ZOOLOGICAL SCIENCE **23**: 999–1008 (2006)

Proximate Mechanisms Causing Morphological Variation in a Turban Snail Among Different Shores

Takeo Kurihara[1]*, Mayu Shikatani[2], Kouji Nakayama[3] and Mutsumi Nishida[2]

[1]*Ishigaki Tropical Station, Seikai National Fisheries Research Institute, Ishigaki Island, Okinawa 907-0451, Japan*
[2]*Department of Marine Bioscience, Ocean Research Institute, University of Tokyo, 1-15-1 Minamidai, Nakano, Tokyo 164-8639, Japan*
[3] *Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*

In many benthic organisms with a planktonic larval stage, local populations have different morphology. Such difference may arise from some of the following proximate mechanisms. "Local recruitment (LR)": no larvae move between local populations, and segregated populations possess alleles coding for locally adaptive morphology. "Intragenerational selection (IS)": larvae move between local populations, and individuals with alleles for locally adaptive morphology survive after recruitment. "Phenotypic plasticity (PP)": larvae move between local populations and show phenotypic plasticity to adapt to a locality after recruitment. We examined which mechanism explains our finding that a planktonic developer *Turbo coronatus coronatus* (Gastropoda) had significantly longer spines on its shell on more exposed shores at scales of < 2 km. Experiments at Ishigaki Island, Okinawa, Japan, showed the following results. (a) Shorter- and longer-spined populations occurring within 2 km showed non-significant low φ_{ST} values (–0.0040 to 0.00095) for the mitochondrial DNA COI region. This suggests no segregation of the local populations, supporting the mechanisms IS and PP. (b) *T. c. coronatus* generated significantly longer spines 70 days after being transplanted to the habitat of a longer-spined population, supporting IS and PP. (c) Individuals caged in the sea for 79 days generated longer spines than individuals in the laboratory, supporting PP. In conclusion, shore-specific morphology of *T. c. coronatus* arises most likely from phenotypic plasticity and possibly from intragenerational selection.

Key words: plasticity, intragenerational selection, morphology, subtropical turban snail, wave action

INTRODUCTION

Phenotypes of diverse groups of marine animals show intraspecific variation between habitats. Morphology, behavior, and life history traits of species of Anthozoa, Gastropoda, Crustacea, Echinoidea, and Osteichthyes have been reported to show such variation (*e.g.* Wainwright and Dillon, 1969; Vermeij, 1978; Boidron-Metairon, 1988; Ogura *et al.*, 1991; Spitze, 1992; Bruno and Edmunds, 1997; Parsons, 1997). Especially, morphologies of snails in the genera *Littorina* and *Nucella* have been frequently reported to vary between exposed and protected shores (Ino, 1953; Kitching *et al.*, 1966; Kitching and Lockwood, 1974; Newkirk and Doyle, 1975; Heller, 1976; Vermeij, 1978; Etter, 1988; Johannesson and Johannesson, 1990; Frid and Fordham, 1994; De Wolf *et al*., 1997; but see also Crothers, 1984).

Such morphological variation in snails has been often attributed to shore-to-shore variation in two proximate factors (*sensu* Calow, 1998): the composition of genes coding for morphology and/or a pattern of morphological plasticity depending on shore-specific environmental factors (Kitching and Lockwood, 1974; Crothers, 1984; Etter, 1988; Boulding and Hay, 1993; Frid and Fordham, 1994; De Wolf *et al*., 1997). Such hypotheses have been examined through artificial crossing (Newkirk and Doyle, 1975; Palmer, 1985), allozyme analysis (Johannesson and Tatarenkov, 1997), rearing snails in various environments (Palmer, 1990; Trussell, 1996; Delgado *et al*., 2002; Trussell and Nicklin, 2002), and transplanting marked snails onto various shores (Etter, 1988; Chapman, 1997; De Wolf *et al*., 1997). As a result, the hypotheses of shore-specific genetic composition and morphological plasticity have been generally supported or at least not refuted for each species examined (Newkirk and Doyle, 1975; Etter, 1988; De Wolf *et al*., 1997).

The shore-specific genetic composition has been attributed to the mechanism shown in Fig. 1A: snail populations on different shores are segregated, and the larvae recruiting onto each shore have different genes coding for different morphologies (hereafter termed the "local-recruitment mechanism"; *e.g.*, Parsons, 1997). On the other hand, the shore-specific morphological plasticity has been attributed to the mechanism in Fig. 1C: snail populations on different shores are not segregated, and each individual after recruitment

* Corresponding author. Phone: +81-980-88-2866;
Fax : +81-980-88-2573;
E-mail: takeo@affrc.go.jp
doi:10.2108/zsj.23.999

