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Genetic differentiation in two sibling species of the brackish-water polychaete *Hediste japonica* complex (Nereididae)

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Abstract Genetic divergence among ten populations of small- and large-egg forms of the brackish-water polychaete *Hediste japonica* complex was investigated on 14 isozyme loci by electrophoretic analysis. The two forms were distinguishable by complete allele substitutions at five loci, resulting in high genetic differentiation (Nei's *D*: 0.533 to 0.662). No genetic evidence of hybridization between the two forms was detected in sympatric populations in three rivers. These results indicate that the two forms are reproductively isolated, clearly showing that the two forms are distinct species. The genetic differentiation among populations was higher in the large-egg form (*D*: 0.005 to 0.111, G_{ST}: 0.435) than that in the small-egg form (*D*: 0.000 to 0.001, G_{ST}: 0.020). This genetic difference between the two forms seems to be attributable to a difference in their life histories. The average expected heterozygosity was low in populations of both the large-egg form (0.005 to 0.068) and the small-egg form (0.014 to 0.038) in comparison with other marine invertebrates.

Introduction

The nereidid polychaete *Hediste japonica* (Izuka 1908) (= *Neanthes japonica*) commonly inhabits brackish waters in and around Japan (Imajima 1972). This species constitutes an allopatric sibling species complex together with *H. diversicolor* inhabiting Europe and the east coast of North America and *H. limnicola* inhabiting the west coast of North America (Smith 1958; Fong and Garthwaite 1994).

Our previous studies have suggested that *Hediste japonica* consists of two distinct species (small- and large-egg forms) which differ in egg diameter and developmental pattern (Sato and Tsuchiya 1991), reproductive behavior, salinity preference for early development (Sato and Tsuchiya 1987), and karyotype (Sato and Ikeda 1992). For further investigation to clarify the taxonomic status of the two forms of *H. japonica*, allozyme electrophoretic analysis was carried out.

Electrophoretic analysis of allozymes has been useful to demonstrate genetic isolation between morphologically similar cryptic species in various aquatic organisms including polychaetes (Grassle and Grassle 1976; Britton-Davidian and Amoureux 1982; Cadman and Nelson-Smith 1990; Manchenko and Radashevsky 1993), shrimps (Chow and Fujio 1985), bivalves (Seed 1992), echinoderms (Matsuoka and Hatanaka 1991), nemerteans (Manchenko and Kulikova 1996), corals (Stobart and Benzie 1994) and fishes (Masuda et al. 1989; Yamazaki and Goto 1996), and also useful to quantify the genetic differentiation among local populations in a species (e.g., Chow et al. 1988; Bristow and Vadas 1991; Grant and Lang 1991; Shimizu et al. 1993; Fong and Garthwaite 1994; Ward and Andrew 1995; Williams and Benzie 1996). By electrophoretic analysis, we have compared the genetic bases of ten Japanese populations of the small- and large-egg forms of *H. japonica*, both of which occurred sympatrically in three rivers. Here we report on genetic differentiation between the two forms and among populations within each form, providing strong evidence that the two forms are genetically isolated and constitute separate species.

Materials and methods

Collection of samples

The small- and large-egg forms are well distinguishable in sexually mature worms by reproductive behavior and egg size (Sato and Tsuchiya 1987, 1991). Mature worms of the small-egg form were obtained from five localities of brackish waters in Japan (Fig. 1;

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Table 1). The worms were collected by scoop net during reproductive swarming near water surface at night during spring tides of the cold season (February to April). Mature eggs obtained from females in these populations were 140 to 170 μm in diameter, and had translucent ooplasm. These characteristics are consistent with the small-egg form reported previously (Sato and Tsuchiya 1987, 1991). On the other hand, reproductive swarming does not occur in the large-egg form (Sato and Tsuchiya 1987). Mature worms of the large-egg form were obtained from three brackish-water habitats in Kagoshima Prefecture from September to October, and one more habitat in Aomori Prefecture in May (Fig. 1; Table 1). They were collected by picking them out of sediment samples which were dug from intertidal flats with a shovel. The larger size of oocytes (more than 170 μm in diameter) and opaque ooplasm were used as diagnostic characters for the large-egg form. Mature males collected together with females of the large-egg form were also regarded as the large-egg form.

