To Dr. Patrick Scaps With best wishes Masanori Sato

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Divergence of reproductive and developmental characteristics in *Hediste* (Polychaeta: Nereididae)

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Abstract

Comparative studies on reproductive and developmental features in brackish water nereidid polychaetes of the genus *Hediste* (especially two Asian species, a small- and a large-egg form) are reviewed. General characteristics of the gamete ultrastructures of the two forms are very similar, though some minor differences were detected between them. The mode of reproduction, body size of mature adults, egg size, fecundity and mode of development are markedly different between the two forms. Not surprisingly, the two forms have contrasting life histories: the small-egg form with planktonic development migrates between brackish waters and the sea, while the whole life history of the large-egg form is usually completed in brackish waters within a river. The different life histories are reflected in different levels of genetic differentiation between geographically separated populations of the two forms. The life history characteristics of the above two Asian forms are compared with those of three other *Hediste* species with special reference to a relationship between developmental pattern and speciation.

Introduction

In polychaetes, reproductive and developmental modes are conspicuously variable even among morphologically similar congeneric species (Åkesson, 1973; Grassle & Grassle, 1976; Levin & Bridges, 1995). It is an interesting problem why so great a variety exists, i.e. what is the ecological or evolutionary significance of the various modes. To clarify this problem, comparisons of various aspects in closely related species with the different modes may be useful.

Many examples of poecilogony, defined as intraspecific variability of reproductive and developmental modes, were reported in polychaetes in the past (e.g. Thorson, 1950). However, most of these seem to represent the existence of sibling species complexes, the members of which have species-specific modes of reproduction and development (Clark, 1977; Hoagland & Robertson, 1988).

Important findings have been made in comparative studies on reproduction or development in sibling-species groups of *Capitella* (e.g. Grassle & Grassle, 1976; Eckelbarger & Grassle, 1987a,b), *Microphthalmus* (Westheide & Rieger, 1987), *Ophryotrocha* (e.g. Åkesson, 1973; Sella et al., 1995), *Chaetozone* (Christie, 1985), *Platynereis* (e.g. Hauenschild, 1951; Schneider et al., 1992) and in a poecilogonous species, *Streblospio benedicti* (e.g. Levin & Creed, 1986; Levin et al., 1991).

In this paper, reproductive and developmental features in brackish-water nereidid polychaetes of the genus *Hediste* Malmgren, 1867 (especially two Asian sibling species, a small- and a large-egg form) are reviewed, with special reference to the relationship between developmental pattern and speciation.

Taxonomy and distribution of species of *Hediste*

Three allopatric species of *Hediste* Malmgren, 1867 have been described from shallow brackish waters in the North Temperate Zone. *H. diversicolor* (O. F. Müller, 1776) from both the European and the North American coast of the Atlantic (Smith, 1977), *H. limnicola* (Johnson, 1903) from fresh waters of the North American Pacific coast (Smith, 1958) and

H. japonica (Izuka, 1908) from East Asia (Ushakov, 1955; Imajima, 1972; Wu et al., 1985) (Figure 1). Recently, Kirkegaard (1995) described *H. kermadeca* from 4410 m depth in the Kermadec Trench. This species is only known from a single specimen and is not considered in the present paper. The most important characteristic of the genus *Hediste* is the presence of heavily fused falcigers in the posterior neuropodia (Fong & Garthwaite, 1994). The three brackish-water *Hediste* species are morphologically very similar to one another (Smith, 1958).

In Asia, the distributional range of *Hediste* species extends north to Sakhalin in Russia and south to the Amami-Oshima Island in Japan (Ushakov, 1955; Imajima, 1972; Wu et al., 1985; Sato, unpubl.). Previously, the Asian *Hediste* worms were regarded to belong to a single species, *H. japonica*. However, we found that '*H. japonica*' is a sibling species complex and consists of three distinct species, namely, true *H. japonica* and two additional undescribed species (Sato & Tsuchiya, 1987, 1991; Sato, 1992; Sato & Ikeda, 1992; Sato & Masuda, 1997; Sato & Nakashima, unpubl.). Here, these three Asian species are designated as 'small-egg form', 'large-egg form' and 'Ariake form'. Though the three forms are morphologically very similar to one another, mean numbers of paragnaths on the proboscis (especially in area II) are different, and chaetal morphology of the Ariake form is clearly different from that of the other forms (Sato & Nakashima, unpubl.). However, morphological distinction between the large-egg form and the small-egg form is difficult except for mature adults.