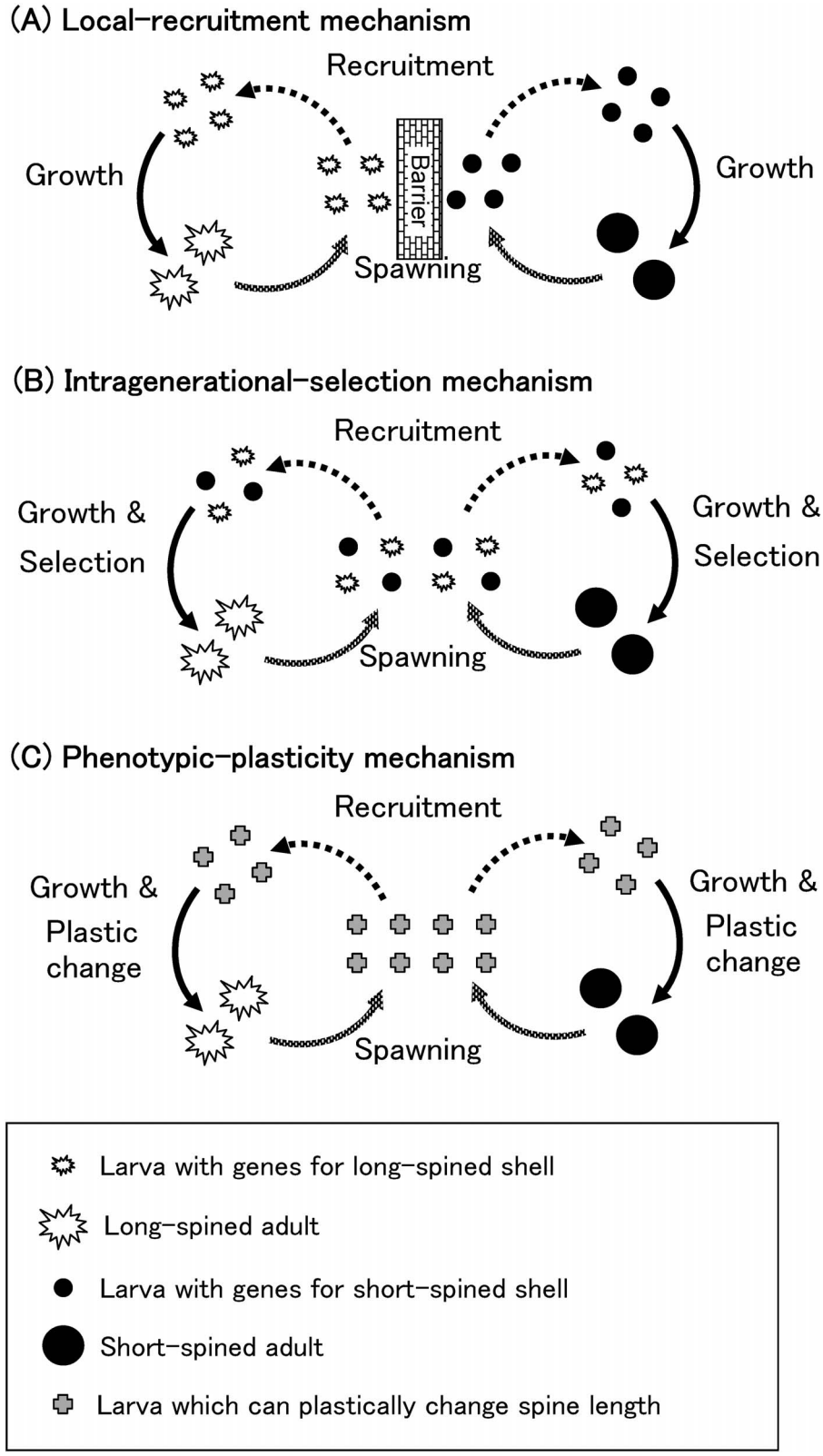

Fig. 1. Three possible mechanisms causing habitat-to-habitat variation in the phenotype of a marine animal. Variation in the shell morphology of a snail is taken as an example. Left and right circles represent life cycles on different shores. Long- and short-spined adults are assumed to occur on the left and right shores, respectively. **(A)** Local-recruitment mechanism: larvae with genes for long- and short-spined shells recruit onto the left and right shores, respectively. **(B)** Intragenerational-selection mechanism: larvae with genes for long- and short-spined shells survive on the left and right shores, respectively. **(C)** Phenotypic-plasticity mechanism: larvae become long- and short-spined adults on the left and right shores, respectively, due to morphological plasticity of each individual.

onto each shore changes its morphology due to shore-specific environmental factors ("phenotypic-plasticity mechanism"; *e.g.*, Etter, 1988).

The two mechanisms are, however, not applicable in some situations. Shore-specific genetic composition might arise from differences not only in the genotypes of recruiting larvae (Fig. 1A) but also in the post-recruitment survival of each genotype of the larvae (Fig. 1B). In addition, the morphological plasticity specific to each shore might be caused not only by the phenotypic change of each individual (Fig. 1C) but also by shore-specific survival patterns of various morphotypes (Fig. 1B). Considering these situations, we further propose the "intragenerational-selection mechanism" (Fig. 1B). In this mechanism, we assume that: larvae recruiting onto each shore have a similar composition of genes coding for morphology; the individuals whose genes code for the more adaptive morphology on a shore survive better on the shore; as a result, genotypes for morphology differ between shores; and from these shores larvae are spawned and mixed in the sea. The intragenerational-selection and local-recruitment mechanisms are somewhat similar in involving shore-specific genes but are different in that snail populations on different shores are segregated in the local-recruitment mechanism only. The intragenerational-selection and phenotypic-plasticity mechanisms are somewhat similar in involving morphological plasticity but are different in that shore-specific morphology is independent of shore-specific genes in the phenotypic-plasticity mechanism only.

Our aim is to examine which of these mechanisms causes shore-to-shore morphological variation in the turban snail *Turbo* (*Lunella*) *coronatus coronatus* (Gmelin, 1791). We expected the spines on its shell to be longer on more exposed shores as reported for other turban snails (Ino, 1953; Vermeij, 1978). No studies, however, have reported how and why the morphology of *T. c. coronatus* varies.

We first observed the variation in the lengths of the spines of *T. c. coronatus* between protected and exposed shores. We then conducted four experiments (Table 1) to examine the causes of the variation: (1) Development-mode observation to determine whether *T. c. coronatus* is a direct or planktonic developer. Confamilial species include both types of developers (Kono and Yamakawa, 1999), and the type of *T. c. coronatus* has not been reported. If *T. c. coronatus* is a direct developer, the larvae would rarely migrate from shore to shore (Parsons, 1997), which would support the local-recruitment mechanism. (2) Mitochondrial DNA analyses to compare the nucleotide sequence between *T. c. coronatus* populations on exposed and protected shores. If the sequences differs, this would indicate low gene flow between the populations (Hartl and Clark, 1997), which would support the local-recruitment mechanism. (3) Transplant experiment to examine the morphological changes in *T. c. coronatus* translocated between shores. If the snails transplanted to a more exposed shore generate longer spines, then spine length may possess plasticity, which would support the intragenerational-selection and phenotypic-plasticity mechanisms. In addition, if the snails originating from populations with different spine length generate spines of similar length after transplantation, then spine length would is unlikely to be hereditary in the broad sense (Hedrick, 1999), which would support the phenotypic-plasticity mechanism. (4) Caging experiment in which *T. c. coronatus* was caged in various environments. If each individual survives and generates longer spines in the environment more similar to exposed shores, as suggested for the congeneric snail *T. cornutus* (Ino and Kametaka, 1943), this would support the phenotypic-plasticity mechanism. Finally,

Table 1. Possible results of each experiment and the inference. The results actually obtained are denoted by screened bold letters. "+": plausible, "0": neutral, "–": implausible.