Immature worms which were collected from Gamo Lagoon near the mouth of the Nanakitagawa River in Miyagi Prefecture in July 1994 were also used as material of the large-egg form because the large-egg form is known to be common there (Kikuchi personal communication), and because all of the worms collected had diagnostic alleles of the large-egg form (see "Results").

Living materials were transported to the laboratory, put into small vinyl bags individually, and deep-frozen at −80 °C until used for electrophoretic analysis.

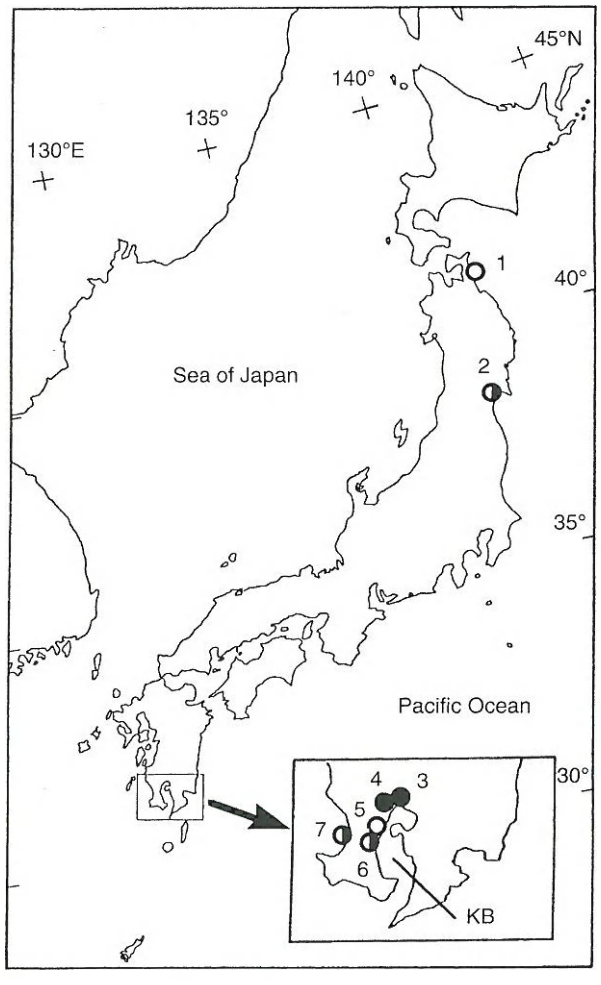

Fig. 1 *Hediste japonica.* Map showing sampling sites (*1* Obuchinuma Swamp, Aomori Prefecture; *2* Nanakitagawa River, Miyagi Prefecture; *3* Beppugawa River, Kagoshima Prefecture; *4* Omoigawa River, Kagoshima Prefecture; *5* Kotsukigawa River, Kagoshima Prefecture; *6* Nagatagawa River, Kagoshima Prefecture; *7* Kaminokawa River, Kagoshima Prefecture). Specimens of large-egg form (○), small-egg form (●) or both forms (◑) were collected (*KB* Kagoshima Bay)

Table 1 *Hediste japonica.* Sample data. Sexally mature adults were used for samples, except for immature adults of the large-egg form in Nanakitagawa River (*) (*Numbers in parentheses* site numbers, see Fig. 1)

Form, locality	Period of collection	No. of individuals
Large-egg form		
Obuchinuma Swamp, Aomori Pref. (1)	31 May 1992	28
Nanakitagawa River, Miyagi Pref. (2)	14 July 1994	51*
Kotsukigawa River, Kagoshima Pref. (5)	Sep–Nov 1991	22
Nagatagawa River, Kagoshima Pref. (6)	Sep–Oct 1991	41
Kaminokawa River, Kagoshima Pref. (7)	Sep–Oct 1991	14
Small-egg form		
Nanakitagawa River, Miyagi Pref. (2)	26 Apr 1994	52
Beppugawa River, Kagoshima Pref. (3)	13 Mar 1994	40
Omoigawa River, Kagoshima Pref. (4)	1 Mar 1991	11
Nagatagawa River, Kagoshima Pref. (6)	Feb–Mar 1992	44
Kaminokawa River, Kagoshima Pref. (7)	Feb–Mar 1992	10

