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Variation and Abnormality of Genital System in *Littorina sitkana* Philippi (Mollusca, Gastropoda) in Northern Japan

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ABSTRACT—The anatomy of *Littorina sitkana* was examined using specimens from nine localities in northern Japan. These localities are known to be genetically clustered into two geographic groups; the first group consists of localities on the northern coast of Hokkaido along the Seas of Japan and Okhotsk and the second one comprises localities on the Pacific coasts of Hokkaido and Honshu. Anatomical surveys revealed that the mean numbers of penial glands differ significantly between the northern and Pacific sites, in correlation with the genetic variation. On the other hand, the mean relative size of the capsule gland within the pallial oviduct was positively correlated with mean sea-surface temperature, almost independently of the genetic difference. In addition, imposex was found for the first time in *L. sitkana*. Females frequently lacked the capsule gland in the imposex-present populations. The mean relative size of capsule gland to the whole pallial oviduct was significantly smaller there than in the imposex-free populations. The number of penial glands in males tended to be small in the imposex-present populations. The penis of imposex females was much shorter than that of males, in contrast to *Nucella* species occurring sympatrically on these coasts, in which the female penis is nearly as long as the male one. Although the pallial oviduct is reported to be replaced by a prostate gland in imposex females of *Littorina littorea*, such replacement was not found in any imposex female of *L. sitkana*.

INTRODUCTION

The genus *Littorina* includes marine gastropods that are common member of rocky epibenthic communities on all temperate seashores of the northern hemisphere (Reid, 1996). Two developmental modes, planktotrophic and nonplanktotrophic development, are known in this genus. Species with the latter mode have been well studied to examine the morphological and genetic variation that may suggest with their restricted gene flow (McQuaid, 1996; Reid, 1996). One of these direct developers is *Littorina sitkana* Philippi, 1846, which is widely distributed on North Pacific coasts. Boulding *et al.* (1993), Zaslavskaya (1995), and Zaslavskaya *et al.* (1994) found significant genetic differences among populations in this species. In northern Japan, Ohgaki (1983) and Nohara (1999) reported the geographic variation of shell sculpture in this snail. Further, Nohara (1999) showed that 19 examined populations on Japanese coasts are genetically clustered into two northern and southern groups; the former comprises populations on the Sea of Japan and Sea of Okhotsk sides of Hokkaido (abbreviated as JO subgroup), and the latter consists of populations in southeastern Hokkaido (SE subgroup), on the eastern side of southern Hokkaido's Oshima Peninsula (OP subgroup), and on the Pacific coasts of southern Hokkaido and the Tohoku District of Honshu (ST subgroup) (Fig. 1).

Great interspecific variation in the penis and pallial oviduct is recognized in the genus *Littorina*, probably related to speciation (Reid, 1989, 1990, 1996). The pallial oviduct is very different among *L. sitkana* and its four sibling species *L. kasatka* Reid, Zaslavskaya and Sergievsky, 1991, *L. subrotundata* (Carpenter, 1864), *L. natica* Reid, 1996, and *L. aleutica* Dall, 1872, despite their similarity in shell form (Reid, 1996; Reid and Golikov, 1991; Reid *et al*, 1991). On the other hand, the differences in penis morphology are relatively small among *L. sitkana* and the sibling species except *L. kasatka* (Reid, 1996; Reid and Golikov, 1991; Reid *et al.*, 1991). Although many researchers have been aware of a close relationship between speciation and the diversification of genital anatomy in this genus, the correlation between anatomical and genetic variation has not been closely studied.

In the present study, the penis and pallial oviduct of specimens of *L. sitkana* from northern Japanese coasts were anatomically surveyed. Populations of the northern (JO) and southern (SE+OP+ST) groups were compared to examine whether anatomical variation shows any correlation with the genetic variation found by Nohara (1999).

During the course of this study, imposex was found for the first time in *L. sitkana*. Imposex is the abnormal occurrence of penis-bearing females in some dioecious marine

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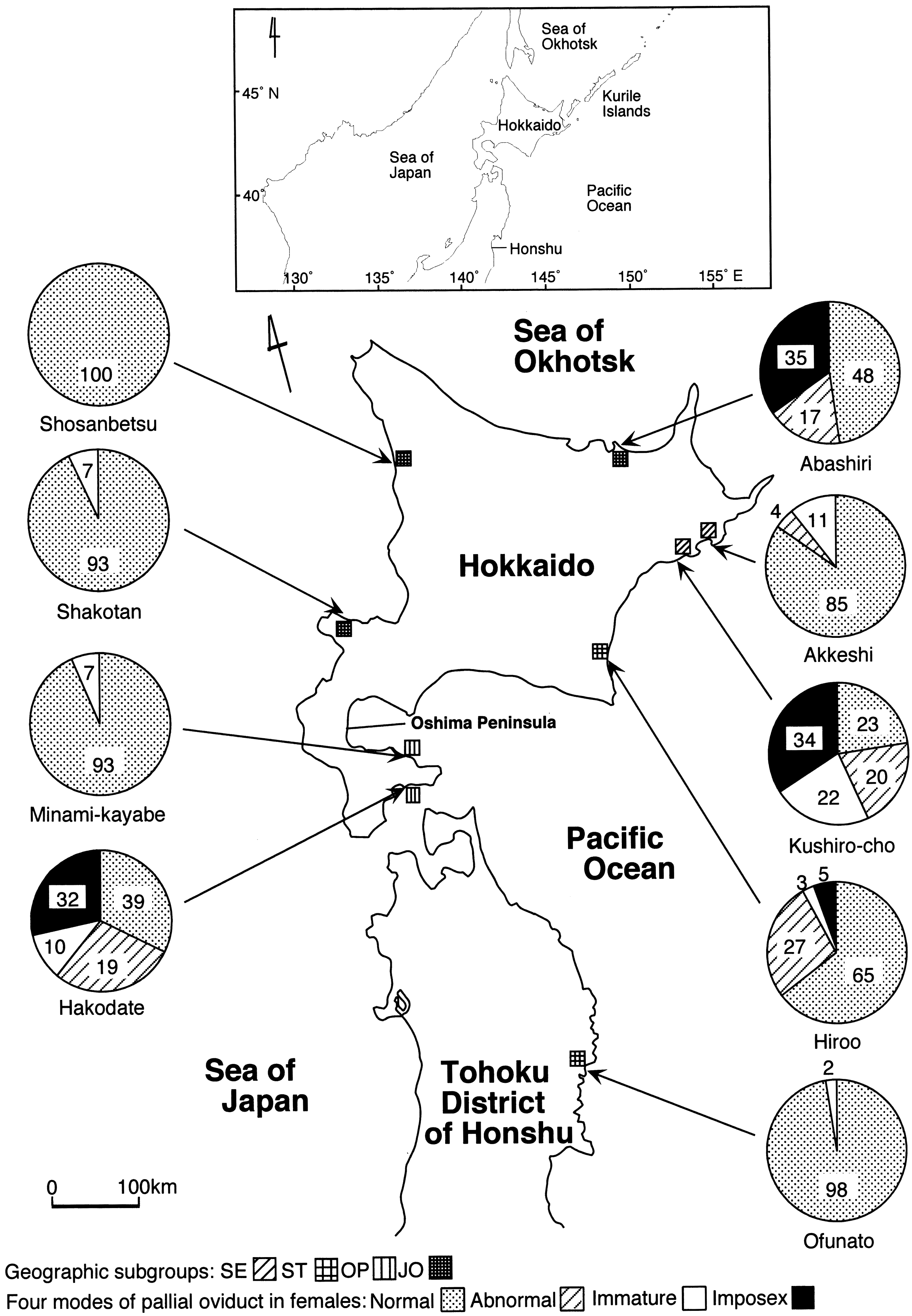

