Conchological and Genetic Characteristics of Three Forms of the True Limpet *Cellana nigrolineata* (Gastropoda: Nacellidae) Mainly Around Shikoku, Japan

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Abstract

Conchological and genetic features of three forms (radially striped, dappled, and complex forms) of the nacellid limpet *Cellana nigrolineata* were examined using six sample lots from localities mainly around Shikoku, Japan. Conchological examination by principal component analysis (PCA), which evaluated data for several shell measurements analytically, created distribution graphs plotted with principal component scores 1 and 2. In the graphs, plots of individuals of the three forms were distributed randomly in all samples, indicating no conchological differences among the three forms. Conversely, in the PCA result for whole samples, plots were well clustered one another by samples, showing conchological peculiarity by samples. Genetic examination of isozyme analysis presumed 37 loci. Regarding dendrograms of individual genetic distances (*Di*), individuals of the three forms were connected randomly in all samples and no specific clades with a certain form were recognized, indicating no genetic peculiarity of the three forms. On the other hand, allelic frequencies of the samples differed from one another at some loci including *EST-3**, *MDH-1**, and *MPI**. Such differences raised the values of population genetic distances (*D*). Consequently, each sample was regarded as an independent local population. In conclusion, the three forms of *C. nigrolineata* represent intra-specific phenotypic variation because they show no conchological or genetic differences from one another. However, some morphologically and genetically diverged local populations exist in *C. nigrolineata*.

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Introduction

The true limpet *Cellana nigrolineata* (Gastropoda: Nacellidae) occurs in coastal rocky areas around Japan, being a representative of the Japanese reef shells. It is utilized for human consumption in some regions[1] and has some value for fisheries. This species includes three forms in shell color patterns, *i.e.*, radially striped (or radial ray[2]) (Fig. 1A), dappled (or concentric[2]) (Fig. 1B) patterns, and a complex (or both[2]) of the two patterns (Fig. 1C). These forms have been traditionally regarded as an intra-specific phenotypic variation[3-5], and a recent molecular study using mitochondrial DNA as a genetic marker gave a positive suggestion for such taxonomic treatment[2]. Subsequently, the present study employed additional approaches to investigate this issue, including examination of conchological characteristics among the three forms, which have never been examined for this species, and examination of genetic characteristics using other genetic markers, isozymes. Further, geographic, conchological, and genetic variations of this species were demonstrated.

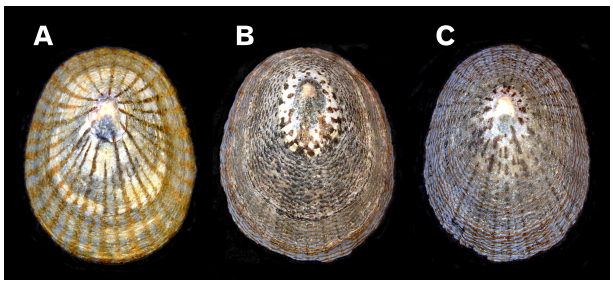

Fig. 1. Apical views of three forms of *Cellana nigrolineata*. A－Radially striped form, B－Dappled form, C－Complex form.

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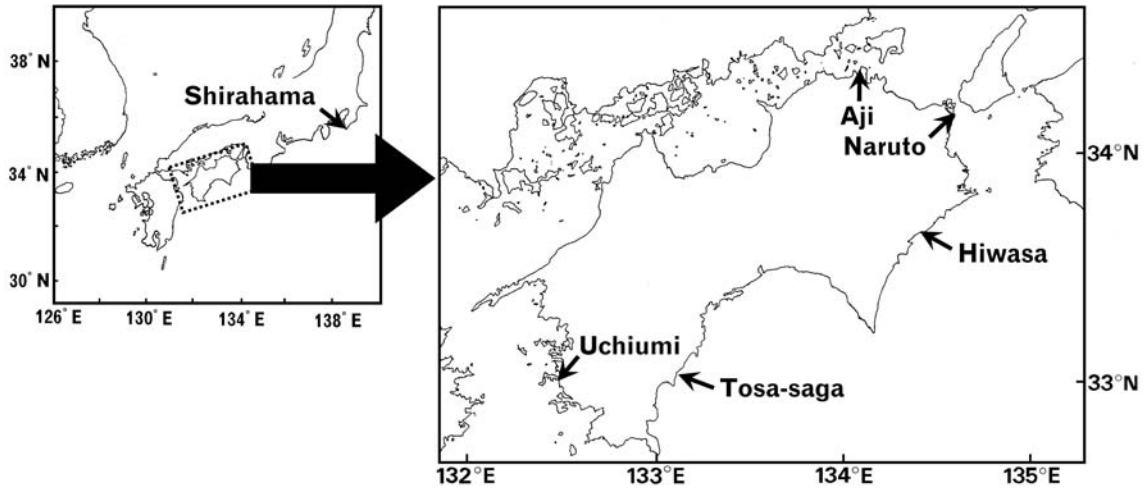

Fig. 2. Localities where the samples were collected.