	Possible result	Suggestion	Plausibility of three mechanisms		
			Local-recruitment	Intragenerational -selection	Phenotypic-plasticity
Development-mode observation	Development mode	Migration of larvae from shore to shore			
	: planktonic	**: active**	**–**	**+**	**+**
	: direct	: inactive	+	–	–
mtDNA analyses	Haplotype composition of populations with different AL of spines	Gene flows between populations with different AL of spines			
	: similar	**: high**	**–**	**0**	**+**
	: dissimilar	: low	+	0	–
Transplant experiment	AL of spines (comparison between release shores)	Influence of shore-specific environments on spine length			
	: similar	: weak	+	–	–
	: longer for the release shore harboring longer-spined population	**: strong**	**–**	**+**	**+**
	AL of spines (comparison between origin shores)	Influence of genetic factors on spine length			
	: similar	: weak	–	–	+
	: longer for the origine shore harboring longer-spined population	**: unclear**	**0**	**0**	**0**
Caging experiment	Plastic change in AL of spines of each individual	Plastic change in spine length of each individual			
	: present	**: plausible**	**0**	**0**	**+**
	: absent	: implausible	0	0	–

we evaluated shore-to-shore variation in wave height to examine its correlation with spine-length variation.

MATERIALS AND METHODS

Observations on shore-to-shore variation in spine length

Turbo coronatus coronatus inhabiting intertidal cobbled shores in subtropical Japan (Fuse, 1993; Kurihara *et al*., 2000) is herein treated as taxonomically distinct from the temperate subspecies *Turbo coronatus coreensis* (Récluz, 1853). *T. c. coronatus* were collected from seven cobbled shores, S1 to S7, at Ishigaki Island (124°10'E, 24°30'N), subtropical Japan (Fig. 2; see Kurihara *et al.*, 2000 for the environmental factors). The snails generally possessed longer spines in the order of S1< S2 <… < S7 in a preliminary survey. Only S1 and S2 were located near the innermost part of a bay. In both summer (17–19 September 1997) and spring (29–31 March 1998), snails were haphazardly collected between the mean-water and mean-low-water levels. For each individual, shell width and the lengths of three spines on the rib (Fig. 3A) were measured with calipers (± 0.01 mm) in the laboratory. After this measurement, each individual was dissected. Only those that could be sexed were analyzed in the present study.

In this and all subsequent analyses comparing spine length among *T. c. coronatus* populations, we divided spine length by shell width (hereafter "adjusted length", or "AL") to eliminate the influence of between-population difference in body size (Reyment, 1984). Previous researchers have often eliminated such influence by analysis of covariance (ANCOVA), comparing the Y-intercepts of regression lines with body size as a covariate and target trait as a criterion variate (*e.g.*, Etter, 1988; Trussell and Nicklin, 2002). However, we did not perform ANCOVA because we often used very small individuals of *T. c. coronatus*, for which the Y-intercept of such regression lines (*i.e.*, spine length) is assumed a priori to be zero. Our adjustment was also useful in general to achieve homogeneity of variances among *T. c. coronatus* populations, which is necessary for many statistical tests (Zar, 1999).

In the morphological observations only, the AL of spines showed heterogeneity of variances ($p<0.05$; Cochran's test). To cope with this problem, we used balanced data (Zar, 1999): four snails with similar shell widths (15.7 to 25.6 mm) were resampled from each combination of shore, season, and sex. The resampled data were analyzed by an orthogonal three-way analysis of variance (ANOVA) (shore and sex, fixed factors; season, a random factor). We compared this ANOVA with an ANOVA of the same design using all the data and confirmed that the resampling of data did not decrease statistical power (see Results). Because the ANOVA of resampled data showed a significant shore × season interaction, AL was further compared among shores by a one-way ANOVA for each season. AL was divided into subgroups within which it did not significantly differ in Tukey tests (5% level; Zar, 1999).

Development-mode observation

Following Okabe's (1982) experiments on *Turbo* (*Batillus*) *cornutus*, we observed the development mode of *T. c. coronatus*. At noon during the spring tides in August 2002, snails (shell width ≈ 20–40 mm) were collected from shore S3. They were put into six laboratory aquaria (26 cm W×37 cm L×23 cm H; 24 snails per aquarium) filled with natural seawater (29.7°C). From the following morning, UV-treated water was run into each aquarium. The temperature was regulated at 29.8°C for three of the aquaria and 32.2°C for the remaining three. The water flowing out of each aquarium was filtered through a screen (mesh size 150 μm×150 μm). This screen was examined under a binocular microscope several times a day for four days to determine whether planktonic eggs and larvae had been released.