Fig. 1. Sampling localities of *Littorina sitkana* in northern Japan. Circle graphs show proportions of females with four genital modes at respective localities, with numbers indicating percentages of these modes. Localities are divided into four geographic subgroups based on genetic similarity (Nohara, 1999).

gastropods and is attributed to tributyltin (TBT) or triphenyltin (TPT) compounds used in antifouling coatings (*e.g.*, Gibbs *et al.*, 1987; Horiguchi *et al.*, 1994). It has already been recorded in 38 marine gastropods in Japan (Horiguchi *et al.*, 1997) and in three species of the *Littorina*, *L. kasatka*, *L. littorea* (Linnaeus, 1758), and *L. saxatilis* (Olivi, 1792) (Reid, 1996). Recently, Bauer *et al.* (1997) discovered that a high TBT concentration in tissue causes the reduced number of penial glands as well as imposex in *L. littorea*. A similar tendency was found in the populations displaying imposex in the present survey. The expression of morphological abnormality in the present material was also described in detail.

MATERIALS AND METHODS

Specimens

Two or three localities were chosen from each of four geographic subgroups of *L. sitkana* (Fig. 1): Abashiri, Shosanbetsu, and Shakotan on the northern side of Hokkaido facing the Seas of Japan and Okhotsk (JO subgroup); Akkeshi and Kushiro-cho on the Pacific coast of southeastern Hokkaido (SE); Minami-kayabe and Hakodate on the eastern half of the Oshima Peninsula in southwestern Hokkaido (OP); Hiroo and Ofunato on the Pacific coasts of southern Hokkaido and the Tohoku District of Honshu, respectively (ST). Sampling was carried out from middle to end of February in 1997 at all the localities and also at the end of March in 1995 at some of them (Table 1). Mating of *L. sitkana* was often observed at these localities during the sampling. More than 50 individuals, grown to mature size, were randomly collected at each locality. Most samples collected in 1997 were kept in different aquariums until laying eggs. Specimens were preserved in a freezer at –80°C until electrophoresis to examine the MDH locus where *L. sitkana* and its sibling species are distinguishable from one another (Nohara, 1999; Zaslavskaya, 1995). After electrophoresis, the specimens were kept frozen for later anatomical analysis.

All the specimens were clearly assigned to *L. sitkana* in the present study because the MDH locus was completely fixed with a single allele clearly different from the diagnostic alleles for the sibling species (Nohara, 1999).

Anatomy of penis and pallial oviduct

The soft parts of the snails were observed with a stereoscopic microscope after removal of the shells by a hammer. Shell heights of more than half of the examined snails were measured by a pair of vernier calipers before breaking the shells. The penis or pallial oviduct of each individual was drawn with the aid of a camera lucida. The figures were digitized by a scanner (resolution at 144 dpi) and analyzed by the public domain NIH Image program (available on the internet at http://rsb.info.nih.gov/nih-image/).

In males and females with a penis, penis length was measured along the edge opposite to the penial glands on the basis of digitized figures (Fig. 2b). The number of penial glands was also counted. Kendall's coefficients of rank correlations (τ) (Sokal and Rohlf, 1995) between shell height, penis length, and gland number were calculated. The mean numbers of penial glands were compared using mixed ANOVA (Sokal and Rohlf, 1995) within and between the northern (JO

Table 1. Measurements of shells and genital organs of *Littorina sitkana*. Relative size of capsule gland is calculated by dividing the area of the gland by the area of the whole pallial oviduct. N indicates the number of examined snails, followed by the number, in the parentheses, of individuals with an unusual penis or pallial oviduct within the examined snails. Geographic groups and subgroups are defined on the basis of genetic similarity (Nohara, 1999).

Geographic group		Southern						Northern		
Subgroup		SE		ST		OP		JO		
Locality		Akkeshi	Kushiro -cho	Hiroo	Ofunato	Minami -kayabe	Hakodate	Shakotan	Shosan-betsu	Abashiri
Male										
Shell height (mm)	Mean (SD)	12.0 (1.4)	11.8 (1.8)	12.6 (1.1)	12.2 (1.4)	10.5 (2.0)	13.6 (1.5)	13.6 (1.6)	9.2 (1.4)	11.2 (1.9)
	Range	9.5–13.8	9.1–14.1	9.6–14.7	9.6–16.1	7.3–14.0	9.7–16.7	11.0–16.9	6.3–11.7	6.7–14.2
Penis length (mm)	Mean (SD)	3.59 (0.43)	3.48 (0.77)	4.24 (0.84)	3.84 (0.47)	3.50 (0.45)	3.70 (0.49)	4.44 (0.54)	3.47 (0.61)	3.42 (0.73)
	Range	2.80–4.46	1.29–5.10	1.89–5.14	3.08–4.82	2.78–4.72	2.63–4.68	3.31–5.19	1.48–4.52	1.96–4.97
No. of penial glands	Mean (SD)	9.6 (1.9)	9.1 (4.1)	10.5 (2.3)	10.6 (1.8)	8.6 (2.4)	7.5 (4.9)	12.7 (2.4)	13.7 (3.6)	10.7 (2.0)
	Range	3–14	0–17	7–16	7–15	4–13	0–18	7–17	8–24	7–15
N		35 (1)	29 (5)	23	30 (1)	30 (1)	32 (12)	35 (2)	21 (1)	33 (2)
Female										
Shell height (mm)	Mean (SD)	12.8 (0.9)	13.1 (0.6)	12.3 (0.9)	12.7 (1.4)	12.6 (1.3)	12.9 (1.0)	13.6 (1.5)	10.7 (1.4)	12.0 (1.2)
	Range	10.4–14.1	11.9–14.0	10.0–14.5	9.4–15.6	9.8–14.3	10.5–14.8	10.4–16.6	6.3–13.3	10.2–13.8
Area of pallial oviduct (mm^2)	Mean (SD)	19.5 (5.2)	11.7 (2.8)	15.2 (5.8)	21.3 (6.1)	20.1 (6.1)	16.2 (4.1)	18.4 (5.7)	23.4 (6.6)	16.6 (4.6)
	Range	9.2–32.0	7.2–18.3	3.9–24.8	14.0–34.4	8.6–37.3	10.6–26.8	9.9–37.2	6.6–40.0	9.5–28.3
Area of capsule gland (mm^2)	Mean (SD)	3.13 (1.27)	1.06 (1.46)	1.55 (1.35)	5.55 (1.85)	4.56 (1.71)	1.33 (1.26)	4.10 (1.30)	5.16 (1.46)	1.60 (1.47)
	Range	0–5.44	0–5.01	0–4.18	3.03–10.2	1.55–9.41	0–4.13	1.78–7.35	1.99–9.14	0–5.87
Relative size of capsule gland	Mean (SD)	0.16 (0.05)	0.08 (0.11)	0.11 (0.08)	0.26 (0.03)	0.23 (0.04)	0.08 (0.07)	0.23 (0.04)	0.22 (0.03)	0.10 (0.08)
	Range	0–0.27	0–0.42	0–0.23	0.19–0.32	0.16–0.31	0–0.23	0.14–0.34	0.13–0.30	0–0.28
N		46 (7)	44 (34)	37 (13)	45 (1)	46 (3)	31 (19)	43 (3)	40	46 (24)
Sampling year and month		Mar. 95 Feb. 97	Mar. 95 Feb. 97	Feb. 97	Feb. 97	Mar. 95 Feb. 97	Feb. 97	Feb. 97	Feb. 97	Mar. 95 Feb. 97