Electrophoresis

Horizontal starch gel electrophoresis was used to determine allele frequencies at 14 loci coding for ten enzymes (Table 2). Frozen samples of a whole body or a piece of body were placed in a plastic multiwell plate with a few drops of distilled water, and then cut into pieces with scissors. Crude protein extracts of thawing samples were absorbed onto filter paper wicks (No. 51A, Toyo Filter Paper Co., Tokyo, Japan) and subjected to electrophoresis. Gels were prepared by using 12.5% Starch-Hydrolysed (Connaught Laboratories Limited, Canada) and two buffer systems, i.e., citric acid, *N*-(3-aminopropyl) diethanolamin (CAEA, pH 7), and citric acid, *N*-(3-aminopropyl) morpholine (CAPM, pH 6) (Clayton and Tretiak 1972). Staining methods followed those cited in Table 2.

Multiple loci for a given enzyme were numbered from the most anodal locus. At each locus, the most common allele in the small-egg form was named **100* (for anodal migration) or **−100* (for cathodal migration), and the other alleles were named according to their relative mobility to the **100* allele.

Allele frequencies were calculated from observed genotypes. A locus was assumed to be polymorphic in each population if the frequency of the most common allele did not exceed 0.95. Observed heterozygosity (h_o) was obtained from direct counts of heterozygotes, and expected heterozygosity (h_e) was calculated as $h_e = 1-\Sigma x_i^2$, where x_i is the frequency of the i-th allele at each locus. Mean heterozygosities (H_o and H_e) for each population were obtained from the formulas, $H_o = \Sigma h_o/r$ and $H_e = \Sigma h_e/r$, where r is the number of loci. A coefficient of gene differentiation (G_{ST}) (Nei 1975) was calculated to estimate interpopulational diversity in both the large- and small-egg forms. Genetic distances (D) (Nei 1972) were used for evaluation of genetic differentiation among populations. A dendrogram based on Nei's D was constructed using the

Table 2 *Hediste japonica*. Enzymes, loci detected, buffer systems used and references for staining procedure. Enzyme commission numbers are given in parentheses

Enzyme	Abbreviation	Locus	Buffer system	Reference, staining procedure
Asparate transaminase (2.6.1.1)	ATA	*Ata-1**	CAPM	Taniguchi and Numachi (1978)
		*Ata-2**	CAPM	
Adenylate kinase (2.7.4.3)	AK	*Ak**	CAEA	Shaw and Prasad (1970)
Glycerol-3-phosphate dehydrogenase (1.1.1.8)	G3PDH	*G3pdh-1**	CAEA	Numachi (1971)
		*G3pdh-2**	CAEA	
Glucose-6-phosphate isomerase (5.3.1.9)	GPI	*Gpi**	CAPM	Shaw and Prasad (1970)
Isocitrate dehydrogenase (1.1.1.42)	IDHP	*Idhp-1**	CAEA	Taniguchi and Numachi (1978)
		*Idhp-2**	CAEA	
Lactate dehydrogenase (1.1.1.27)	LDH	*Ldh-1**[1]	CAPM	Numachi (1970)
		*Ldh-3**	CAPM	
Malate dehydrogenase (1.1.1.37)	MDH	*Mdh-1**[1]	CAPM	Numachi (1970)
Phosphogluconate dehydrogenase (1.1.1.44)	PGDH	*Pgdh**	CAPM	Taniguchi and Numachi (1978)
Phosphoglucomutase (5.4.2.2)	PGM	*Pgm**	CAEA	Shaw and Prasad (1970)
Superoxide dismutase (1.15.1.1)	SOD	*Sod**	CAPM	Numachi (1972)

[1] *Ldh-2** and *Mdh-2** loci appeared to exist but could not be detected clearly

unweighted pair-group method with arithmetic averaging clustering procedure (UPGMA, Sneath and Sokal 1973).