Table 1. Data from the examined *Cellana nigrolineata* specimens

Sample name	Locality of collection	Date	*n*	Range of shell length (mm)
Aji	Aji, Takamatsu City, Kagawa Pref.	Aug. 15, 1997	90	24.4–75.6
Naruto	Iwashiyama, Naruto City, Tokushima Pref.	Dec. 15, 1997	89	21.6–48.8
Hiwasa	Hiwasaura, Minami Town, Tokushima Pref.	Nov. 21, 1997	64	22.6–52.3
Tosa-saga	Shirahama, Kuroshio Town, Kochi Pref.	June 13, 1999	48	15.0–34.0
Uchiumi*	Motonoko, Ainan Town, Ehime Pref.	June 13, 1998	30	11.2–49.3
Shirahama*	Shirahama, Minamiboso City, Chiba Pref.	Mar. 31, 1998	20	25.0–57.7

*Genetic examination was not performed.

Materials and Methods

Conchological characters

Six local sample lots of *C. nigrolineata* were collected mainly from Shikoku, Japan (Fig. 2, Table 1) by random sampling in the field. Each individual from the samples was classified into the three forms (Fig. 1) by its shell color patterns. Subsequently, the shell of each individual was removed and various portions (Fig. 3) and its weight were measured. The projective pre-apex length (PPRAL), projective post-apex length (PPOAL), and apex angle (AA) were calculated using trigonometric functions[6].

In order to evaluate the conchological data analytically, principal component analysis by the usual method[7] was performed. For the analysis, although all measured items shown in Fig. 3 plus shell weight were adopted as variates, shell length (SL) was not included because it was a sum of PPRAL and PPOAL (Fig. 3). In addition, the measured dimension values were transformed into natural logarithms because most of the shell proportions change with growth in *C. nigrolineata*[6].

Genetic characters

Isozymes detected by horizontal starch-gel electrophoresis were adopted as genetic markers. Soft parts of the collected samples except the Uchiumi and Shirahama samples were preserved in a freezer at −80℃ prior to analysis. Using the methodologies of Yokogawa[8], electrophoresis was performed

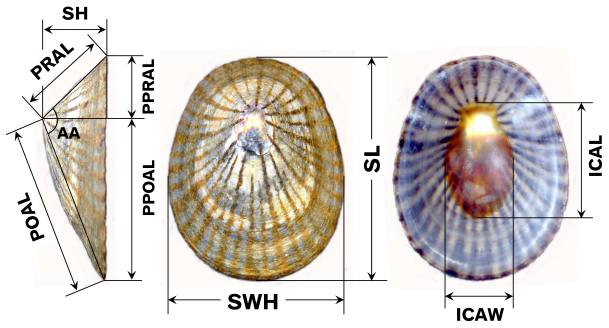

Fig. 3. Measured portions of the shell. SL – Shell length, SWH – Shell width, SH – Shell height, PRAL – Pre-apex length, POAL – Post-apex length, PPRAL – Projective pre-apex length, PPOAL – Projective post-apex length, AA – Apex angle (degrees), ICAL – Internal colored area length, ICAW – Internal colored area width.

to examine isozymes. For detection of esterases (the *EST-1**, *EST-2**, *EST-3** and *EST-5** loci) in the extract prepared from the visceral mass, thin polyacrylamide-gel electrophoresis after Taniguchi and Tashima[9] was performed. The gene nomenclature followed Shaklee *et al.*[10], and the alleles are presented as relative mobility percentages compared with the most dominant alleles (**100* in the anodal zone, **−100* in the cathodal zone) at each locus.

For the polymorphic loci, correspondence to the Hardy-Weinberg equilibrium was tested (H-W exact tests) with the GENEPOP software (ver. 4.0.7)[11]. The H-W exact tests were performed by samples, which contained all three forms. Genetic relatedness between individuals (*Rxy*) by samples was calculated following Queller and Goodnight[12], and it was converted into distance between individuals (*Di*) following Noguchi *et al.*[13] ($Di = 1 - Rxy$). Subsequently, dendrograms were constructed from the *Di* values by using the unweighted pair group method with arithmetic mean (UPGMA).

Wright's *Fst* (fixation indices) between samples and tests for a null hypothesis ($Fst = 0$) were calculated with the Arlequin software (ver. 3.5.1.2)[14]. For the tests, risk percentages were corrected according to total test counts using the Holm-Bonferroni method[15]. Population genetic distances (*D*), following Nei[16], were calculated with the allelic frequencies of the samples. Dendrograms of genetic relationships between the samples were constructed from the genetic distances by UPGMA, and bootstrap estimates to test the reliability of the dendrogram were computed with the Populations genetic software (ver. 1.2.30)[17].