Mitochondrial DNA analyses

On 14 June 1999 we collected *T. c. coronatus* from S2 (mean±SD shell width=26.5±5.1 mm; AL of spines=0.018±0.020;

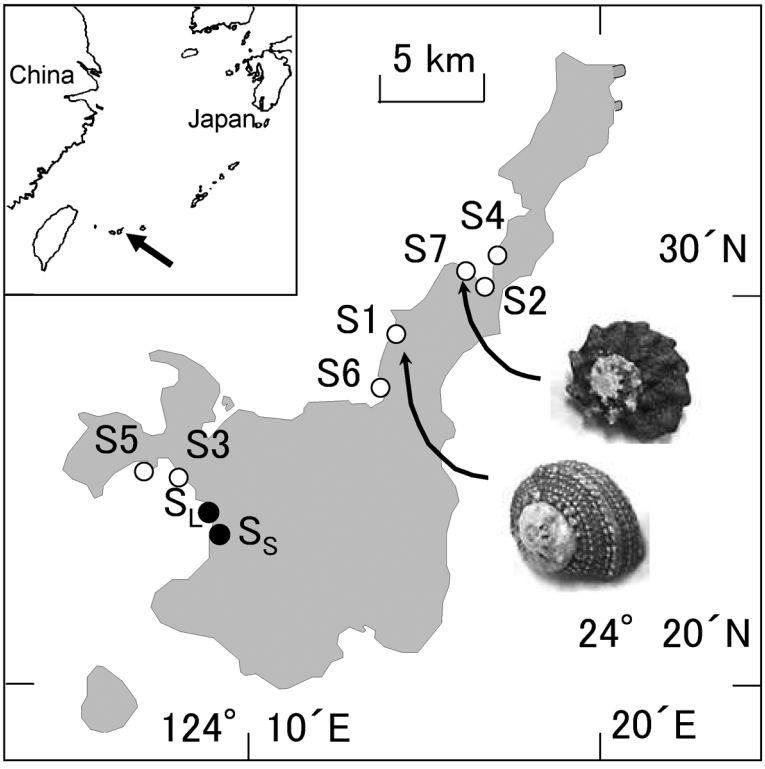

Fig. 2. Map of Ishigaki Island showing the locations of the shores studied and indicating the typical morphology of *Turbo coronatus coronatus* on shores S1 and S7. Shores with open circles (S1 to S7) and closed circles (SL and SS) were used in different experiments (see Materials and Methods).

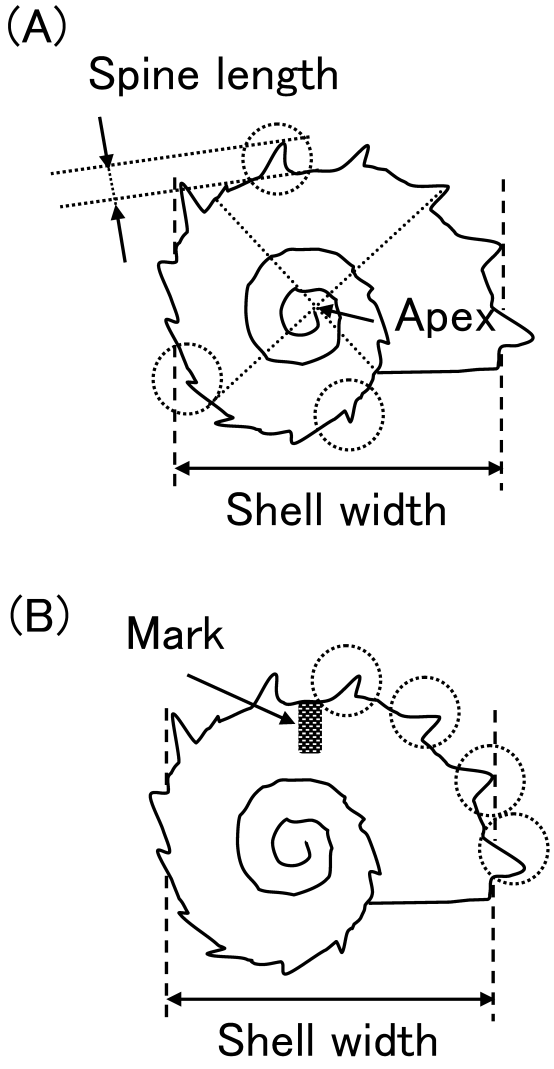

Fig. 3. Measurements of the AL of spines. **(A)** For measurements of shore-to-shore variation in AL, we drew two virtual lines that orthogonally crossed at the apex, with one of the lines running through the inner lip. These lines divided the shell into four sectors. In three of these sectors, the spine nearest to the apex was chosen to measure spine length (*i.e*., the distance between the spine pinnacle and the shoulder rib). **(B)** To determine AL during the transplant and caging experiments, we measured the lengths of all spines between the paint mark and outer lip.

N=20) and S4 (21.2±1.7; 0.086±0.019; N=33). Total genomic DNA was extracted from the foot of each individual with a DNeasy Tissue Kit (Qiagen). The first half of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified by the polymerase chain reaction (PCR) with primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*., 1994). PCR amplifications were carried out in a GeneAmp™ PCR System 9700 thermal cycler (PE Applied Biosystems) with an initial 2 min at 94°C followed by 35 cycles of 15–20 s at 94°C, 15–20 s at 45–55°C, and 40–60 s at 72°C. To verify the amplified fragment length, PCR products were subjected to 1.5% agarose gel electrophoresis and visualized with ethidium bromide under UV light. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen). These purified products were used as the template DNA for cycle sequencing reactions with an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems), and the reaction products were separated on an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems). The primers used for sequencing were the same as those for PCR amplification. Sequences 621 bp long were obtained and aligned with DNASIS ver. 3.5 software (Hitachi Software Engineering).

To examine further a 321-bp segment that had varied within the 621-bp region sequenced in the above-mentioned anlyses, we collected *T. c. coronatus* again on 8–10 February 2004 from shores S2 (N=71), S4 (N=71), S_S (N=67), and S_L (N=71; Fig. 2). Spines of *T. c. coronatus* from S_S were shorter (mean±SD AL= 0.12±0.11; specimen N=10) than those from S_L (0.22±0.09; N=10). We used the methods described above, except for the kit to purify PCR products (ExoSAP-IT, Amersham Biosciences) and the sequencing primer (TCO-CO1L01, 5-ACTGGGACAACCGGGTGCTTT-3).