Results

Allele frequencies in ten populations of two forms are shown in Table 3. The two forms did not share alleles at five loci out of 14 loci tested, i.e., *G3pdh-1**, *G3pdh-2**, *Idhp-2**, *Ldh-3**, *Pgdh**. At an additional locus (*Ak**), nearly complete substitution of allele between the two forms was also observed. No hybrids between the two forms were found in three rivers where both forms occur sympatrically. Four loci (*Ata-1**, *Ata-2**, *Idhp-1**, *Sod**) were monomorphic ($P > 0.95$), fixed in the same allele in all populations of both forms. The observed number of genotypes seemed to be consistent with the expectations of Hardy–Weinberg equilibrium at all polymorphic loci in all populations, though significancy of the difference between them could not be assessed by χ^2-test because of the small sample sizes.

Nei's genetic distance (D) was calculated for each pair of populations (Table 4). Based on the D-values, a dendrogram was drawn by the UPGMA clustering (Fig. 2). The dendrogram showed two major clusters, clearly separating the two forms. Between the two forms, the D-values ranged from 0.533 to 0.662 (average: 0.581).

The genetic differentiation among populations of the large-egg form was relatively large (D: 0.005 to 0.111, average: 0.050). The large-egg form was subdivided into two groups, i.e., a northern-Japan population (Nanakitagawa) and the other populations including three southern-Japan populations and another northern-Japan population (Obuchinuma). As for the large-egg form, allele frequencies were locally specialized at *Pgm** and *Idhp-2** loci in two populations located in Kagoshima Bay, and at *Mdh-1** locus in a northern-Japan population (Nanakitagawa) (Fig. 3).

On the other hand, such a locally specialized allele frequency was not observed in any populations of the small-egg form. Thus, the genetic differentiation among populations of the small-egg form was extremely small (D: 0.000 to 0.001, average: 0.0005), even between pairs of a northern-Japan population (Nanakitagawa) and the other southern-Japan populations.

The lower genetic variation among populations of the small-egg form was also shown by a lower G_{ST} value. The five populations of the small-egg form had a lower G_{ST} value (0.020) than those of the large-egg form (0.435) (Table 5).

The small-egg form exhibited relatively low genotypic diversity within a population (Table 5). Percentage of loci polymorphic ($P_{0.95}$: 7.1 to 14.3%) and average expected heterozygosity (H_e: 0.014 to 0.038) in the five populations of the small-egg form were lower than those in populations of the large-egg form except in the Obuchinuma population ($P_{0.95}$: 14.3 to 21.4%, H_e: 0.048 to 0.068). The $P_{0.95}$ value (0%) and the H_e value (0.005) in the Obuchinuma population were much lower than those in any other populations. The differences among the H_e values of all populations of both forms were not significant (one-way ANOVA; $F_{9,130} = 0.83$, $P = 0.59$).

Discussion

Species status for the two forms of *Hediste japonica*

The small-egg form and the large-egg form are very similar in adult morphology to each other, and identified as *H. japonica* according to the key of Imajima (1972). But, the two forms differ markedly in their life-history

Table 3 *Hediste japonica*. Allele frequencies in ten populations of the large-egg and small-egg forms

