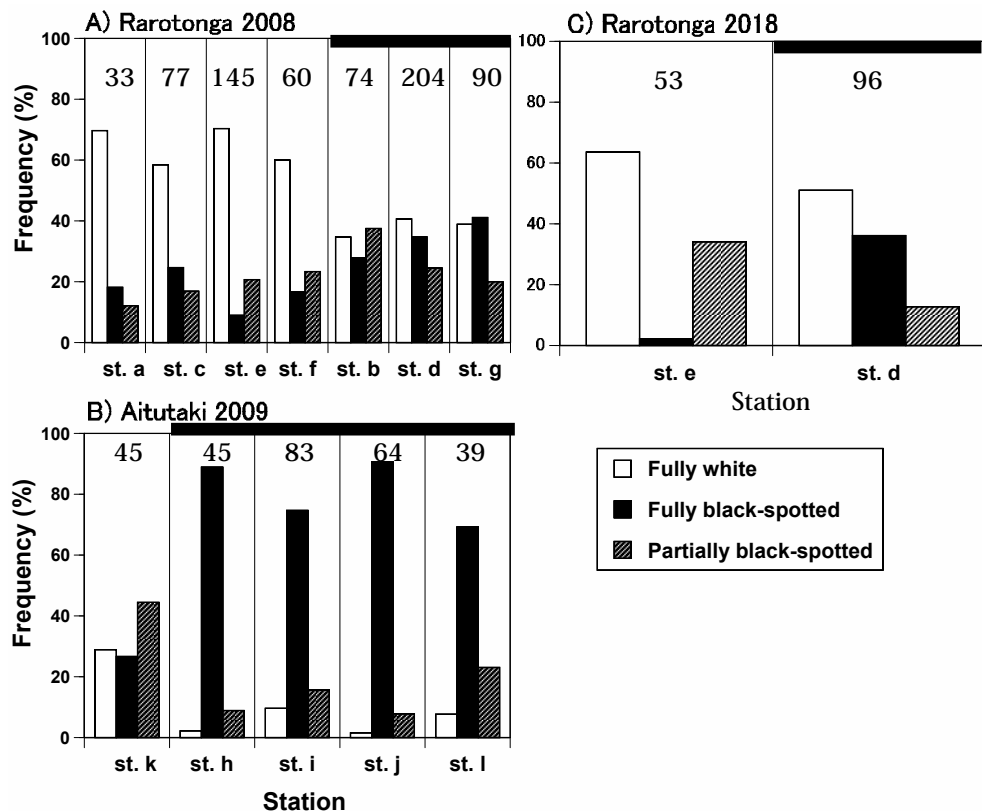


Fig.3. Frequency distributions of fully white, fully black-spotted and partially black-spotted morphs on (A) Rarotonga Island in 2008, (B) Aitutaki Island in 2009, and (C) Rarotonga Island in 2018. Numbers indicate sample size. The basal rock's research areas indicate presence of black bands above the number of sample size within each station.

frequencies of Rarotonga Island in 2008 and Aitutaki Island in 2009 regarding both beach ($\chi^2 = 16.63$, $df = 1$, $p < 0.001$) and basalt rocks ($\chi^2 = 139.66$, $df = 1$, $p < 0.001$) (Fig. 3A and B).

In 2018, on Rarotonga Island's basalt rocks of station d, the fully white, fully black-spotted, and partially black-spotted morphs comprised 51%, 36%, and 13% of individuals, respectively (Fig. 3C). No significant differences were found between the morph frequencies observed in 2008 and 2018 at that station ($\chi^2 = 0.009$, $df = 1$, $p > 0.05$). On Rarotonga Island's beach rocks of station e, the fully white, fully black-spotted, and partially black-spotted morphs comprised 64%, 2%, and 34% of individuals, respectively (Fig. 3C). No significant differences were found between 2008 and 2018 regarding the morph frequencies observed at station e ($\chi^2 = 0.007$, $df = 1$, $p > 0.05$).