Results

Conchological characters

Proportions of the three forms differed considerably by sample. In particular, the Aji sample comprised mostly the radially striped form only, while the other samples included the dappled and complex forms in certain proportions, in which case the radially striped form was the most dominant (Table 2).

In the results of principal component analysis (PCA) by samples, because cumulative contribution ratios exceeded 0.9 within principal component 2 (PC-2) in most samples (Table 3), the conchological data were explained well by PC-1 and PC-2. Regarding distribution graphs plotted with PC-1 and PC-2 scores, plots of individuals of the three forms were distributed randomly in all samples (Fig. 4), indicating no conchological differences among the three forms throughout the samples. On the other hand, regarding the PCA result for whole samples, plots were well clustered one another by samples (Fig. 5), showing conchological peculiarity by samples.

Table 2. Frequency (%) of forms by samples

	Radial striped	Dappled	Complex
Aji	97.8	1.1	1.1
Naruto	44.9	39.3	15.7
Hiwasa	64.1	12.5	23.4
Tosa-saga	64.6	29.2	6.3
Uchiumi	56.7	23.3	20.0
Shirahama	50.0	25.0	25.0

Table 3. Cumulative contribution ratios in principal component analysis by samples

	PC-1*	PC-2*	PC-3*	PC-4*
Aji	0.903	0.958	0.977	0.986
Naruto	0.826	0.931	0.968	0.980
Hiwasa	0.863	0.951	0.976	0.985
Tosa-saga	0.783	0.891	0.939	0.971
Uchiumi	0.929	0.977	0.984	0.991
Shirahama	0.842	0.959	0.986	0.992
Pooled	0.899	0.970	0.986	0.990

* PC substitutes for principal component.

Genetic characters

By electrophoresis, 19 enzymes were detected and 37 loci were presumed (Table 4). Concerning the correspondence to Hardy-Weinberg equilibrium, the H-W exact tests indicated no significance at any loci with any sample; all corresponded to the equilibrium. Values indicating genetic features were similar throughout the samples (Table 5). Among these, the observed heterozygosity (*Ho*) ranged from 0.129 to 0.142 (Table 5), showing the somewhat high genetic variability of this species.

Regarding dendrograms of individual genetic distances (*Di*), individuals of the three forms were connected quite randomly in all samples and no specific clades with a certain form were recognized (Fig. 6), indicating no genetic peculiarity of the three forms. On the other hand, allelic frequencies of the samples somewhat differed from one another at some