Locus	Allele	Large-egg form					Small-egg form				
		Obuchinuma	Nanakitagawa	Kotsukigawa	Nagatagawa	Kaminokawa	Nanakitagawa	Beppugawa	Omoigawa	Nagatagawa	Kaminokawa
*Ata-1**	**169*	0	0	0	0	0	0.010	0	0	0	0
	**146*	0	0	0	0.024	0	0	0	0	0.011	0
	**100*	1.000	1.000	1.000	0.976	1.000	0.980	0.988	1.000	0.977	1.000
	**58*	0	0	0	0	0	0.010	0	0	0.011	0
	**42*	0	0	0	0	0	0	0.013	0	0	0
*Ata-2**	*–100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Ak**	**100*	0	0	0	0	0	1.000	0.988	1.000	0.988	1.000
	**66*	1.000	1.000	1.000	1.000	1.000	0	0.013	0	0.013	0
*G3pdh-1**	**105*	1.000	1.000	1.000	1.000	1.000	0	0	0	0	0
	**100*	0	0	0	0	0	1.000	1.000	1.000	1.000	1.000
*G3pdh-2**	**132*	1.000	0.971	1.000	1.000	1.000	0	0	0	0	0
	**100*	0	0	0	0	0	0.971	1.000	1.000	0.989	1.000
	**95*	0	0.029	0	0	0	0	0	0	0	0
	**64*	0	0	0	0	0	0.029	0	0	0.011	0
*Gpi**	**132*	0	0.137	0	0	0	0.020	0	0	0.011	0
	**116*	0	0	0	0	0	0	0.013	0	0	0
	**100*	0.982	0.863	1.000	1.000	1.000	0.980	0.988	0.955	0.977	0.950
	**79*	0	0	0	0	0	0	0	0.045	0.011	0
	**58*	0.018	0	0	0	0	0	0	0	0	0.050
*Idhp-1**	**121*	0.018	0	0	0	0	0.010	0	0	0	0
	**110*	0	0	0	0	0	0.010	0	0	0	0
	**100*	0.982	1.000	1.000	1.000	1.000	0.951	0.975	1.000	0.989	1.000
	**86*	0	0	0	0	0	0	0.013	0	0	0
	**81*	0	0	0	0	0	0.010	0.013	0	0.011	0
	**76*	0	0	0	0	0	0.020	0	0	0	0
*Idhp-2**	**154*	1.000	0.990	0.238	0.183	0.615	0	0	0	0	0
	**123*	0	0.010	0.762	0.817	0.385	0	0	0	0	0
	**100*	0	0	0	0	0	1.000	1.000	1.000	1.000	1.000
*Ldh-1**	**111*	0	0	0	0	0	0.082	0	0	0.023	0
	**100*	1.000	1.000	1.000	1.000	1.000	0.908	0.988	0.909	0.942	0.950
	**87*	0	0	0	0	0	0	0.013	0.091	0.035	0.050
	**83*	0	0	0	0	0	0.010	0	0	0	0
*Ldh-3**	**159*	0	0	0	0	0	0.020	0	0	0	0
	**100*	0	0	0	0	0	0.970	1.000	1.000	1.000	1.000
	**95*	1.000	0.990	1.000	1.000	1.000	0	0	0	0	0
	**88*	0	0	0	0	0	0.010	0	0	0	0
	**46*	0	0.010	0	0	0	0	0	0	0	0
*Mdh-1**	**147*	0	0.765	0	0	0	0	0	0	0	0
	**100*	1.000	0.235	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Pgdh**	**156*	0	0	0	0	0	0	0	0	0.013	0
	**133*	0	0	0	0	0	0.010	0.025	0	0.013	0
	**100*	0	0	0	0	0	0.960	0.925	1.000	0.938	1.000
	**81*	1.000	0.989	0.950	0.868	0.885	0	0	0	0	0

Locus	Allele	Large-egg form					Small-egg form				
		Obuchinuma	Nanakitagawa	Kotsukigawa	Nagatagawa	Kaminokawa	Nanakitagawa	Beppugawa	Omoigawa	Nagatagawa	Kaminokawa
	**72*	0	0	0	0	0	0.020	0.050	0	0.013	0
	**50*	0	0.011	0	0	0	0	0	0	0	0
	**44*	0	0	0	0	0	0.010	0	0	0.025	0
	**22*	0	0	0.050	0.132	0.115	0	0	0	0	0
*Pgm**	**104*	0	0	0	0	0	0	0.013	0	0.011	0
	**100*	1.000	1.000	0.545	0.780	1.000	1.000	0.963	1.000	0.989	1.000
	**92*	0	0	0.455	0.220	0	0	0.013	0	0	0
	**85*	0	0	0	0	0	0	0.013	0	0	0
*Sod**	**100*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 4 *Hediste japonica*. Nei's genetic distance (Nei 1972) between each pair of populations is shown (*Numbers in parentheses* site numbers, see Fig. 1)