For each of the years 1999 and 2004, we calculated population pairwise φ_{ST}, which defines genetic distance as the number of different nucleotides between two haplotypes (Excoffier *et al.*, 1992). For each φ_{ST} we did a permutation test with 1,023 randomizations. For the year 2004, we lowered the significance level from 0.05 to 0.0085 with the Dunn-Sidàk procedure (Underwood, 1997), because we repeated the permutation test six times.

Transplant experiment

We conducted a transplant experiment on shores safe from vandalism. Snails were collected in March 2000 from shores S_S and S_L (hereafter "origin shores"; Fig. 2). The outer lip of each snail was painted with Mitsubishi Paint Marker, which differentiated S_S (N=74) and S_L (N=80). Half of these snails were released to each of shores S2 and S3 (hereafter "release shores") and were recaptured 70–71 days later. The recaptured snails were measured under a binocular microscope to calculate the AL of (1) old spines that had already been generated before the release day and (2) new spines generated after the release day. To determine (1), we measured three spines and shell width for the old part of the shell, that is, the part nearer to the apex than the paint mark (Fig. 3B). To determine (2), we measured all spines between the paint mark and outer lip and the shell width on the recapture day. AL was analyzed by an orthogonal two-way ANOVA, with the origin and release shores as fixed factors (Zar, 1999).

Caging experiment

Nine or 10 snails (mean±SD shell width=12.6±2.6 mm; N=38) collected from shore S3 were marked and reared in each of four unroofed cages (≈ 40 cm×40 cm×40 cm) from 25 January to 13 April 2000. Two of the cages consisted of 2 mm×2 mm mesh and were set in the sea where wave height was about 1–20 cm. The other two consisted of resin board and were set in the laboratory protected from wave action. Nearly weekly, the snails were fed microalgae attached to pebbles. At the end of the experiments, the AL of newly generated spines was determined in a similar manner as described for the transplant experiment. AL was compared between the sea and laboratory by a nested ANOVA, with cage nested within each treatment (Zar, 1999). Because AL was not significantly affected by the nested factor ($p>0.25$), the nested components were pooled (Underwood, 1997).

Measurements of wave height

We measured the wave height on shores S1 to S7, using a ruler perpendicular to the surface of the water. We took measurements nine to 12 times between February and September 1998 at low tide. At this tidal stage, waves tend to be smaller because they break on the coral reef offshore. Although this leads to an underestimate of the wave action, the shore-to-shore variation may be correctly evaluated. We compared this method with that of Komatsu and Kawai (1992), which estimates wave action for two days from the decrease in the weight of a plaster ball. Wave action evaluated

for 24 experimental plots was significantly correlated between the two methods (r^2=0.345, p=0.003). The relationship between wave height and the AL of spines was analyzed by a Spearman rank-correlation coefficient (Zar, 1999).

RESULTS

Shore-to-shore variations in adjusted length of spines and wave height

In each season on each shore, 29 to 72 *Turbo coronatus coronatus* were collected (shell width 4.6–36.2 mm; total N=714). Of these, 26 to 71 snails could be sexed (shell width 7.9–36.2 mm; N=620). From each combination of shore, season, and sex, four snails (shell width 15.7–25.6 mm) were resampled to obtain balanced data.

The adjusted length (AL) of spines was significantly affected by only a shore × season interaction among all interaction terms for both the resampled data ($F_{6, 84}$=3.23, p=0.007) and original data ($F_{6, 592}$=4.70, p<0.001). In a one-way ANOVA for each season, AL significantly varied from shore to shore for the resampled data ($F_{6, 49}$=9.87 to 9.90, p<0.001). In Tukey tests for each season, shores S1 and S2 belonged to the subgroups with the shortest or second shortest AL, whereas the other shores generally belonged to some of the subgroups with the second to fourth shortest AL. These results closely reflected Fig. 4, in which mean AL was mostly smaller for S1 and S2 than for the other shores in each season. Overall, although the shore-to-shore variation was gradual, the AL of spines was generally shorter for

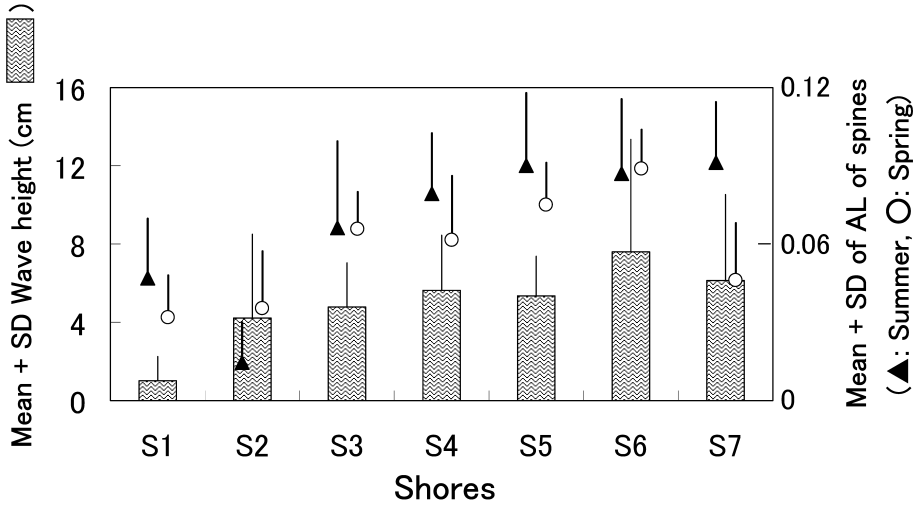

Fig. 4. Shore-to-shore variations in wave height and the AL of spines (mean + SD, calculated from resampled seasonal morphological measurements).