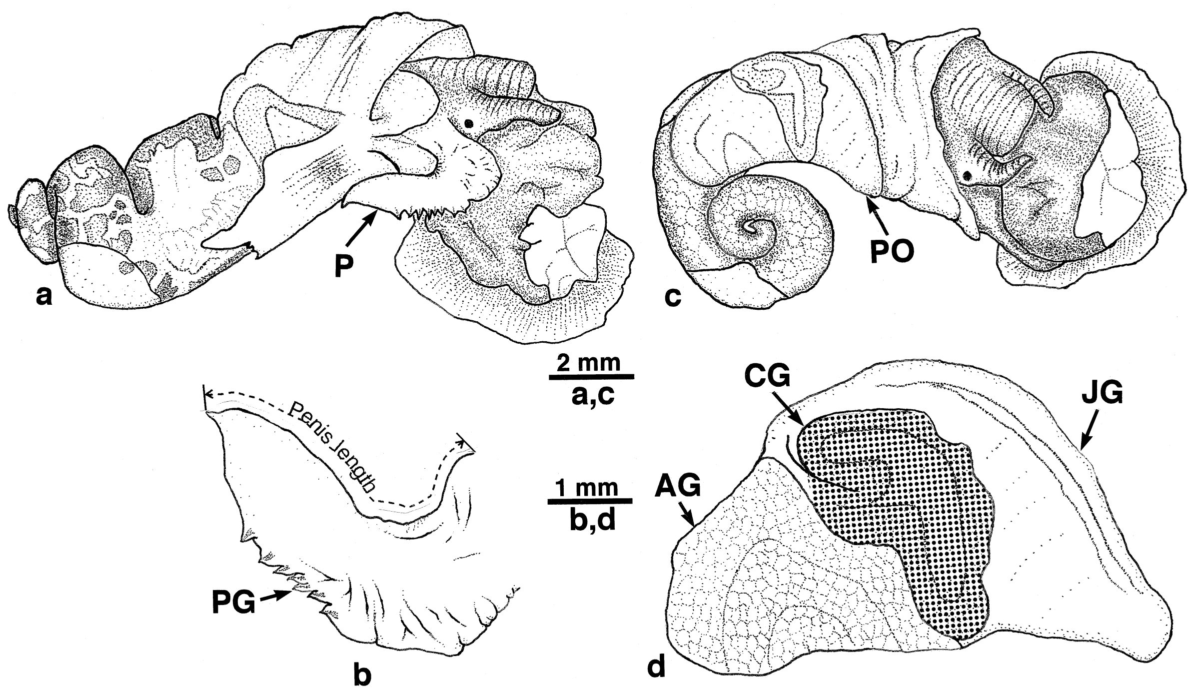

Fig. 2. Diagrams of male removed from shell (a), penis (b), female removed from shell (c), and pallial oviduct (d) of *Littorina sitkana* (right side view). Penis length was measured along the margin opposite to the penial glands. The shaded area in the pallial oviduct indicates the measured area of the capsule gland. AG: albumen gland; CG: capsule gland; JG: jelly gland; P: penis; PG: penial gland; PO: pallial oviduct.