Form	Population	Large-egg form					Small-egg form				
		Obuchinuma	Nanakitagawa	Kotsukigawa	Nagatagawa	Kaminokawa	Nanakitagawa	Beppugawa	Omoigawa	Nagatagawa	Kaminokawa
Large-egg form	Obuchinuma (1)										
	Nanakitagawa (2)	0.045									
	Kotsukigawa (5)	0.060	0.111								
	Nagatagawa (6)	0.055	0.106	0.005							
	Kaminokawa (7)	0.012	0.059	0.027	0.018						
Small-egg form	Nanakitagawa (2)	0.565	0.659	0.588	0.561	0.538					
	Beppugawa (3)	0.559	0.652	0.578	0.553	0.533	0.001				
	Omoigawa (4)	0.570	0.662	0.592	0.565	0.543	0.001	0.001			
	Nagatagawa (6)	0.561	0.654	0.582	0.556	0.534	0.000	0.000	0.000		
	Kaminokawa (7)	0.567	0.659	0.589	0.563	0.541	0.001	0.001	0.000	0.000	

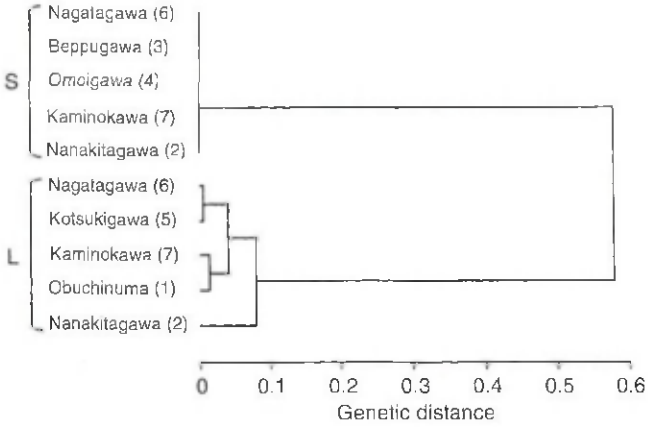

Fig. 2 *Hediste japonica*. Dendrogram showing UPGMA clustering by Nei's genetic distances among populations of the large- and small-egg forms (*Numbers in parentheses* site numbers, see Fig. 1; *S* small-egg form; *L* large-egg form)

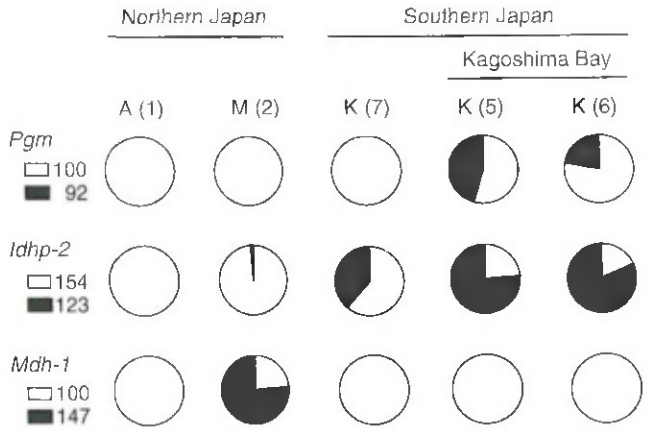

Fig. 3 *Hediste japonica*. Geographic variation in allele frequencies of three loci (*Pgm**, *Idhp-2**, *Mdh-1**) in five populations of the large-egg form (*Numbers in parentheses* site numbers, see Fig. 1; *A* Aomori Prefecture; *M* Miyagi Prefecture; *K* Kagoshima Prefecture)

traits, i.e., reproductive cycle, reproductive behavior (Sato and Tsuchiya 1987) and developmental pattern (Sato and Tsuchiya 1991). Slight differences in karyotype (Sato and Ikeda 1992), adult morphology (Sato 1992, unpublished data), and ultrastructure of gametes (Sato unpublished data) have also been found between the two forms. These previous studies have suggested that the two forms may be separate species.