The three forms are clearly distinguishable by complete allele substitutions at several isozyme loci, which are detectable by electrophoretic analysis (Sato & Masuda, 1997; Sato & Nakashima, unpubl.). The electrophoretic pattern of lactate dehydrogenase (LDH) is available as a diagnostic character for identification of the three forms.

Figure 1 shows the geographical distributions of the three forms. The small- and large-egg forms are often sympatric in Japan. No genetic evidence of hybridization between the two forms has been detected even in localities where both occur, indicating that they are reproductively isolated, and that they are distinct species. The Ariake form, just recently discovered, occurs exclusively in the inner part of the Ariake Sea, a closed large bay in western Kyushu, Japan. In the following sections, reproductive and developmental features are compared mainly between the small- and large-egg forms.

Gamete morphology and fertilization in the two Japanese species of *Hediste*

Sperm morphology of the small- and large-egg forms was compared utilizing phase-contrast microscopy and electron microscopy (Figures 2 & 3). The 'primitive type' of spermatozoa with cone-shaped acrosome was observed in both forms. Shape of the sperm head was slightly different between the two forms: The sperm head was angular at the base of acrosome in the small-egg form, and rather round in the large-egg form (Figures 2 & 3). General characteristics of the sperm ultrastructure of the small- and large-egg forms were very similar to each other and to those described in *Hediste diversicolor* (Bertout, 1976). In all the three *Hediste* species, a prominent acrosomal rod is contained in the axial portion of the subacrosomal space (anterior half) and the deep central indentation of the nucleus (posterior half).

Egg morphology is markedly different between the small- and large-egg forms (Sato & Tsuchiya, 1991). Fully mature unfertilized eggs are much larger in the large-egg form than in the small-egg form (see below). Ooplasm is relatively transparent in the small-egg form, and opaque in the large-egg form (Figure 4). The egg surface structure of the two forms is generally similar, but some differences have been detected between them (Table 1). In both forms, the egg is surrounded by a chorion (0.6–0.7 μm thick) consisting of three layers, and egg microvilli are inserted into the cavities of the chorion (Figure 5). A specialized structure, the microvillus tip vesicle (MTV), is situated on the