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                                30                                  60                                  90
CTGGTTGGAACTGCTTTGAGTCTTTTGATT CGGGCTGAACTGGGACAACCGGGTGCTTTG TTAGGTGATGATCAGCTGTATAATGTAATT

                               120                                 150                                 180
GTYACTGCTCATGCTTTTGTRATAATTTTT TTTCTGGTGATGCCTCTTATGATTGGRGGG TTTGGAAATTGACTTATYCCTTTAATGTTA

                               210                                 240                                 270
GGRGCTCCRGATATAGCGTTTCCTCGACTT AATAATATGAGATTTTGGTTACTTCCACCT TCTTTAACTTTACTTCTAACTTCGGCTGCR

                               300                                 330                                 360
GTTGAGAGTGGAGCTGGGACAGGGTGGACT GTTTATCCGCCTTTAGCTGGTAATTTAGCT CATGCCGGGGCTTCTGTRGATYTAGYRATT

                               390                                 420                                 450
TTTTCTCTTCATCTTGCTGGTRTTTCTTCT ATTTTGGGGGCTGTTAATTTTATYACTACT GTAATTAATATGCGATGACAGGGRATAAAA

                               480                                 510                                 540
TTTGAACGATTGCCTTTATTTGTGTGGTCA GTGAAAATTACAGCTATTTTGCTTCTTCTG TCCCTTCCAGTTTTAGCTGGTGCTATTACA

                               570                                 600                                 621
ATACTTTTGACTGATCGGAATTTTAATACT TCTTTTTTTGATCCTGCTGGTGGGGGGGAT CCYATTTTATACCAGCACTTG
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Fig. 5. Partial mtCOI nucleotide sequence for *Turbo coronatus coronatus* collected in 1999 (621 bases, variable at positions indicated by R) and 2004 (321 bases from T to G, variable at positions indicated by R and Y).

Table 2. Variable nucleotides in the mtDNA COI region (denoted by the numbers presented in Fig. 5), determined for *Turbo coronatus coronatus* collected in 2004. Dots correspond to nucleotides in Haplotype 04.

Haplotype	Nucleotide position										Number of snails			
	93	111	168	183	270	348	352	356	357	382	S_S	S_L	S2	S4
04	T	A	T	G	A	A	T	C	A	A	57	63	67	70
05	C	.	.	.	.	.	C	.	.	.	6	3	0	1
06	C	.	.	.	.	.	.	.	.	.	1	2	1	0
07	.	.	.	.	.	G	.	.	.	.	2	0	0	0
08	.	.	.	.	.	.	.	.	.	G	1	0	0	0
09	.	.	.	.	G	.	.	.	.	.	0	1	0	0
10	.	.	C	.	.	.	.	.	.	.	0	1	0	0
11	C	G	.	.	.	.	.	.	.	.	0	1	0	0
12	C	.	.	A	.	.	.	.	.	.	0	0	1	0
13	.	.	.	.	.	.	.	T	.	.	0	0	1	0
14	.	.	.	.	.	.	.	.	G	.	0	0	1	0

Table 3. Population pairwise ϕ_{ST} for *Turbo coronatus coronatus* collected in yr 2004 (significance level in parenthesis).

	S_S	S_L	S2
SL	–0.0031 (0.392)		
S2	0.0381 (0.020)	0.0123 (0.139)	
S4	0.0447 (0.013)	0.0190 (0.116)	–0.0040 (0.999)

S1 and S2.

Similarly, mean wave height was small for S1 and S2 and great for shores S6 and S7 (Fig. 4). The correlation between AL and wave height was significant for summer (Spearman's ρ=0.786; p=0.036) and was high for spring (ρ=0.679; p=0.094).

Development-mode observation

Three days after being put into aquaria, *T. c. coronatus* spawned planktonic eggs (diameter 0.10–0.13 mm) in only the three aquaria with the higher water temperature. Twenty-four hours later, some of the eggs developed into trochophore-stage planktonic larvae.

Mitochondrial DNA analyses

For *T. c. coronatus* collected in 1999, the COI sequences obtained varied at nucleotide positions 147 and 189 (Fig. 5; accession no. AB088067). Based on such variations, we defined Haplotypes 01 (A and A at positions 147 and 189, respectively), 02 (G and A), and 03 (A and G). Haplotype 01 accounted for all snails from S2 (N=20) and most from S4 (N=30). Haplotypes 02 and 03 accounted for only a few snails from S4 (N=2 and 1, respectively). Population pairwise φ_{ST} was 0.00095, not significant (df_1=1, df_2=51, p=0.292; permutation test).

For *T. c. coronatus* collected in 2004, the sequences varied at 10 nucleotide positions (Fig. 5), and Haplotypes 04 to 14 were recognized (Table 2). Haplotype 04 appeared to be identical to Haplotype 01, since both haplotypes were dominant on all the shores and showed the same sequence within, at least, the segments sequenced. Population pairwise φ_{ST} for geographically neighboring shores (S_S vs. S_L, and S2 vs. S4) were low (–0.0031 and –0.0040) and not significant (p=0.392 and 0.999; permutation test with the Dunn-Sidàk procedure; Table 3). In contrast, some φ_{ST} for distant shores (S_S vs. S2, and S_S vs. S4) were high (0.0381 and 0.0447) and nearly significant (p=0.020 and 0.013).