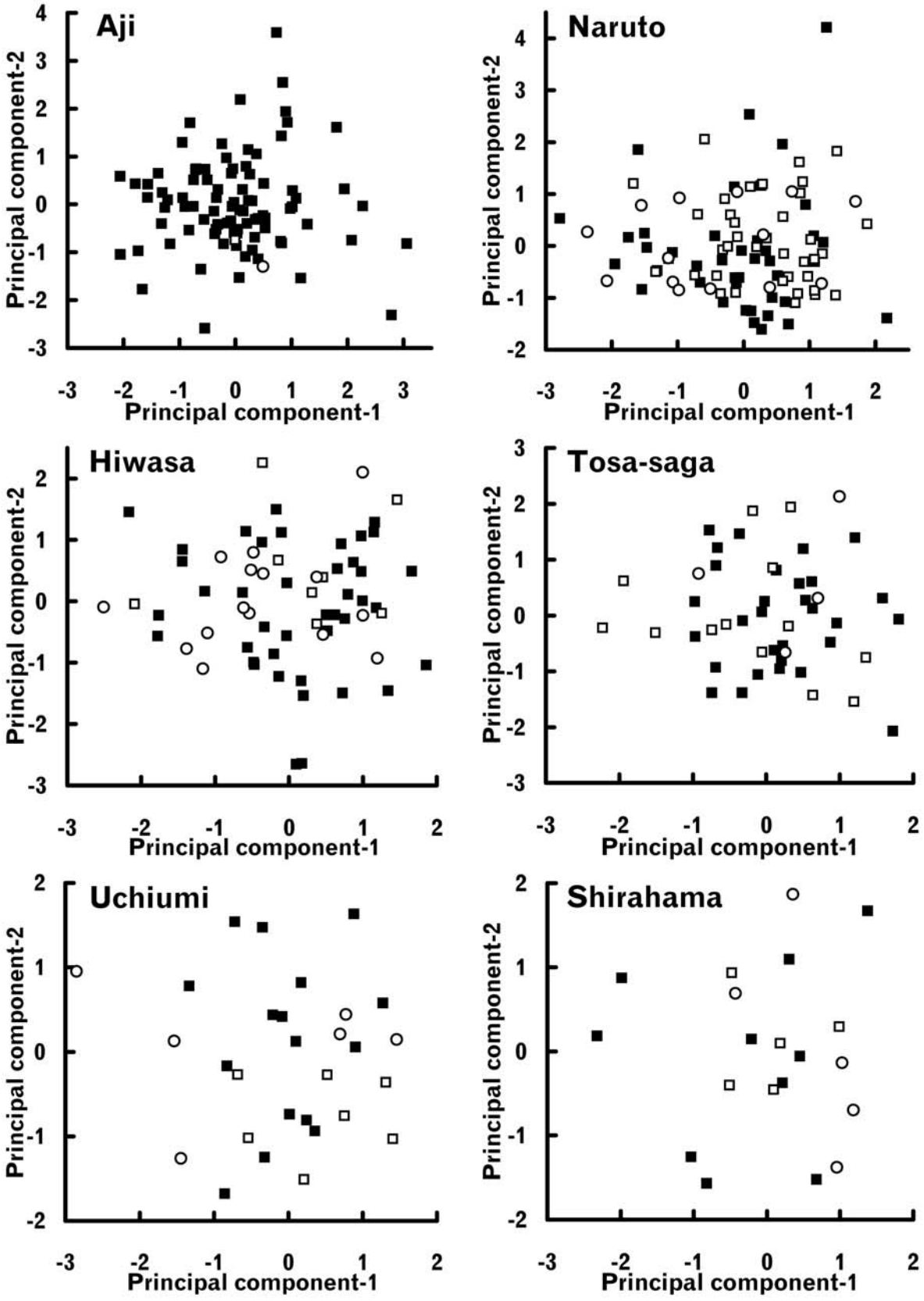

Fig. 4. Results of principal component analysis for each *Cellana nigrolineata* sample: distribution graphs plotted with principal component scores 1 and 2 for conchological data. Dark squares − Radially striped form, Open squares − Dappled form, Open circles − Complex form.

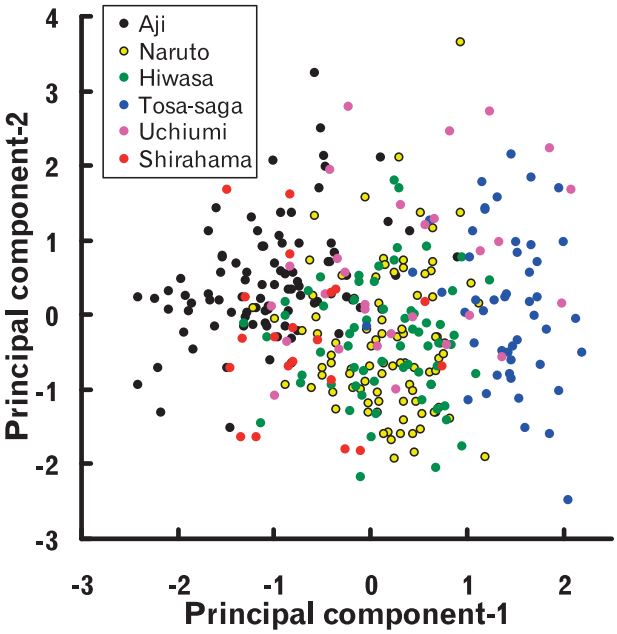

Fig. 5. Results of principal component analysis for whole *Cellana nigrolineata* samples: a distribution graph plotted with principal component scores 1 and 2 for conchological data.

loci including *EST-3**, *MDH-1**, and *MPI** (Table 5). Such differences raised the values of population genetic distances (*D*) and *Fst* values in some combinations (Table 6). In particular, the Aji sample was the furthest from the other samples in the *D* value mostly over 0.005 (Fig. 7, Table 6), which is significant at the inter-population level[18], and it showed a high level of significant differences from the other samples in *Fst* (Table 6).

Discussion

The three forms of *C. nigrolineata* showed no conchological differences in principal component analysis (Fig. 4). Genetically, the examined samples, which comprised individuals of the three forms, were all regarded as corresponding to Hardy-Weinberg equilibrium. Suppose the three forms genetically differ from one another, some mismatch from the equilibrium could occur like the case of the two forms of pen shell, *Atrina pectinata*, in which the two forms were clarified to be distinct species[19]. Also, such a mismatch has been reported for a complex of a cryptic species pair of common West Coast limpets, *Lottia digitalis* and *L. austrodigitalis*[20].