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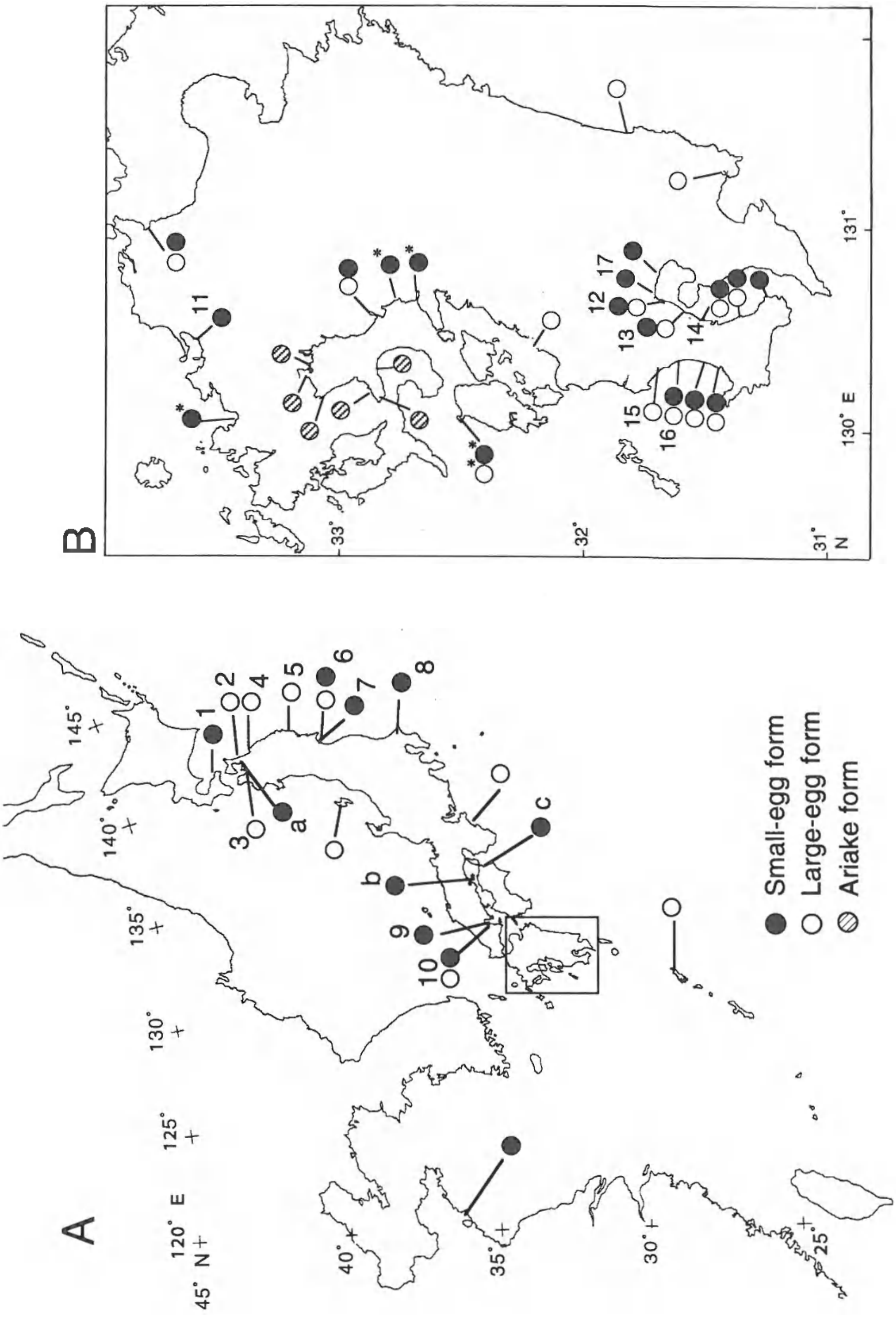
Figure 1. Distribution and collection sites of three species of the *Hediste japonica* group in Japan (A), in Kyushu (B) (Izuka, 1908; Kagawa, 1955; Sato & Tsuchiya, 1987, 1991; Sato & Ikeda, 1992; Sato & Masuda, 1997; Sato & Nakashima, unpubl.) and China (Sun et al., 1980). In most cases, species were distinguished by diagnostic characters of electrophoretic patterns of LDH (between the three forms), reproductive and developmental features (between the small-egg and the large-egg form) or chaetal morphology (between the Ariake form and the others). In some localities($^+$), small-egg and large-egg form distinguished tentatively by numbers of paragnaths in the area II of proboscis. Numbers (1–17, a–c) mean localities for the studies of interpopulational variations in the small-egg and large-egg form (see below). 1: off the coast of Usujiri; 2: Obuchinuma Swamp; 3: Shinjogawa River; 4: Niidagawa River; 5: a small creek in Otsuchi; 6: Nanakitagawa River; 7: a small creek in Yuriage; 8: Tonegawa River; 9: Kyobashigawa River; 10: Yahatagawa River; 11: Nakagawa River; 12: Omoigawa River; 13: Kotsukigawa River; 14: Nagatagawa River; 15: Osatogawa River; 16: Kaminokawa River; 17: Beppugawa River; (a) Kominatogawa River; (b) Kojima Bay; (c) Shinmachigawa River.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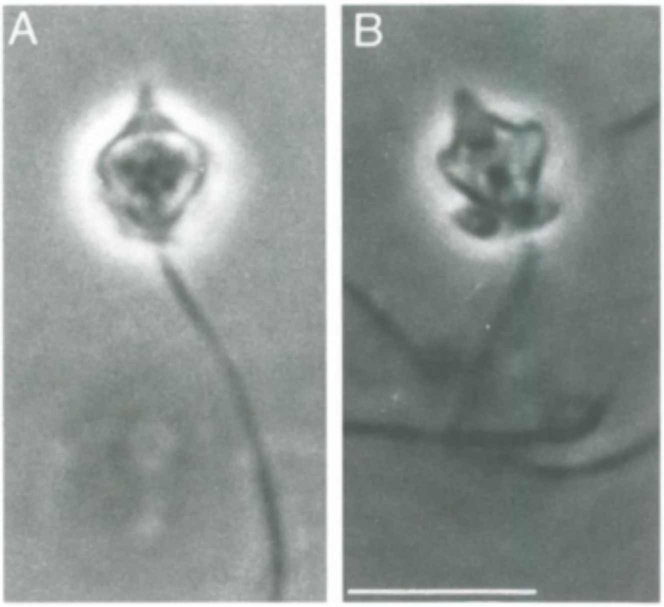

Figure 2. Phase-contrast micrographs of spermatozoa of the large-egg form collected from the Kotsukigawa River (A) and of the small-egg form collected from the Omoigawa River (B). Scale bar: 5 μm.