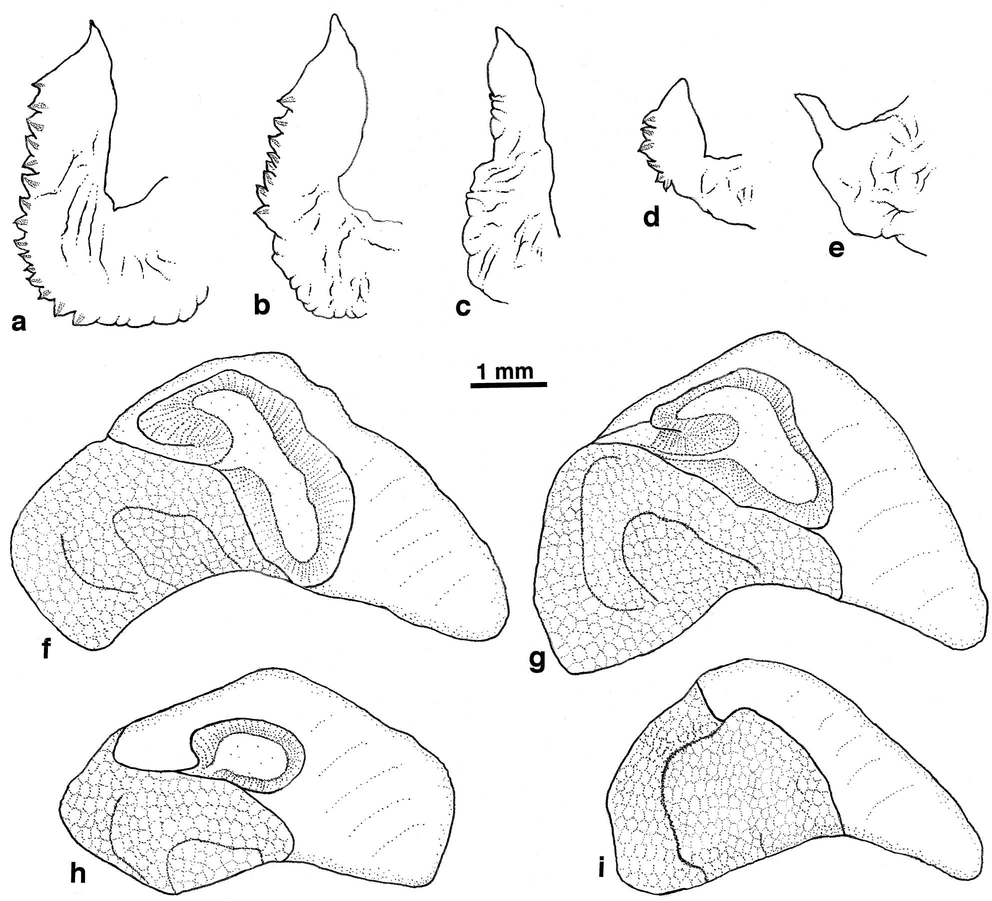

Fig. 3. Penes and pallial oviducts of specimens from (a) Shakotan, 12.9 mm in shell height; (b) Minami-kayabe, 12.8 mm; (c) Hakodate 11.9 mm; (d) and (h) Hakodate, 13.2 mm, imposex; (e) Kushiro-cho, 14.0 mm, imposex; (f) Ofunato, 11.5 mm; (g) Akkeshi, 12.1 mm; and (i) Hiroo, 12.3 mm. The penis in (c) has no penial glands. The pallial oviduct in (i) lacks a capsule gland.

subgroup only) and southern groups (SE+ST+OP). A posteriori comparisons between populations in the northern and southern groups were carried out by the Tukey-Kramale method (Sokal and Rohlf, 1995). Some males were excluded from the statistical analyses because of their unusual penes; they had sporadic or double-rowed glands instead of a usual sequential array of penial glands in a single line (Table 1). The Kushiro-cho, Hiroo, Hakodate, and Abashiri samples were completely removed from the statistical tests because imposex females occurred at these localities and Bauer *et al*. (1997) found that in *L. littorea* tributyltin (TBT) compounds caused not only imposex but also a significant reduction in gland number of males. The mean numbers of penial glands in males were compared using the Mann-Whitney U-test (Sokal and Rohlf, 1995) between the imposex-present and imposex-free populations within the two groups. Differences in penis morphology between imposex females (see below) and males of imposex-free populations were also examined by the Mann-Whitney U-test.

In females, a normal pallial oviduct is triangular and consists of three visibly distinct organs, the albumen gland, capsule gland, and jelly gland (Figs. 2d and 3f–h); abnormal oviducts lacked the capsule gland (Fig. 3i), which is an essential organ for the encapsulation of eggs (Reid, 1996). On the other hand, some females exhibited an immature pallial oviduct which was shrunk in contrast to the normal or abnormal pallial oviducts which looked well-swollen in the present samples. Penes were found in some females, which were treated as imposex in the statistical analyses regardless of whether their pallial oviducts were normal, abnormal, or immature. The proportions of specimens with the four modes of female genital tract (normal, abnormal, immature, and imposex) were calculated for each populations.

In specimens with a normal pallial oviduct, the areas of the capsule gland and the whole pallial oviduct were measured on digitized figures (Fig. 2d). In the specimens without a capsule gland, only the area of the whole oviduct was measured. Kendall's τ was calculated for the relationships among shell height, pallial oviduct area, and relative size of capsule gland. The mixed ANOVA and Tukey-Kramale methods were used to compare the mean relative sizes of the capsule gland. Some females were excluded from these statistical tests because of immaturity or abnormality of their pallial oviducts (Table 1). The Abashiri, Kushiro-cho, Hiroo, and Hakodate samples were not included in the statistical analyses because females with a penis or abnormal pallial oviduct appeared frequently. The mean areas of the pallial oviduct were compared using the Kruskal-Wallis test (Sokal and Rohlf, 1995) among three categories: the first category (Imposex-free—Normal) comprised females with a normal pallial oviduct in the five imposex-free populations; the second (Imposex-present—Normal) and third (Imposex-present—Abnormal) comprised females with normal and abnormal oviducts, respectively, in the four populations exhibiting imposex. The difference in the relative size of the capsule gland was examined between the former two categories by the Mann-Whitney U-test. The abnormal females found in the five imposex-free populations were excluded from comparisons because of their small numbers.

In each of Akkeshi, Kushiro-cho, Minami-kayabe, and Abashiri, where specimens were twice sampled, significant morphological differences in the penis and pallial oviduct were not detected between the two samples. Therefore, the those samples from each population were pooled in the statistical analyses.

Validity of mixed ANOVA

Normality and homoscedasticity were examined by Shapilo-Wilk's test (Takeuchi *et al.*, 1993) and Bartlett's test (Sokal and Rohlf, 1995), respectively. These tests proved that those assumptions of ANOVA were not violated.

RESULTS

Penis morphology

The penis length and the number of penial glands were positively correlated with shell height (Figs. 4a and 4b). The number of penial glands showed a positive correlation with penis length (Fig. 4c). Most males had a penis with penial glands ranging in number from 2 to 24 (Fig. 5). The modes of gland number were smaller than 12 in the southern populations, but 12 or greater in the northern group (Fig. 5). The mixed ANOVA revealed that the mean numbers of penial glands were significantly different between the two groups, although significant differences of the means were also detected among populations within the groups (Table 2). A posteriori tests also showed that the mean gland number was significantly larger at Shakotan and Shosanbetsu, two of the northern sites, than in any compared southern population (Table 3).

At Kushiro-cho and Hakodate some males lacked penial glands. An absence of penial glands is characteristic of *L. kasatka*, one of the sibling species of *L. sitkana*. However, these males were electrophoretically identified as *L. sitkana* because of the absence of the diagnostic allele of *L. kasatka* at the MDH locus (Nohara, 1999). Further, some of these unusual males had sculptured shells, which have never been recorded in *L. kasatka*. Tables 1 and 4 show that the mean number of penial glands tends to be smaller in the imposex-present populations than in the populations without imposex females. A significant difference in gland number was detected in the northern group (Table 4, Mann-Whitney U-test: $U=402$, $p<0.01$) while the statistical test was not significant in the southern group ($U=3638$, $p=0.50$).

Variation of pallial oviduct among imposex-free populations

Most females exhibited the "normal" condition of the pallial oviduct at Shakotan, Shosanbetsu, Akkeshi, Minami-kayabe, and Ofunato, where imposex was not found at all (Fig. 1). The area of the whole pallial oviduct varied greatly within populations (Table 1). The area of the pallial oviduct showed a correlation with shell height (Fig. 4d). The capsule gland forms a single loop in a normal pallial oviduct, with a large variation in loop size from a small circular one (Fig. 3h) to one broadly extending ventrally and dorsally (Fig. 3f). The relative size of the capsule gland ranged widely within populations (Fig. 6). The relative size had little correlation with shell height (Fig. 4e) nor with the area of the pallial oviduct (Fig. 4f). The mean relative size of the capsule gland was not significantly different between the northern and southern groups while the differences were significant among populations within each group (Table 2). A posteriori comparisons proved that the mean relative size of the capsule gland was significantly smaller at Akkeshi (0.16) than at any the other four populations surveyed (Table 5). At Ofunato, the mean relative size (0.26) was the largest in the five imposex-free localities.