The correspondence to the equilibrium suggests no genetic difference among the three forms. This is also supported by the result that the three forms were connected randomly in the dendrograms for individual genetic distances (Fig. 6). These conchological and genetic results indicate that the three

Table 4. Enzymes and tissues examined for *Cellana nigrolineata*

Enzyme or protein name	Enzyme number	Locus	Tissue
Aspartate aminotransferase	2.6.1.1	*AAT-1**	FM
		*AAT-2**	FM
Acid phosphatase	3.1.3.2	*ACP-1**	VM
		*ACP-2**	VM
		*ACP-3**	VM
Aconitate hydratase	4.2.1.3	*AH-1**	VM
		*AH-2**	VM
Adenylate kinase	2.7.4.3	*AK-1**	FM
		*AK-2**	FM
		*AK-3**	FM
Alkaline phosphatase	3.1.3.1	*ALP**	VM
Diaphorase	1.6.-.-	*DIA**	VM
Esterase	3.1.1.-	*EST-1**	VM
		*EST-2**	VM
		*EST-3**	VM
		*EST-5**	VM
Fructose biphosphate aldolase	4.1.2.13	*FBALD**	FM
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH**	VM
Glucose-6-phosphate isomerase	5.3.1.9	*GPI**	FM
L-Iditol dehydrogenase	1.1.1.14	*IDDH**	FM
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	*IDHP-1**	VM
		*IDHP-2**	VM
		*IDHP-3**	VM
Leucine aminopeptidase	3.4.11.1	*LAP-1**	VM
		*LAP-2**	VM
		*LAP-3**	VM
Lactate dehydrogenase	1.1.1.27	*LDH-1**	FM
		*LDH-2**	FM
		*LDH-3**	FM
Malate dehydrogenase	1.1.1.37	*MDH-1**	VM
		*MDH-2**	VM
Malic enzyme ($NADP^+$)	1.1.1.40	*MEP-1**	FM
		*MEP-2**	FM
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	FM
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	FM
Phosphoglucomutase	5.4.2.2	*PGM-1**	FM
		*PGM-2**	FM

FM: Foot muscle; VM: Visceral mass

Table 5. Allelic frequencies and values indicating the genetic features of the examined *Cellana nigrolineata* samples

Locus	Allele	Aji	Naruto	Hiwasa	Tosa-saga
*AAT-1**	**130*	0.050	0.084	0.078	0.021
	**100*	0.950	0.916	0.914	0.979
	**85*	0	0	0.008	0
*AAT-2**	**-30*	0	0.017	0.016	0.010
	**-100*	0.994	0.983	0.984	0.990
	**-135*	0.006	0	0	0
*ACP-1**	**100*	1.000	1.000	1.000	1.000
*ACP-2**	**160*	0.056	0.063	0.040	0.053
	**100*	0.556	0.511	0.540	0.543
	**50*	0.388	0.420	0.421	0.404
	**-50*	0	0.006	0	0
*ACP-3**	**-100*	0.911	0.893	0.883	0.813
	**-135*	0.089	0.107	0.117	0.188
*AH-1**	**150*	0	0.011	0	0
	**100*	1.000	0.979	1.000	1.000
	**80*	0	0.011	0	0
*AH-2**	**-100*	1.000	1.000	1.000	1.000
*AK-1**	**100*	1.000	1.000	1.000	1.000
*AK-2**	**100*	1.000	0.983	1.000	1.000
	**75*	0	0.017	0	0
*AK-3**	**-100*	1.000	1.000	1.000	1.000
*ALP**	**0*	0.098	0.067	0	0
	**-100*	0.727	0.758	0.813	0.792
	**-150*	0.121	0.163	0.156	0.125
	**-200*	0.053	0.011	0.031	0.083
*DIA**	**-100*	1.000	0.994	0.983	1.000
	**-200*	0	0.006	0.017	0
*EST-1**	**100*	1.000	1.000	1.000	1.000
*EST-2**	**100*	1.000	1.000	1.000	1.000
*EST-3**	**100*	0.910	0.831	0.836	0.719
	**90*	0.077	0.107	0.094	0.240
	**80*	0.013	0.062	0.070	0.042
*EST-5**	**100*	0.375	0.436	0.325	0.415
	**50*	0.479	0.314	0.377	0.330
	**0*	0.146	0.250	0.298	0.255
*FBALD**	**135*	0.006	0.034	0	0
	**100*	0.994	0.961	1.000	1.000
	**55*	0	0.006	0	0
*G3PDH**	**125*	0.012	0.006	0	0
	**100*	0.952	0.983	0.984	0.979
	**65*	0.036	0.011	0.016	0.021
*GPI**	**100*	0.994	1.000	1.000	1.000
	**75*	0.006	0	0	0
*IDDH**	**180*	0.213	0.194	0.347	0.155
	**100*	0.747	0.775	0.621	0.750
	**0*	0.039	0.031	0.032	0.095
*IDHP-1**	**100*	1.000	0.989	1.000	1.000
	**50*	0	0.011	0	0
*IDHP-2**	**140*	0.041	0.011	0.008	0
	**100*	0.959	0.989	0.992	1.000
*IDHP-3**	**100*	0.988	1.000	1.000	1.000
	**50*	0.012	0	0	0
*LAP-1**	**100*	1.000	1.000	1.000	1.000
*LAP-2**	**100*	0.866	0.803	0.786	0.888
	**80*	0.134	0.197	0.214	0.113
*LAP-3**	**100*	1.000	1.000	1.000	1.000
*LDH-1**	**100*	1.000	1.000	0.992	1.000
	**80*	0	0	0.008	0
*LDH-2**	**100*	1.000	1.000	1.000	1.000
*LDH-3**	**100*	0.994	1.000	1.000	0.979
	**30*	0.006	0	0	0.021
*MDH-1**	**120*	0.259	0.079	0.023	0
	**100*	0.741	0.921	0.977	1.000
*MDH-2**	**100*	1.000	1.000	1.000	1.000
*MEP-1**	**200*	0	0.006	0	0
	**100*	1.000	0.994	1.000	1.000
*MEP-2**	**-100*	0.989	0.972	0.945	0.948
	**-150*	0.011	0.022	0.047	0.052
	**-200*	0	0.006	0.008	0
*MPI**	**130*	0.034	0	0.039	0
	**100*	0.358	0.506	0.617	0.583
	**35*	0.602	0.489	0.344	0.417
	**15*	0.006	0.006	0	0
*PGDH**	**-10*	0	0	0	0.011
	**-100*	0.994	0.994	0.992	0.968
	**-175*	0.006	0.006	0.008	0.021
*PGM-1**	**110*	0.079	0.045	0.016	0.042
	**100*	0.449	0.455	0.381	0.469
	**85*	0.331	0.348	0.484	0.375
	**75*	0.107	0.140	0.071	0.094
	**65*	0.034	0.011	0.048	0.021
*PGM-2**	**110*	0.080	0.019	0.080	0.056
	**100*	0.866	0.971	0.900	0.833
	**90*	0.054	0.010	0.020	0.111
Alleles/Locus		1.919	2.054	1.838	1.703
Percentage of polymorphic loci	P*	0.324	0.297	0.324	0.297
	P	0.243	0.297	0.189	0.108
	P+P*	0.568	0.595	0.514	0.405
Average Heterozygosity	Ho	0.131	0.129	0.132	0.142
	He	0.127	0.125	0.124	0.124
	Ho/He	1.032	1.028	1.065	1.144