Our electrophoretic results in the present study show that the two forms share no common allele at five out of 14 isozyme loci studied, even in sympatric populations. This result suggests that the two forms are reproductively isolated, providing evidence for the existence of two sibling species in *Hediste japonica* according to the biological species concept (Mayr 1969). Taxonomical description of the two forms will be presented in a later paper with a detailed comparison of adult morphology.

Fong and Garthwaite (1994) examined the extent of genetic differentiation among three allopatric sibling species, *Hediste diversicolor*, *H. limnicola* and the large-egg form of *H. japonica*, and reported *D*-values of 0.50 to 1.18 among them. These *D*-values are comparable to our results for *D*-values between the two forms of *H. japonica* (0.533 to 0.662). The *D*-values between the two forms of *H. japonica* are also comparable to typical levels among congeneric species in various taxa (0.616; Thorpe 1982) and among sibling species in many marine taxa (0.5 or greater; Knowlton 1993), though not as high as among the "*Nereis acuminata*" species group (1.36 to 1.76; Rodriguez-Trelles et al. 1996).

Genetic differentiation among populations within a form

The genetic differentiation and variability among populations were higher in the large-egg form (*D*: 0.005 to 0.111, G_{ST}: 0.435) than the small-egg form (*D*: 0.000 to 0.001, G_{ST}: 0.020).

Table 5 *Hediste japonica*. Genetic variability at 14 loci in ten populations and coefficient of gene (G_{ST}) and Nei's genetic distance (*D*) within each form [*Numbers in parentheses* site numbers, see Fig. 1; H_T average expected heterozygosity in the total populations in each form; H_S average expected heterozygosity within a population in each form; G_{ST} is equal to $(H_T - H_S)$]

Population	Mean sample size per locus ± SD	Polymorphic loci (%)	Mean heterozygosity ± SD		H_T	H_S	G_{ST}	Mean *D* ± SD
			Observed	Expected				
Large-egg form								
Obuchinuma (1)	27.5 ± 1.3	0.0	0.005 ± 0.013	0.005 ± 0.013				
Nanakitagawa (2)	49.9 ± 2.2	14.3	0.056 ± 0.125	0.051 ± 0.109				
Kotsukigawa (5)	21.6 ± 0.8	21.4	0.067 ± 0.152	0.068 ± 0.157	0.0842	0.0476	0.435	0.050 ± 0.037
Nagatagawa (6)	40.1 ± 2.2	21.4	0.069 ± 0.131	0.066 ± 0.125				
Kaminokawa (7)	13.3 ± 0.6	14.3	0.060 ± 0.171	0.048 ± 0.134				
Small-egg form								
Nanakitagawa (2)	50.7 ± 0.6	7.1	0.037 ± 0.046	0.038 ± 0.050				
Beppugawa (3)	40.0 ± 0.0	7.1	0.027 ± 0.042	0.026 ± 0.040				
Omoigawa (4)	10.6 ± 0.8	7.1	0.019 ± 0.053	0.018 ± 0.048	0.0257	0.0249	0.020	0.0005 ± 0.0005
Nagatagawa (6)	43.3 ± 1.4	14.3	0.030 ± 0.042	0.029 ± 0.040				
Kaminokawa (7)	9.8 ± 0.6	14.3	0.014 ± 0.036	0.014 ± 0.035				

This difference seems to be attributable to the differences in life-history strategies between the two forms. Adults of *Hediste japonica* (perhaps both forms) show euryhaline distributions in estuaries (Kagawa 1955; Wu et al. 1985; Sato unpublished data). Mature adults of the small-egg form spawn around the mouth of a river, where the salinity approaches full-strength seawater, following reproductive swarming (the conspicuous migration toward the sea) (Sato and Tsuchiya 1987). Their smaller eggs (diameter: 140 to 170 μm) develop into free-swimming larvae with a pelagic life around the river mouth (Sato and Tsuchiya 1991). The larvae gain tolerance to the lower salinity at the six-setiger stage, when they move upstream into the adult habitat with the rising tide and settle there (Kagawa 1955). It seems that the larvae of the small-egg form easily migrate from river to river through the sea, resulting in frequent gene flow among populations.