Table 1. Comparison of egg surface structures between two species of the *Hediste japonica* group. mtv – microvillus tip vesicles; ca – cortical alveoli; pta – phosphotungstic acid

	Small-egg form	Large-egg form
Before fertilization		
Thickness of chorion (μm)	0.6–0.7	0.6–0.7
Electron density of mtv	low	high
Thickness of ca layer (μm)	10–20	4–5
pta-staining of ca	positive	negative
After fertilization		
Thickness of jelly layer (μm)	100–150	50–60

tip of each microvillus. Electron density of the MTV is higher in the large-egg form than in the small-egg form.

In the egg cortex of both forms, there are numerous cortical alveoli which contain a fibrous material. After fertilization, the fibrous material is secreted as jelly to the outside (Sato & Osanai, 1986). The content of these cortical alveoli was intensively stained by phosphotungstic acid in the small-egg form, but not in the large-egg form, suggesting that the chemical nature is different in the two forms. The cortical layer filled with cortical alveoli was thicker in the small-egg form (10–20 μm) than in the large-egg form (4–5 μm).

The mode of sperm entry during fertilization was shown in the small-egg form (Sato & Osanai, 1986). The MTV is known to function as a sperm receptor, which induces the acrosome reaction and adheres to the sperm acrosomal process. Spermatozoa can not penetrate the chorion proper, but can fuse with the tip of an egg microvillus, which penetrates the chorion. At least two other species of *Hediste*, i.e. the large-egg form and *H. diversicolor* (Pasteels, 1966), probably have the same manner of sperm entry as the small-egg form, because their eggs have the MTV, and the adhesion of the sperm acrosomal process just above an egg microvillus was also observed in the large-egg form (Figure 3C). All the MTV disappeared from the egg surface within 5 min of insemination in the small-egg form (Sato & Osanai, 1986).

In unfertilized eggs of the *Hediste* small- and large-egg forms, the available area for sperm fusion was estimated from the values of mean cross-section area and density of microvilli: Total area of the microvillus tips occupies 2–3% of the whole egg surface in both of the two forms (Table 2).