P*: Polymorphism less than 0.95. Ho : Observed heterozygosity.
P : Polymorphism greater than 0.95. He : Expected heterozygosity.

color pattern forms of *C. nigrolineata* represent intra-specific phenotypic variation, which is consistent with the conclusion from using another genetic marker, mitochondrial DNA[2].

Such shell color variation is also known in many other shells[21], and is separated into ecophenotypic and genophenotypic variation. In patellogastropods, including the nacellids, ecophenotypic conchological variation has been reported for some taxa including *Acmaea digitalis* (=*Lottia digitalis*)[22], *Lottia asmi* and *L. digitalis*[23], and *Notoacmea parviconoidea*[24]. Although shell color variation in these species has been considered to be due to ecological factors (differences in diet or microhabitat), such factors are hardly inferred for *C. nigrolineata* because the three phenotypic forms appeared in all samples from various localities (Table 2). Therefore, the three forms in *C. nigrolineata* are regarded as genophenotypic variation, for which some cases have been revealed by genetic and breeding studies. In particular, it is known that the shell color variation obeys Mendelian inheritance in some taxa[25].

However, even though emergence of the color pattern phenotypes in *C. nigrolineata* was genophenotypic, it might not inherit simply. When hypothesizing the allele responsible for the radially striped and dappled forms, respectively, each form is a homozygote of the responsible allele, while the complex form is hypothesized as a heterozygote between the two responsible alleles. Based on this hypothesis, when the H-W exact tests were performed for the samples, mismatch from the equilibrium was observed in some samples. This implies that there may be some other factors such as polygenes and incomplete dominance, as well as some environmental factors that might affect the emergence of the phenotypes.