Transplant experiment

Seven and 15 *T. c. coronatus* originating from shores S_S and S_L, respectively, were recaptured on shore S2. Eight snails originating from each of S_S and S_L were recaptured

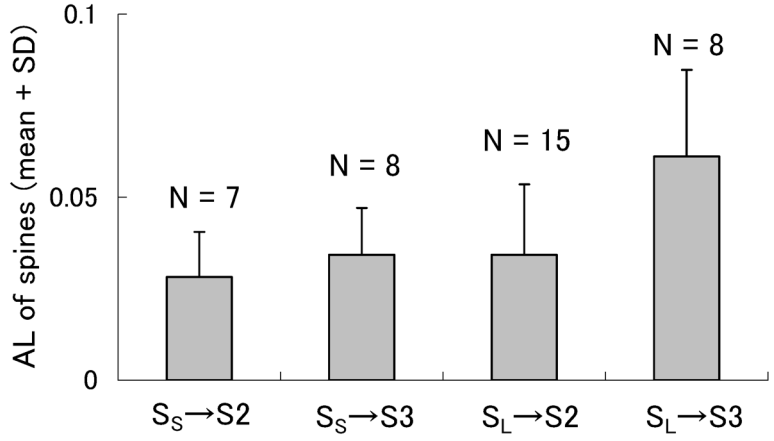

Fig. 6. AL of spines of *Turbo coronatus coronatus* transplanted from S_S and S_L to S2 and S3.

on shore S3. The AL of their spines on the release day was not significantly dependent on the origin shore ($F_{1, 34}$= 0.597, p=0.445), release shore ($F_{1, 34}$=0.205, p=0.653), or the interaction ($F_{1, 34}$=0.115, p=0.737). In contrast, the AL of spines newly generated during the experiment was significantly affected by the origin shore ($F_{1, 34}$=7.26, p=0.011) and release shore ($F_{1, 34}$=7.23, p=0.011), with no significant interaction ($F_{1, 34}$=2.87, p=0.099) (Fig. 6). As expected, mean AL was longer for the snails originating from S_L than for those from S_S, and longer for snails recaptured on S3 than for those on S2. The range in AL in the snails originating from the same shore overlapped between the release shores.

Caging experiment

All snails survived. The AL of spines generated during the experiment was not significantly affected by the nested factor, cage ($F_{2, 34}$=0.16, p=0.86). As expected, AL was longer in the sea (mean±SE=0.088±0.006, N=19) than in the laboratory (0.074±0.004, N=19) at a marginally significant level ($F_{1, 36}$=4.14, p=0.049).

DISCUSSION

Mechanisms of shore-to-shore variation in spine length

We interpret whether the results of each experiment favor the local-recruitment, intragenerational-selection, and phenotypic-plasticity mechanisms (Table 1) as follows. (1) Development-mode observation. *Turbo coronatus coronatus* was found to spend one day from hatching to the trochophore stage. After this stage, *T. c. coronatus* possibly has a longer planktonic existence, considering that the subspecies *T. c. coreensis* spends 2.5 days as planktonic larvae (Amio, 1963). Although it has been suggested that some molluscs with planktonic development (*e.g. Adalaria proxima*, *Littorina plena*) are unable to disperse for even ≈ 3 km (Todd *et al*., 1988; Kyle and Boulding, 2000), *T. c. coronatus* possibly disperses for > 3.5 km, considering its planktonic period (> 1 day) and the mean current velocity near the shores studied (3.5 km day^{-1}; Harii and Kayanne, 2002, 2003). *T. c. coronatus* larvae may disperse even farther under the influence of typhoons, which frequently hit the study area during the spawning season (September to December; Fuse, 1993). Within a distance of 3.5 km, *T. c. coronatus* populations with short and long spines occur (S_S and S_L; S1 and S6; S2 and S4; S2 and S7; Fig. 2). Between these populations, at least, gene flow may occur, which supports the intragenerational-selection and phenotypic-plasticity mechanisms and contradicts the local-recruitment mechanism.

(2) Mitochondrial DNA analyses. *T. c. coronatus* populations showed somewhat large genetic differences between shores 17 km apart. Perhaps *T. c. coronatus* planktonic larvae do not frequently disperse over such a distance. Such limited dispersal ability has been reported for the gastropod *Littorina plena* (planktonic period is 35–70 days; geographical distance between populations with a significant genetic difference is 0.5 km) and the bivalve *Arctica islandica* (32–55 days; <25 km). The genetic difference between the distant shores was, however, not statistically significant and thus should be scrutinized with methods more sensitive than mtDNA analysis (*e.g.*, microsatellite analysis).

In contrast to the geographically distant populations, *T. c. coronatus* populations occurring 1–2 km apart were genetically similar. They comprised both short- and long-spined populations. This indicates frequent genetic interchange and movement of planktonic larvae between short- and long-spined populations (Hartl and Clark, 1997). Such gene flow refutes the local-recruitment mechanism and supports the phenotypic-plasticity mechanism. Whether the gene flow supports the intragenerational-selection mechanism depends on whether the following two situations exist: (1) that the spine length of each individual is scarcely affected by environmental conditions (*i.e*. high heritability in the broad sense; Hedrick, 1999); and (2) that spine length often differs between parents and offspring (*i.e*., low heritability in the narrow sense). Such situations would arise, for example, if spine length is inherited in a Mendelian fashion, with long spines being either dominant or recessive to short ones, as reported for the shell morphology of the snail *Thais emarginata* (Palmer, 1985). In such situations, some *T. c. coronatus* larvae destined to have short spines would be spawned from a long-spined population, possibly recruiting into a short-spined population. Conversely, some larvae destined to have long spines would migrate from short- to long-spined populations. These situations lead to gene flow between these populations, as inferred from computer simulations by Boulding (1990).

(3) Transplant experiment. *T. c. coronatus* generated longer spines when released to the habitat of a longer-spined population. Although this result should be interpreted with caution because of the small sample size, it permits two interpretations: (a) intragenerational selection, where the snails hereditarily destined to generate longer spines survived; and (b) phenotypic plasticity, where each individual showed morphological plasticity, responding to habitat-specific environments. Which interpretation is more likely? Etter (1988) is skeptical of intragenerational selection, considering that the snail *Nucella lapillus*, when released to different shores, attained a phenotypic character (pedal surface area) differing with little overlap. De Wolf *et al*. (1997) have also presented similar results and deductions for the snail *Littorina striata*. In our experiments, however, *T. c. coronatus* released to different shores showed a considerable overlap in a phenotypic character (AL of spines), and thus intragenerational selection cannot be refuted as in the two previous studies. Therefore, the difference in spine length between release shores possibly supports not only the phenotypic-plasticity but also the intragenerational-selection mechanisms. On the other hand, the morphological change after recruitment contradicts the local-recruitment mechanism.