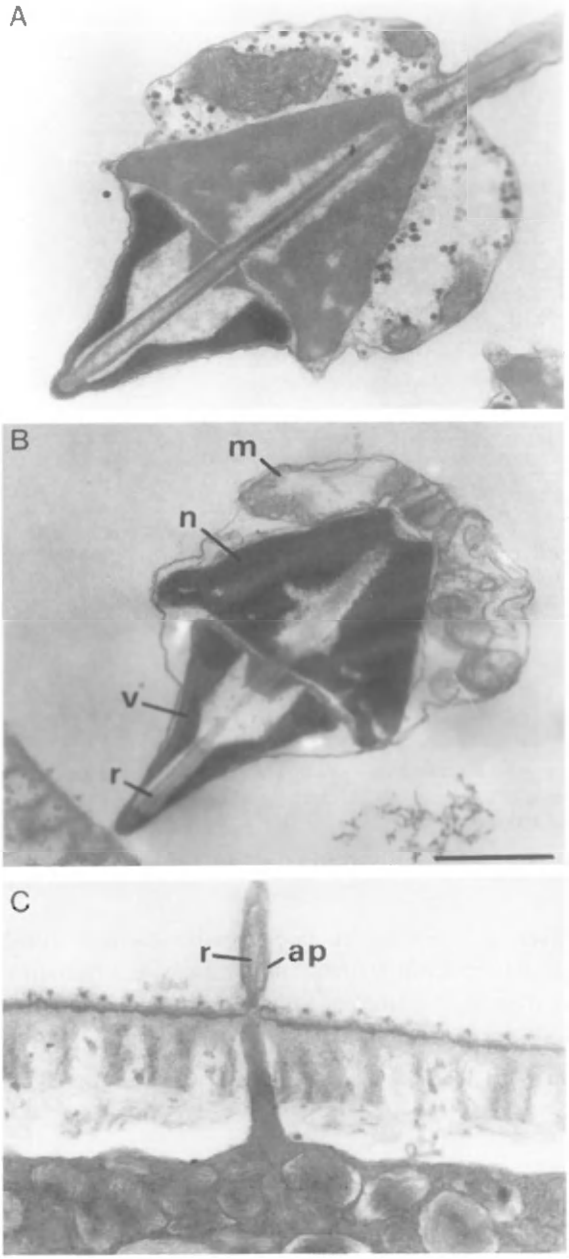

Figure 3. Electron micrographs of spermatozoa of two species of the *Hediste japonica* group. (A) Spermatozoon of small-egg form collected from the Kyobashigawa River. (B) Spermatozoon of large-egg form collected from Niidagawa River. m – mitochondrion; n – nucleus; v – acrosomal vesicle; r – acrosomal rod. (C) Tip of acrosome-reacted spermatozoon of large-egg form collected from Niidagawa River. Specimen fixed 1 min after insemination to eggs pretreated in 8% acetone in 70% seawater for 15 min in order to induce polyspermy. Acrosomal process (ap) containing acrosomal rod (r) adhering to the egg surface just above a microvillus. For TEM methods see Sato & Osanai (1986). Scale bar: 1 μm.

Differentiation of life-history traits in the two Japanese species of *Hediste*

Modes of reproduction

In the small-egg form, simultaneous reproduction occurs as reproductive swarming in the cold season, winter or early spring (Izuka, 1908; Kagawa, 1955; Sun et al., 1980; Wu et al., 1985; Sato & Tsuchiya, 1987). During reproductive swarming, mature adults swim up just after night high tide during spring tides and are transported downstream by water currents of the ebb tide (Figure 6). In Kagoshima, southern Japan, the largest number of adults participate swarming in late February or early March, and swarming of a smaller number of adults continues until April. The body wall of the swimming adults becomes very thin, so that in females, green colour of ripe eggs can be seen, and in males, the whitish colour of the sperm. Epitokous metamorphosis is not so distinct as in the heteronereid forms of many marine species (Izuka, 1908; Imajima, 1972). Eggs or sperm are shed into the ambient water around river mouth, and fertilization occurs there. After spawning, adults die, i.e. this species is semelparous (Sato & Tsuchiya, 1987). The life span (generation time) of the small-egg form seems to be one year or more, because the reproduction is restricted to a short period once a year.

In contrast, spawning of the large-egg form occurs within the brackish waters of the adult habitat without reproductive swarming (Sato & Tsuchiya, 1987). Females spawn within the burrows. A mature male was observed swimming near the bottom and releasing sperm in an aquarium. Therefore, in the large-egg form, males may release sperm near the openings of burrows of females, and sperm may be incorporated into a female burrow by water current made by the wriggling behaviour of a female. Spawning of the large-egg form is not as simultaneous as in the small-egg form. Many mature adults were collected in late September–October in southern Japan, and in June in northern Japan (Sato & Tsuchiya, 1987). But a few mature worms were found throughout almost the whole year. The life span of the large-egg form seems to be less than one year, because a male raised from an egg spawned in an aquarium reached reproductive maturity three months after fertilization. The large-egg form is also semelparous. Within sediments, we can find spent adults which are alive for a while after spawning (Sato & Tsuchiya, 1987). It is unknown whether the spent adults brood the young or not.