Regarding the relationships between the samples, conchological and genetic peculiarities of each sample were found (Figs. 5, 7), suggesting that the samples were independent local populations. The mitochondrial DNA analysis for this species suggested the presence of some genetically distinct groups (Honshu, Shikoku to eastern Kyushu, western Kyushu, and southern Kyushu)[2], which is similar to the results of the present study. Also, similar geographic genetic diversity has been reported for some other marine gastropods that adhere to rocks including

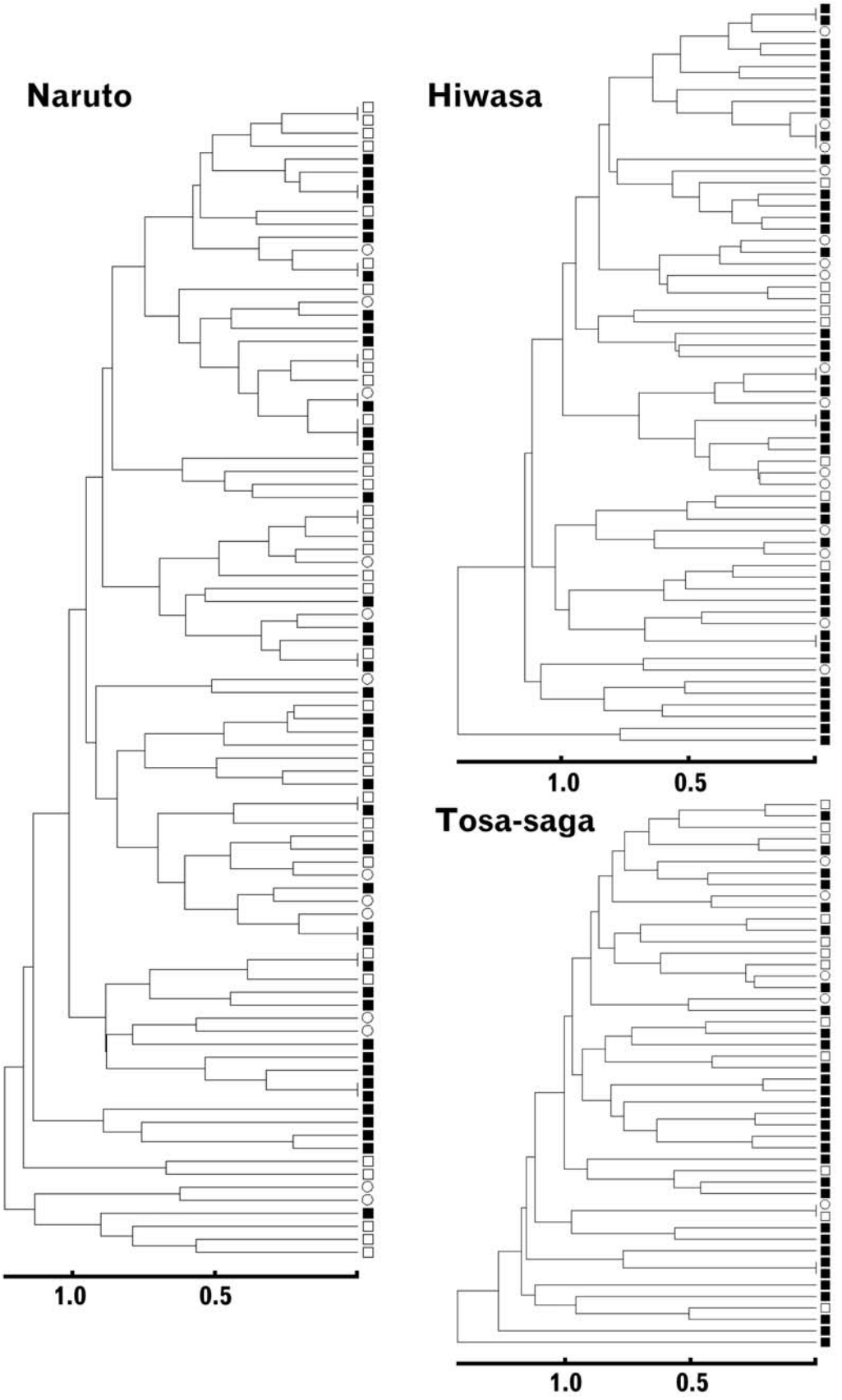

Fig. 6. Dendrograms based on individual genetic distances (*Di*) in some *Cellana nigrolineata* samples (the Aji sample was omitted because it comprised mostly the radially striped form only). Dark squares – Radial striped form, Open squares – Dappled form, Open circles – Complex form. Transverse axes indicate individual genetic distances (*Di*).

the Japanese abalones (*Haliotis discus discus* and *H. d. hannai*)[26] and the pulmonate limpets (*Siphonaria japonica* and *S.* sp.)[27]. This may be caused by the low mobility of those adhesive gastropods.

Cellana nigrolineata has a stable rest site and a home range individually, and does not migrate a long distance[28, 29], which implies low mobility. Although this species has planktonic larval stages (trochophore and veliger), occurrence of local populations suggests that planktonic larvae do not flow far away and rather little genetic exchange occurs among the populations. In addition, the somewhat high genetic variability of this species (Table 5) can be a basis for such independent populations.