Discussion

The present study showed that in *Nerita plicata*, the shell color distribution was closely related to the type of habitat. In fact, for snails on the same island, individuals with fully white shells occurred at a higher frequency on light-colored substrates (beach rocks) than on dark-colored substrates (basalt rocks; Fig. 3). Similarly, black-colored shells occurred at a higher frequency in dark-colored habitats than in light-colored habitats (Fig. 3). This shell color polymorphism may be explained by the visual selection of the snails' predators (BYERS 1990, MCKILLUP and MCKILLUP 2002). Both birds and fish are important visually selective predators of intertidal snails (HUGHES and MATHER 1986, JOHANNESSON and EKENDAHL 2002, REIMCHEN 1979, 1989). Crabs are also thought to prey on these species (HUGHES 1986, LITTLE and KITCHING 1996), although predation by the crab, *Carcinus maenas*, does not influence the color frequency of *Littorina saxatilis* (EKENDAHL 1998). Crabs such as *Chlorodiella cytherea* and *Grapsus tenuicrustatus* (GIBBS *et al.* 1975), and birds such as *Pluvialis dominica* (HOLYOAK 1980), are common predators found on the shores of the Cook Islands. The present study suggests that conspicuous differences between substrate and shell color (for example, a white shell on a dark substrate) would put the gastropods at a disadvantage by making them more easily recognizable. It was therefore suggested that the frequency distribution of shell colors observed in the present study may have been driven by the visual selection of predators. Further studies using both field and experimental observations are required to understand the effect of selective predation on other ecological characteristics such as size distribution.

In some gastropods, shell color polymorphisms have been shown to result from complex interactions between selection forces (JONES *et al.* 1977). Although predation may be an important selection force, predator densities generally fluctuate (ITO *et al.* 1992), which suggests that the intensity of the selection by the predators may also fluctuate. However, at the observed study sites, the morph frequencies

remained relatively stable for 10 years (Fig. 3), indicating that other selection forces may also act upon shell color polymorphism. Temperature is usually an important factor influencing marine gastropods' survival rates and thermal selection is known to drive shell color polymorphism in different microhabitats (COWIE and JONES 1985, HARRIS and JONES 1995). However, VERMEIJ (1971b), WARBURTON (1973), and COLEMAN (1976) have suggested that *N. plicata* is highly adaptable to temperature changes. Other environmental factors such as salinity (SOKOLOVA and BERGER 2000), insolation (HEATH 1975), wave exposure (ETTER 1988), coloration of prey species (MANRÍQUEZ *et al.* 2009), and parasite tolerance (SCHEIL *et al.* 2014) may also affect shell color polymorphism in *N. plicata*. Continued, long-term monitoring of intertidal environments may provide an excellent opportunity to understand the causal relationships between environmental changes and shell color polymorphism.

In the present study, the frequencies of shell color morphs were similarly distributed for snail populations from the same island and rock type (Fig.3). *Nerita plicata* are planktotrophic organisms and their larvae disperse over long distances across the Indo-Pacific Ocean (CRANDALL *et al.* 2008). This suggests that there is a low level of genetic differentiation underlying the shell variation between the studied populations of *N. plicata* from nearby locations. It also suggests that *N. plicata* maintains a relatively similar genotype between populations inhabiting the same island. However, despite the putative genetic similarities, there are likely to be different selection forces acting upon the shell morphology of snails inhabiting different rock types on the same island. In addition to the differences between populations on the same island, differences were also observed between the populations of Rarotonga and Aitutaki Islands regarding the distribution of shell color morph frequencies on the same type of substrate (Fig. 3). Aitutaki Island is located approximately 260 km north of Rarotonga Island and a current flow between the two islands. Together with other environmental selection forces, both the distance and the current may increase the genetic differentiation between the *N. plicata* populations of Rarotonga Island and Aitutaki Island. Further studies with detailed molecular analyses using DNA markers, such as microsatellites, are required to elucidate the effect of genetics on shell color polymorphism.

The Pacific Ocean comprises several types of island, including atolls, almost-atolls, uplifted islands, and young volcanic islands. Many beach rocks on islands such as Rarotonga and Aitutaki, are composed of beach sediments that have been chiefly cemented by calcium carbonate deposits in a process influenced by temperature and seawater (TANAKA 1983). Previous studies have reported that some beach rocks were formed only several decades ago, indicating that beach rocks may have a comparatively short formation time (see TANAKA 1990, for review), although it sometimes takes hundreds or thousands of years to form beach rocks. Basalt rocks exist naturally on the coast, however, their positions are occasionally altered by people residing on volcanic islands. In recent years, climate change has been widely discussed (IPCC 2014) and is expected to increase the frequency of typhoons and lead to a rise in the sea level. This

would lead to a flow of seawater inland with subsequent coastal erosion, especially in island regions. As defense against the rising sea levels and coastal erosion, people construct cement walls and deposit stones along coastal areas. For this reason, many basalt rocks have been deposited along the coastlines of volcanic islands. Therefore, beach rocks have been formed along the coast in a short time and basalt rocks have been deposited relatively recently on the Pacific Islands. This scenario may have greatly influenced the native substrates and habitats of intertidal organisms. Therefore, in addition to any genetic and environmental factors, human-induced changes to coastal areas may also affect shell color morph frequencies in intertidal organisms.

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