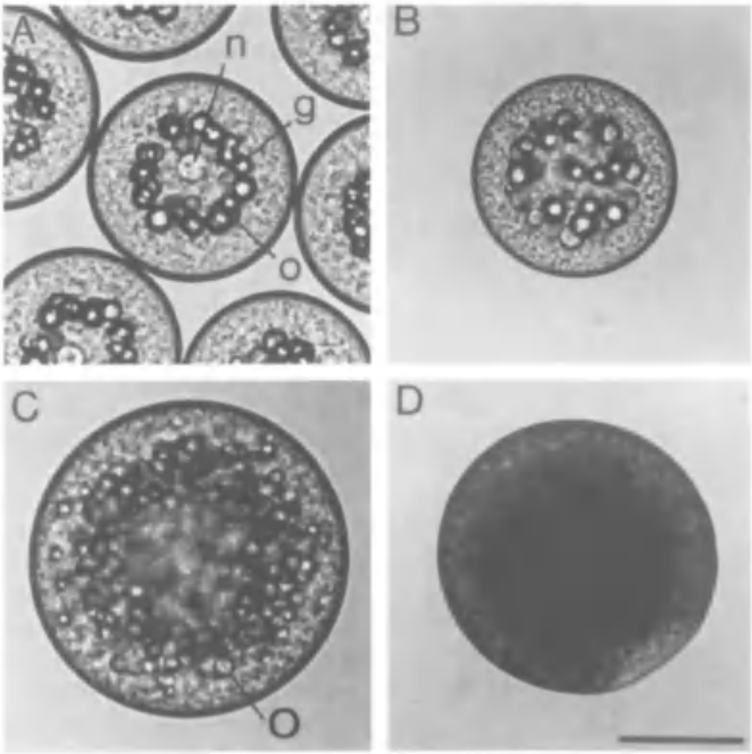

Figure 4. Mature unfertilized eggs. Small-egg form collected from the Yahatagawa River in 15 ‰ salinity (A) and in 23 ‰ salinity (nearly isotonic to the ooplasm) (B). Large-egg form collected from Niidagawa River in 6 ‰ salinity (C) and in 15 ‰ salinity (nearly isotonic to the ooplasm) (D). Germinal vesicle (g) with nucleolus (n). o– oil drops. Scale bar = 100 μm.

Sex determination and sex ratio

Sato & Ikuda (1992) examined karyotypes of the two sexes by obtaining chromosome samples from regenerating tail tips of males and females and from clumps of spermatogonia in males. Their results showed that in both the small- and large-egg forms, the diploid chromosome number is 28 and the karyotype is similar, containing heteromorphic sex chromosomes (Sato & Ikeda, 1992). The sex seems to be determined by a simple system of male heterogamy, i.e. the male genotype is XY, and the female genotype is XX. It is characteristic that the Y chromosome is larger than the X in this case.

According to the chromosomal sex determination, simple out-crossing should produce a 1:1 sex ratio. In fact, a sex ratio of almost 1:1 in both forms of *Hediste* has been observed in the field. Swimming adults of the small-egg form were collected at the peak of reproductive swarming in the Omoigawa River, southern Japan on 1st March 1991. Their sex was easily determined (male:female = 143:134). Benthic adults of the large-egg form were collected in the Kotsukigawa River, southern Japan, in September–October, 1988 just before reproduction, and their sex was examined by observing mature or immature gametes within the body cavity (male:female = 117:121).

Body size of mature adults

In body length of fixed intact specimens collected from many places in Japan, mature adults of the small-egg form (28–150 mm, average: 65 mm, n=185) were significantly larger than those of the large-egg form (25–100 mm, average: 50 mm, n=73) (Mann-Whitney's U-test: $p < 0.0001$).

The adult body size of the two forms was also compared using index of width of anterior body without parapodia (Figure 7). Though adult size varied among populations within a form, mean body width of the small-egg form was significantly larger than that of the large-egg form in the sympatric populations (Mann-Whitney's U-test: $p < 0.001$). In neither form was the difference in size between males and females significant.