It is also notable that color pattern phenotype compositions of the samples differed from one another, in particular the Aji sample, which was genetically the furthest from the others (Fig. 7, Table 6), comprising mostly the radially striped form only (Table 2). This may suggest that there is some linkage between genes that is responsible for isozymes and emergence of the color pattern phenotypes.

Table 6. Wright's *Fst* (above the oblique line) and Nei's genetic distances (*D*) (below the oblique line) between *Cellana nigrolineata* samples

	Aji	Naruto	Hiwasa	Tosa-saga
Aji		0.0123***	0.0302***	0.0261***
Naruto	0.0031		0.0099**	0.0076*
Hiwasa	0.0063	0.0059		0.0106***
Tosa-saga	0.0059	0.0024	0.0030	

Asterisks indicate significance of *Fst* vales for a null hypothesis (*Fst*=0); single, double, and triple asterisks indicate 5%, 1%, and 0.1% levels after Holm-Bonferroni correction, respectively.

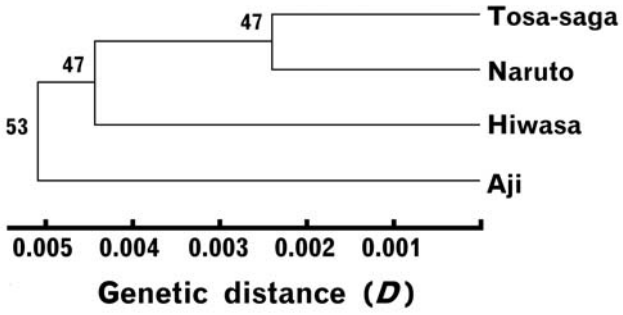

Fig. 7. A dendrogram based on population genetic distances (*D*) calculated from allelic frequencies of *Cellana nigrolineata* samples. Numbers in the dendrogram represent bootstrap probability values based on 1000 replicates.

Hualkasin *et al.*[30] examined mitochondrial and nuclear DNA of three shell coloration phenotypes of ivory shell (*Babylonia areolata*) from the Gulf of Thailand and reported that its genetic and phenotypic divergence were consistent with geography. Their nuclear DNA data also suggested some consistency between the phenotypes and gene structure. This information is so similar to the results of this study that *C. nigrolineata* may have a similar genetic structure.

On the other hand, another possible mechanism for the emergence of the differences in color pattern phenotype compositions by locality may be environmental factors. For example, it is known that two phenotypes (spiny and non-spiny forms) in horned turban, *Turbo cornutus*, are not inheritable but environment-dependent, *i.e.*, individuals grown in a rough coastal environment like the Pacific and Japan Sea coasts are spiny, and those grown in a calm coastal environment like the Seto Inland Sea are non-spiny[31, 32]. The fact that the sample from Aji, which is located in the Seto Inland Sea (Fig. 3), comprised mostly the radially striped form only, unlike the other samples (Table 2), is similar to the case of *T. cornutus*. This suggests the possibility of marine environmental factors being responsible for phenotype emergence of *C. nigrolineata*.

Although the present study suggested that the three shell coloration phenotypes in *C. nigrolineata* were intra-specific genophenotypic or environment-dependent variation, the emergence mechanism of the phenotypes still remains uncertain. In order to resolve this issue, breeding experiments are indispensable for revealing the mechanism. In addition, more detailed examinations of population genetics, conchology, and ecology of *C. nigrolineata* would be necessary, by using more samples from a wider area of distribution.

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主に四国周辺におけるマツバガイ 3 型の殻形態と遺伝的特徴

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ヨメガカサガイ科のマツバガイ 3 型（放射彩型、まだら型、混合型）の殻形態と遺伝的特徴について、主に四国周辺から採集した 6 標本群を材料に用いて調べた。殻形態について、殻の数部位を計測してそのデータを総合的に評価する主成分分析を行なった。第一主成分と第二主成分をプロットした散布図では、すべての標本群で 3 型は全くランダムに分布し、3 型の殻形態に相違がないことが示された。一方、すべての標本群を対象にした主成分分析では、散布図のプロットは標本群ごとによくまとまり、それぞれの形態的独自性が示唆された。遺伝形質としてアイソザイムを調べ37遺伝子座を推定した。個体間の遺伝的距離（*Di* 値）を標本群ごとに計算して分岐図を作成したところ、すべての標本群で 3 型は全くランダムに結合して特定の型によるクレードはなく、3 型に遺伝的な差がないことが示された。一方、標本群ごとの遺伝子頻度は *EST-3**、*MDH-1**、*MPI** などで相互に異なり、そのために標本群間の遺伝的距離（*D* 値）はやや大きく、それぞれ独自の地域個体群とみなされた。結論として、マツバガイ 3 型には形態的および遺伝的相違が全くないことから種内の形態的多型現象とみなされるが、マツバガイには形態的、遺伝的に分化した地域個体群が存在することが明らかになった。