Another result of the transplant experiment is that *T. c. coronatus* collected from a longer-spined population attained longer spines. This might show that the length of spines newly generated was hereditarily determined (supporting the local-recruitment and intragenerational-selection mechanisms). Otherwise, the result might show that the length of the spines was affected by what the snails had experienced before the transplantation (Etter, 1988). This result is inconclusive regarding the mechanisms in Table 1.

(4) Caging experiment. Since all *T. c. coronatus* individuals survived, the difference in the length of newly generated spines demonstrates that at least some individuals showed plasticity in response to cage-specific environ-

ments. This favors the phenotypic-plasticity mechanism.

In conclusion, our results suggest that the local-recruitment mechanism is unlikely, the intragenerational-selection mechanism possible, and the phenotypic-plasticity mechanism likely (Table 1). To further clarify the plausibility of each mechanism, additional experiments should be conducted on the heritability and fitness for spine length.

Environmental factors influencing shore-to-shore variation in spine length

Shore-to-shore differences in the spine length of *T. c. coronatus* are likely due in part to differences in wave action. This is because spines were longer on shores with stronger wave action, and because snails exposed to wave action in cages generated longer spines than did those protected from it. This inference on the effect of wave action can plausibly explain the observation that shore-to-shore variations in both wave action and the AL of spines were gradual.

Although an influence of wave action on shell morphology has been also suggested for many intertidal snails in the genera *Nucella* and *Littorina* (*e.g.*, Kitching *et al*., 1966; Heller, 1976; Kitching, 1976; Vermeij, 1978; De Wolf *et al*., 1997), the pattern differs between these snails and *T. c. coronatus*. That is, stronger wave action is correlated with smoother shells in *Nucella* and *Littorina* snails (Kitching, 1976; De Wolf *et al*., 1997) but with a longer-spined, rougher shell in *T. c. coronatus*. Such interspecific differences are attributable to species-specific strategies to cope with wave action and related environmental factors, as explained below. When wave action is strong, intertidal snails often face the danger of being swept away from their original habitat (Kitching *et al*., 1966; Etter, 1988). To cope with such danger, *Nucella* and *Littorina* snails may utilize their smooth shells for decreasing the drag force of water (De Wolf *et al.*, 1997); in contrast, *T. c. coronatus* may utilize its long spines as cleats to secure itself in the interstices that are abundant in its habitat, namely cobbled shores (for a similar explanation, see Ino and Kametaka, 1943). When wave action is weak, intertidal snails often face severe desiccation due to the lack of splash of water (Raffaelli and Hawkins, 1996). To cope with this stress, *Nucella* and *Littorina* snails are considered to utilize their rough shell surface to increase convection and the area of heat loss (Vermeij, 1973; Britton, 1995; De Wolf *et al*., 1997); in contrast, *T. c. coronatus* may utilize its smooth shell to dig efficiently into the fine sediments under cobbles to acquire moisture and remain cool (Kurihara, personal observation), as observed for the subspecies *T. c. coreensis* (Takada, 1999).

The inference that wave action affects the spine length of *T. c. coronatus*, however, apparently contradicts some other results. That is, between the shores S2 and S3 with similar wave height, AL differed for both the local populations and transplanted individuals. AL should be similar between these shores if wave action mainly affects spine length. One reason for this contradiction might be that we measured wave height for only several seconds. We might not have accurately evaluated the long-term magnitude of wave action. This is indicated by a low correlation coefficient between our wave-height measurements and wave action determined from a decrease in plaster ball weight (r^2=0.345; see Material and Methods); the latter measured wave action over a longer period (*i.e*., 2 days).

Another reason for the contradiction may be that environmental factors other than wave action also strongly affect AL. Such factors possibly include predation (Vermeij, 1978), desiccation (Atkinson and Newbury, 1984; Britton 1995), and water chemicals (Ino and Kametaka, 1943). The influence of predation is especially plausible, because it has often been suggested to cause shore-to-shore variation in anti-predator morphology (*e.g.*, shell thickness; Trussell and Nicklin, 2002) for temperate and boreal snails (Hughes and Elner, 1979; Johannesson, 1986; Appleton and Palmer, 1988; Palmer, 1990; Trussell, 1996). Although predation pressure has been suggested to correlate inversely with wave action because predators can feed on snails more freely on more protected shores (Kitching *et al*., 1966; Palmer, 1990; Trussell, 1996), such a correlation is unlikely in our study. This is because, in comparison with predators on temperate and boreal snails (mainly crabs; Appleton and Palmer, 1988; Palmer, 1990; Trussell, 1996), predators of *T. c. coronatus* may be more diverse (polyclads, mantis shrimps, crabs, predatory gastropods, octopuses and fishes) due to the greater biodiversity of subtropical regions (Vermeij, 1978; Kurihara *et al*., 2000). It is thus possible that the total abundance of predators on *T. c. coronatus* is greater on some shores (*e.g.* S3) than on others (*e.g.* S2), even if the wave action on these shores is similar (Kurihara *et al*., 2000). If this were the case, predation pressure on the former shore would be greater, inducing anti-predator morphology more markedly.

ACKNOWLEDGMENTS

We thank R. Fuseya, M. Katoh, N. Kono, T. Kosuge, H. Kubo, Y. Takada, M. Yamaguchi and reviewers for providing information on the genetics, morphology, and ecology of snails; T. Komatsu for guidance in how to measure wave action; M. Kobayashi, M. Ogura, and the staff of Ishigaki Tropical Station for their logistic support; and C. Norman for correcting our English. This study was financially supported in part by the Japan Ministry of Education, Science, Sports, Culture, and Technology.

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(Received May 13, 2005 / Accepted June 24, 2006)