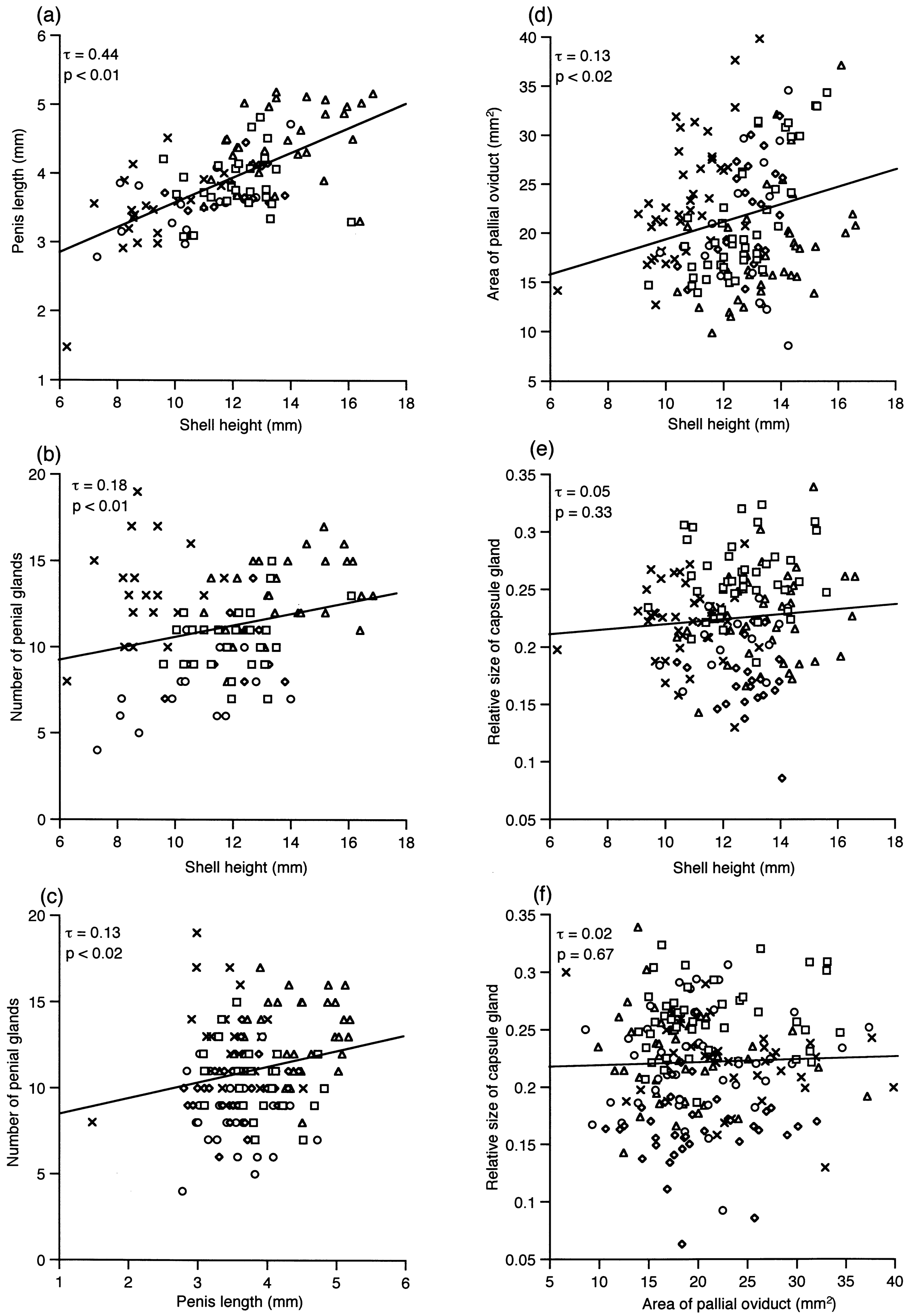

Fig. 4. Relationships among shell height and some anatomical characters: (a) shell height—penis length; (b) shell height—gland number; (c) penis length—gland number; (d) shell height—area of the whole pallial oviduct; (e) shell height—relative size of the capsule gland as part of the whole pallial oviduct; (f) area of the whole pallial oviduct—relative size of the capsule gland. Each relationship was examined using specimens from Akkeshi (◇), Ofunato (□), Minami-kayabe (○), Shakotan (△), and Shosanbetsu (×). Kendall's coefficient of rank correlation (τ) was calculated for each relationship. The probability (p) that the coefficient is significantly different from zero was also calculated.