In contrast, mature adults of the large-egg form spawn where they normally live without long-distance dispersal (Sato and Tsuchiya 1987). Their larger eggs (diameter: 200 to 240 μm) develop directly into benthic juveniles without a true pelagic phase under lower salinity (Sato and Tsuchiya 1991). Thus, the whole life cycle of the large-egg form is usually completed in brackish waters within rivers, resulting in limited gene flow between populations. Therefore, the higher interpopulational genetic differentiation may be expected in the large-egg form.

The mode of life history of *Hediste diversicolor* resembles that of the large-egg form of *H. japonica*; the whole life cycle is completed within brackish waters without a true planktonic stage (Dales 1950; Smith 1964; Bartels-Hardege and Zeeck 1990). In European *H. diversicolor*, relatively high levels of genetic differentiation were found between two North Sea populations (D: 0.31; Fong and Garthwaite 1994) and among four western Mediterranean populations (D: up to 0.356; Abbiati and Maltagliati 1996). Through a study of LDH polymorphisms in English populations of *H. diversicolor*, Hateley et al. (1992) suggested that there is considerable genetic differentiation between populations, even those separated by just 2 km.

It may be generalized in not only brackish-water species but also marine species that modes of larval development influence the scale of gene flow, consequently affecting genetic differentiation between populations. Hoskin (1997) demonstrated that a species producing planktonic larvae exhibits lower levels of genetic differentiation among local populations than species that undergo direct development in intertidal gastropods within the southeast coastal region of Australia.

Genetic variability within a population

The average heterozygosities in populations of both the large-egg form (H_e: 0.005 to 0.068) and the small-egg form (0.014 to 0.038) are low in comparison with other marine polychaetes such as *Polydora* spp. (0.131 to 0.283; Manchenko and Radashevsky 1993) and *Glycera dibranchiata* (0.09 to 0.18; Bristow and Vadas 1991), and other marine invertebrates (mean ± SD for 18 species: 0.124 ± 0.08; Nevo 1978). The low levels of H_e values in two forms of *Hediste japonica* are rather comparable to those in *H. diversicolor* (0.014 to 0.034; Abbiati and Maltagliati 1996) and a freshwater shrimp (0.000 to 0.069; Ikeda et al. 1993). Populations with larger effective sizes tend to have higher values of H_e (Kimura and Crow 1964; Kijima and Fujio 1984). It is probable that effective population sizes are usually smaller in brackish-water and freshwater species inhabiting estuaries and rivers than in marine species.

Our results show that the level of genetic variability within a population of the small-egg form was lower in comparison with most populations of the large-egg form, suggesting that an effective population size was relatively small in the small-egg form. This result may be unexpected, because local populations of the small-egg form seemed to be interconnected by planktonic larvae to constitute a "compound population" (see above). In fact, the percentage of polymorphic loci and H_e values were greater in populations of a species producing planktonic larvae than species that undergo direct development in intertidal gastropods inhabiting the southeast coast of Australia (Hoskin 1997). Low population density of the small-egg form in each locality (Sato unpublished data) may be related to its low levels of genetic variability.

An extremely low level of genetic variability such as 0% of polymorphic loci observed in the Obuchinuma population of the large-egg form may be caused by a population-size bottle-neck. Habitats of the large-egg form throughout its whole life cycle are in estuaries, which are situated in lower reaches of rivers. Japanese estuaries are relatively small, and their environments are sometimes severely damaged by a catastrophe such as a heavy flood.

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