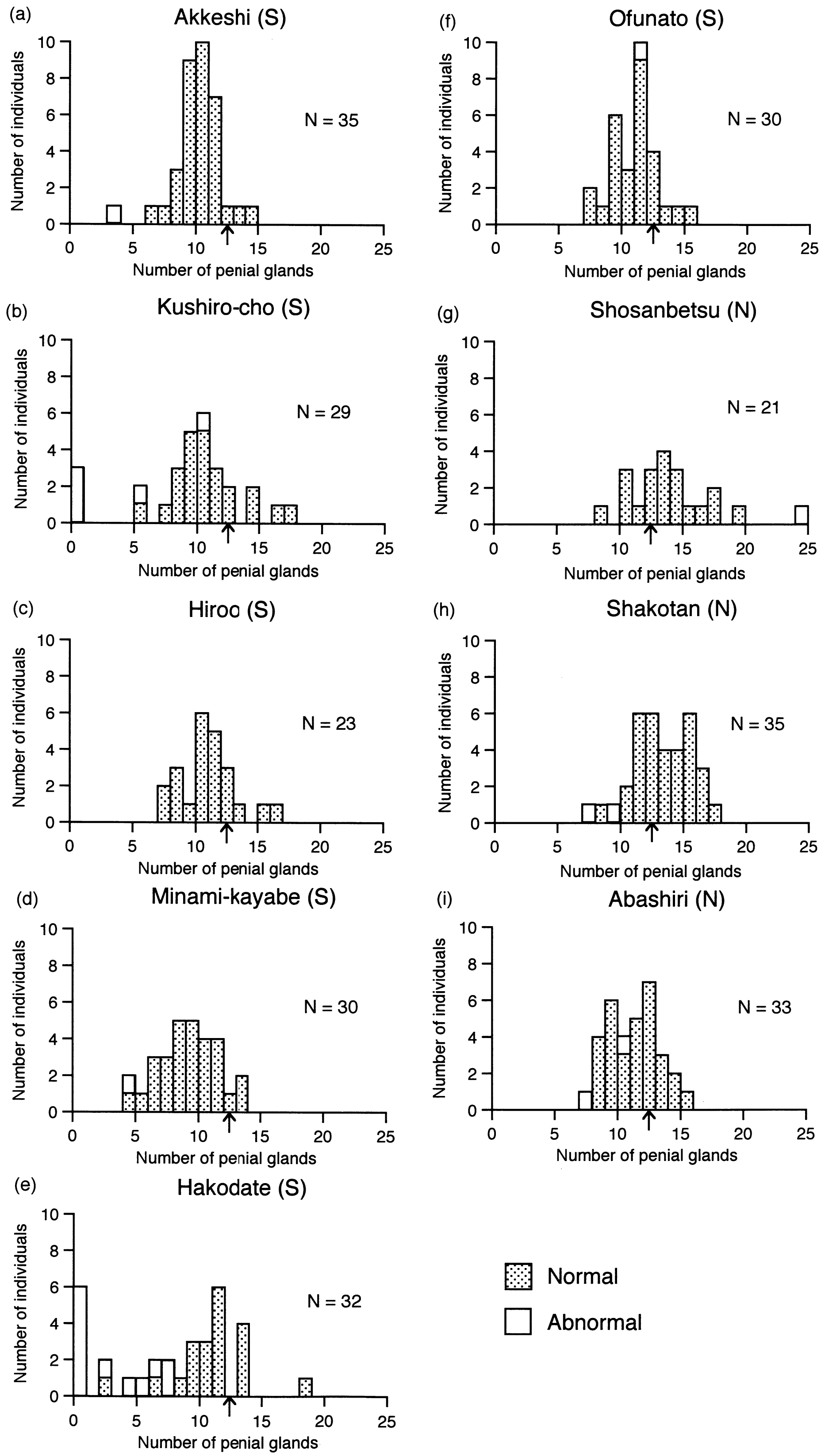

Fig. 5. Histograms of the number of penial glands of individuals from the local populations of Akkeshi (a), Kushiro-cho (b), Hiroo (c), Minami-kayabe (d), Hakodate (e), Ofunato (f), Shakotan (g), Shosanbetsu (h), and Abashiri (i). Individuals with an abnormal penis were included in the histograms. Arrows below the horizontal axis indicate the class of 12 penial glands. N and S following the locality name indicate, respectively, the northern and southern group based on genetic similarity (Nohara, 1999).

Table 2. Results of mixed ANOVA for the number of penial glands and the relative size of the capsule gland.

	df	SS	MS	F	P
Number of penial glands					
Geographic group	1	373	373	85.9	<0.001
Population	3	45.6	15.2	3.50	<0.02
Residual	140	607	4.34		
Relative size of capsule gland					
Geographic group	1	0.001	0.001	1.07	0.302
Population	3	0.178	0.059	45.6	<0.001
Residual	201	0.261	0.001		

Table 3. A posteriori comparisons of the number of penial glands between populations of the northern and southern groups. Significant levels are indicated by * ($p<0.05$).

		Northern group	
		Shakotan	Shosanbetsu
Southern group	Akkeshi	*	*
	Hiroo	*	*
	Minami-kayabe	*	*
	Ofunato	*	*

Table 4. Comparisons of the number of penial glands between imposex-free and imposex-present populations within the two geographic groups. N indicates the number of examined males.

	Imposex-free	Imposex-present
Northern group		
Mean (SD)	13.1 (2.3)	10.8 (2.0)
Range	8–19	7–15
N	53	31
Southern group		
Mean (SD)	9.6 (2.2)	8.9 (4.2)
Range	4–15	0–18
N	92	84

Imposex and abnormality of pallial oviduct

Imposex females were found at Abashiri, Kushiro-cho, Hiroo, and Hakodate (Fig. 1).

The mean area of the whole pallial oviduct was significantly smaller in imposex-present populations than in imposex-free populations (Table 6, Kruskal-Wallis test: $H=64.5$, $p<0.01$). The relative size of the capsule gland, even if the pallial oviduct appeared normal, was significantly smaller in the four populations with imposex females than in the other five populations (Table 6, Mann-Whitney U-test: $U=2741$, $p<0.01$). Penis length in imposex females, ranging from 0.46 to 3.39 mm, was significantly smaller than that in males (Table 7, Mann-Whitney U-test: $U=82.5$, $p<0.01$). The number of penial glands was also significantly smaller in imposex females than in males (Table 7, Mann-Whitney U-test: $U=391$, $p<0.01$).

DISCUSSION

Penis morphology

In some North Atlantic species of *Littorina*, the number of penial glands shows a large intraspecific variation and a significant positive correlation with shell height (Heller, 1975; Janson, 1982; Raffaelli, 1979). In the present study, the number of glands in *L. sitkana* also varied widely among populations and there was a significant correlation between gland number and shell height.

In *L. saxatilis*, variation of gland number is known to be related to the extent of exposure to wave action; the number of penial glands is correlated with the intensity of wave impact, as well as shell height, positively in Nova Scotia (Davis, 1973), or negatively on the west coast of Sweden (Janson, 1982). In the present study, geographic variation of the number of penial glands in *L. sitkana* seems to have something to do with genetic differentiation of this snail rather than with environmental condition (*e.g.* wave regime) since Nohara (1999) revealed that a genetic difference was apparent between the northern coasts of Hokkaido (northern group in the present study) and the Pacific coasts of Hokkaido and the Tohoku District of Honshu (southern group).

Pallial-oviduct morphology

Reid (1996) showed that in *L. sitkana* the capsule gland always forms a large simple loop. It was true of most female specimens in the present study. However, some females exhibited a very small or no capsule gland. A reduction or an absence of the capsule gland may have been induced environmentally by tributyltin (TBT) because such a condition was frequently found in the four populations exhibiting imposex probably due to this organotin compound. On the other hand, abnormal pallial oviducts might instead be caused by parasitic digenean trematodes, which are known to damage the pallial oviduct in *L. littorea* (Reid, 1996; Robson and Williams, 1971). This possibility was rejected in the present case because no trematodes were found in any dissected abnormal pallial oviducts.

In the present study, the relative size of the capsule gland to the whole pallial oviduct differed among the five imposex-free populations. As shown in Fig. 7, the mean relative size tended to become smaller gradually towards the northeast, and this tendency seems to be positively correlated with the mean sea-surface temperature. The largest mean relative size of the capsule gland (0.26) was found at Ofunato with the highest mean sea-surface temperature in February (*ca.* 9°C) and August (*ca.* 23°C) while the smallest mean relative size (0.16) and the lowest mean water temperatures (*ca.* 2°C and 17°C in February and August, respectively) were recorded at Akkeshi (National Astronomical Observatory, 1996). On the other hand, the mean relative size was quite similar among Shakotan, Shosanbetsu, and Minami-kayabe, which have similar mean sea-water temperatures. However, this possible correlation could not be statistically tested because of the lack of local sea water temperature data at the sampling sites.

Imposex in Japanese *L. sitkana*

Horiguchi *et al.* (1994) revealed that in intertidal gastropods *Thais clavigera* (Küster, 1858) and *T. bronni* (Dunker,

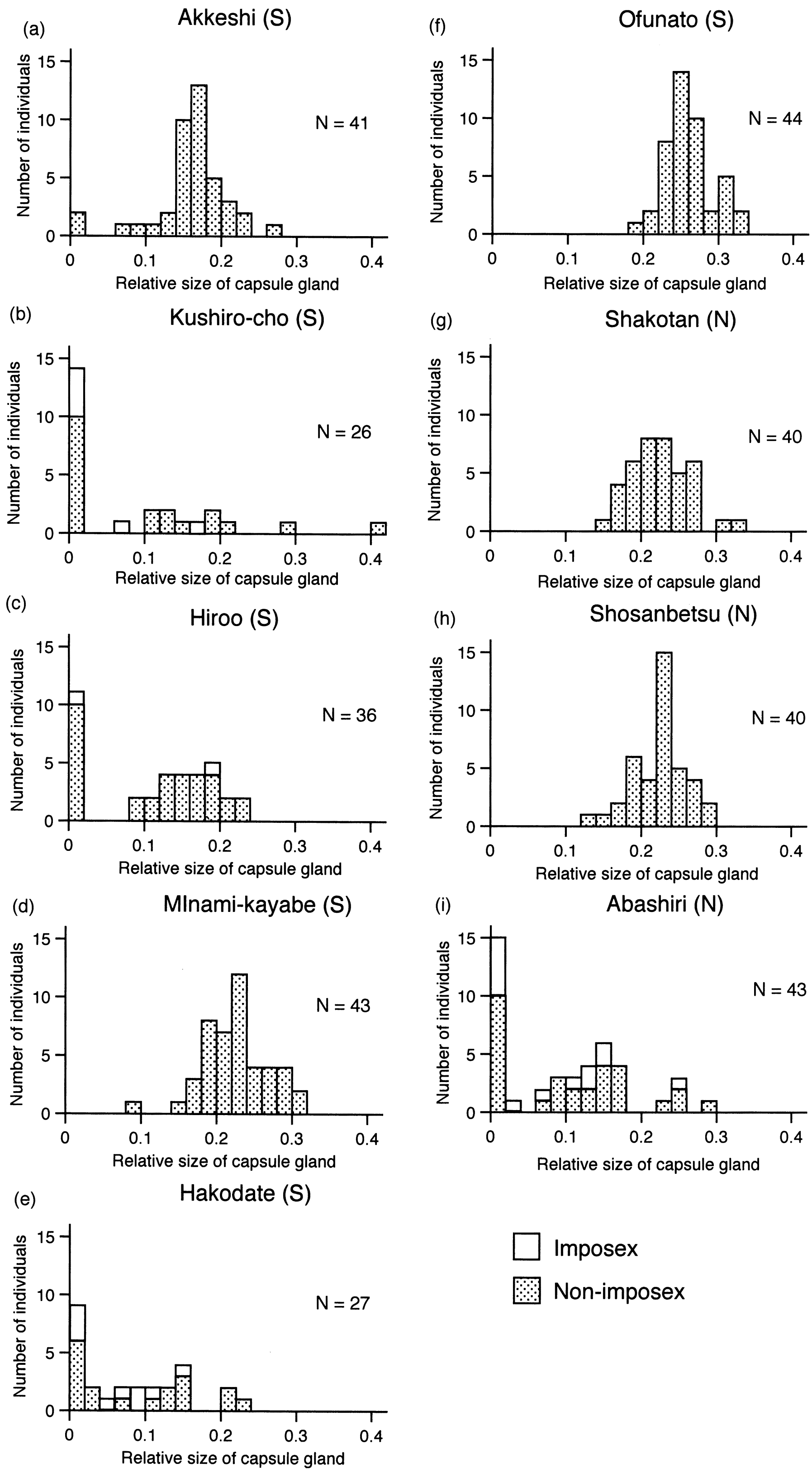

Fig. 6. Histograms of the relative size of the capsule gland as part of the whole pallial oviduct of individuals from the local populations of Akkeshi (a), Kushiro-cho (b), Hiroo (c), Minami-kayabe (d), Hakodate (e), Ofunato (f), Shakotan (g), Shosanbetsu (h), and Abashiri (i). Imposex females were included in the histograms when their pallial oviducts were "normal" or "abnormal" condition. On the other hand, females with an immature pallial oviduct were excluded. N and S following the locality name indicate, respectively, the northern and southern group based on genetic similarity (Nohara, 1999).

Table 5. A posteriori comparisons of the relative size of the capsule gland among five imposex-free populations. Significant levels are indicated by * ($p<0.05$) and – ($p\geq0.05$).

	Akkeshi	Ofunato	Minami-kayabe	Shakotan
Ofunato	*			
Minami-kayabe	*	*		
Shakotan	*	*	—	
Shosanbetsu	*	*	—	—

Table 6. Comparison of pallial oviduct between populations with and without imposex individuals. Females are divided into two categories of the capsule gland expression (Normal: with a complete capsule gland; Abnormal: without the gland). N indicates the number of examined females.

	Imposex-free	Imposex-present	
	Normal	Normal	Abnormal
Whole pallial oviduct (mm^2)			
Mean (SD)	20.6 (6.1)	15.5 (5.2)	14.7 (4.2)
Range	6.6–39.8	3.9–27.6	7.6–28.3
Relative size of capsule gland			
Mean (SD)	0.22 (0.05)	0.15 (0.06)	
Range	0.06–0.34	0.02–0.42	
N	206	68	33

Table 7. Comparison of penis morphology between males and imposex females. N indicates the number of examined snails.

	Males	Imposex females
Penis length (mm)		
Mean (SD)	3.80 (0.61)	2.02 (0.61)
Range	1.48–5.19	0.46–3.39
Number of penial glands		
Mean (SD)	10.9 (2.7)	4.7 (3.2)
Range	4–19	0–12
N	145	43

1860) all females have a penis in almost all the examined populations around Japan except in Hokkaido. Further, Horiguchi *et al*. (1997) defined a relative penis length index, RPL: (mean penis length in female / that in male) ×100. They found that the RPL indices in four dog-whelks *Nucella freycineti heyseana*, *N. freycineti alabaster*, *N. emarginata*, and *N. lima* varied from 80.7 to 92.1 on the coasts of Hokkaido and the Tohoku District of Honshu. In *L. sitkana*, the proportion of imposex females in a population, ranging from 5% to 35%, was much smaller than those in *T. clavigera* and *T. bronni*. Imposex-present populations only rarely occurred in *L. sitkana*; imposex females were found only at four of 19 localities examined electrophoretically by Nohara (1999). The RPL index in *L. sitkana*, estimated from Table 6, was 53.3, much smaller than those of the four dog-whelks species occurring sympatrically on northern Japanese coasts. The different food habits between *L. sitkana* and Japanese muricid species might be one of the possible causes of their differences mentioned above; the former snail is a herbivore while the latter ones are carnivores.

In *L. littorea*, the pallial oviduct is gradually replaced by a prostate gland and a penis in accordance with increased concentration of TBT in tissue, although a penis appears only at higher TBT concentrations (Bauer *et al*., 1995, Bauer *et al*., 1997). On the contrary, this gradual replacement of the reproductive organs was not found in *L. sitkana*: penis-bearing females always had a pallial oviduct, which varied in capsule gland size from complete lack of the gland to its full development. However, females without the capsule gland frequently occurred in the imposex-present populations. Further, the mean relative size of the capsule gland was significantly smaller there than in the imposex-free populations, suggesting a possible influence of TBT compounds on the development of the capsule gland.

Some males completely lacked penial glands at Kushiro-cho and Hakodate where imposex was also seen. In addition, the number of penial glands was significantly smaller at the Abashiri population than any other northern populations, although significant differences were not detected between imposex-present and imposex-free populations in the southern group. These abnormalities in male reproductive organ may be also due to TBT pollution at the imposex-present populations since Bauer *et al*. (1997) recently discovered that a high TBT concentration in tissue caused a significant decrease in gland number as well as imposex in *L. littorea*.

Imposex is also known to prevent reproduction in some snails. In *T. clavigera*, a well-developed penis in females obstructs the genital opening and keeps the females from laying eggs (Horiguchi *et al*., 1994). Matthiessen *et al*. (1995) found that survival of eggs and/or larvae may be impaired by TBT pollution in *L. littorea*. Accordingly, imposex is likely to influence the effective population size and the level of genetic diversity of affected populations. However, the average heterozygosity (Nei, 1987) estimated from Nohara's (1999) allozyme data was not significantly different between imposex-present (H=0.310) and imposex-free populations (H=0.304). This result may indicate that there has been no significant decrease in the effective population size in the four populations displaying imposex. In the present survey, most of the imposex females had a small penis, which may not disturb mating nor laying eggs. In fact, I observed some instances of copulation between imposex females and males in the laboratory. In addition, observations of serial sections showed that encapsulated eggs were produced in the abnormal pallial oviducts. These facts may suggest that females with a penis or an abnormal pallial oviduct can still contribute to reproduction, at least to some extent. However, the population size might progressively get smaller in the imposex-present populations in the future. Therefore, the population size of *L. sitkana* should be carefully surveyed for long term.

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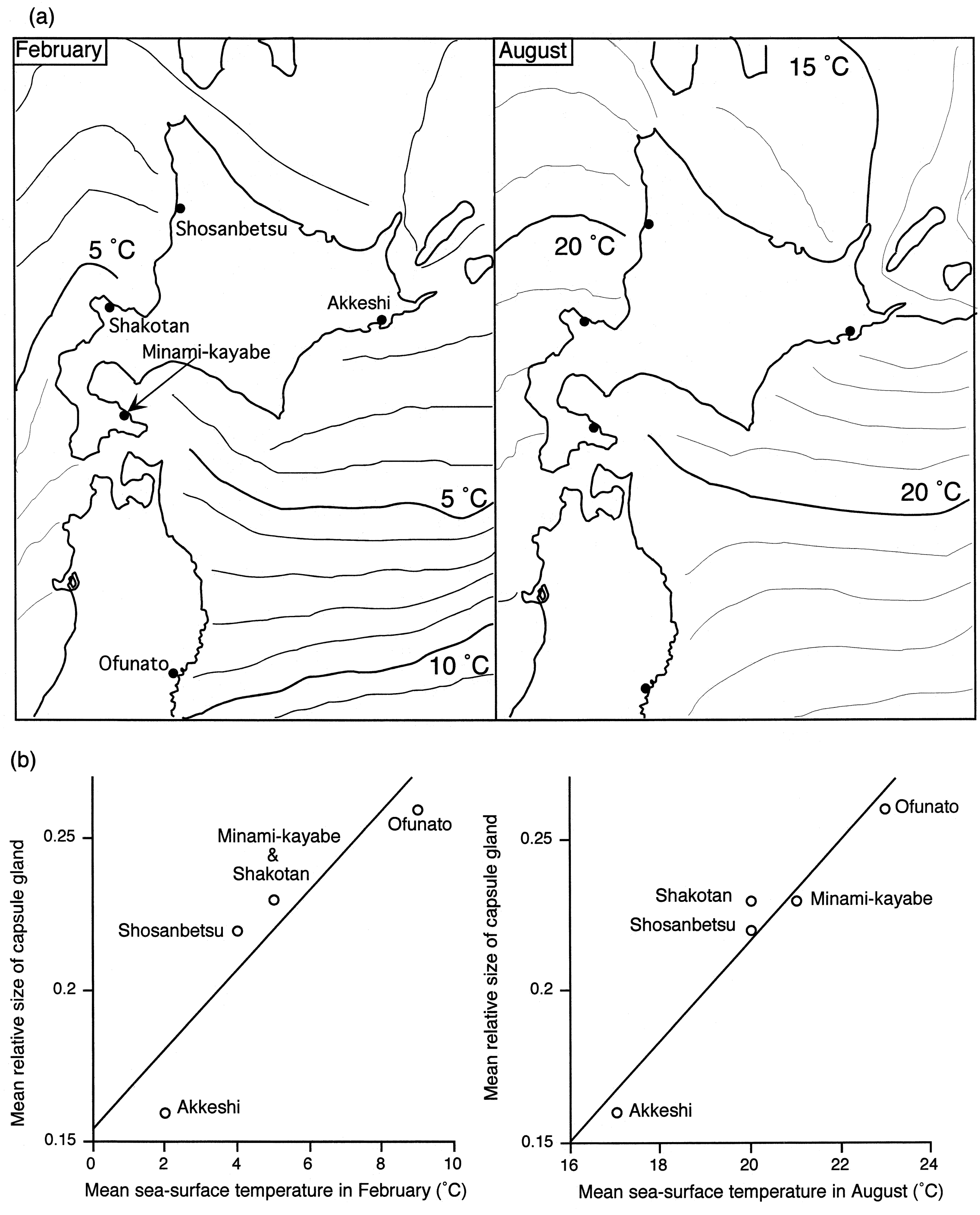

Fig. 7. (a) The mean sea-surface temperature from 1961 to 1990 around Japan in February and August (National Astronomical Observatory, 1996). (b) Relationships between the relative size of the capsule gland as part of the whole pallial oviduct and the mean sea-surface temperature in February and August.

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