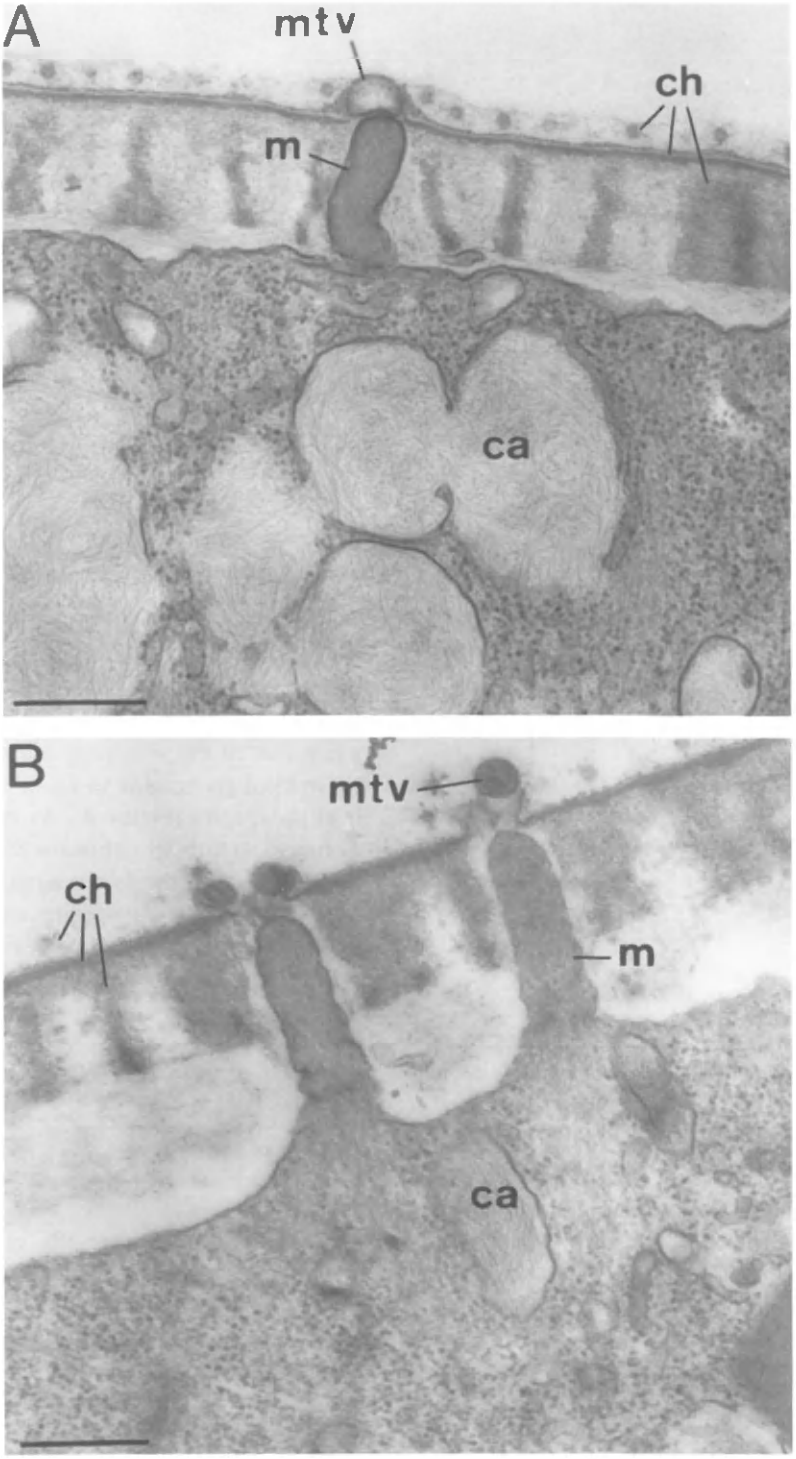

Figure 5. TEM micrographs of unfertilized egg surface of small-egg form collected from Yuriage (A), and large-egg form collected from Niidagawa River (B). mtv – microvillus tip vesicle; m – microvillus; ch – chorion consisting of three layers; ca – cortical alveolus. For TEM methods see Sato & Osanai (1986). Scale bar: 0.5 μm.

Figure 6. Swarming of mature adults of small-egg form at the mouth of the Omoigawa River (25th February, 1986). Females green, males whitish.

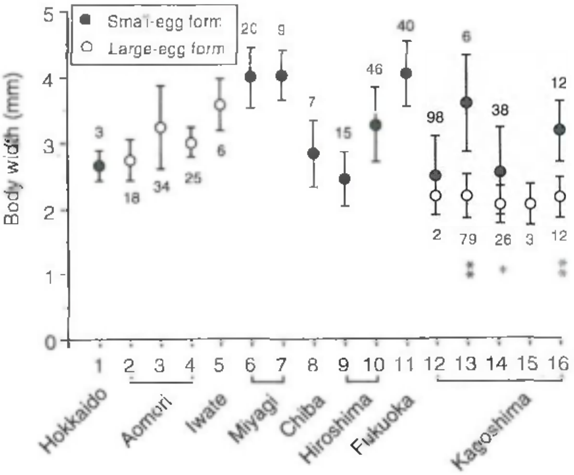

Figure 7. Body width of mature adults of two species of the *Hediste japonica* group. Small-egg form collected from 11 localities, and large-egg form from 9 localities. Average of the width of anterior body without parapodia and SD bar are shown in each locality. The locality numbers (1–16, Figure 1) are arranged from north to south. Number on each datum indicates sample size. Significance of differences between the sympatric two forms in 3 localities (13, 14, 16) in Kagoshima Prefecture tested by Mann-Whitney U-test; for all localities, difference is significant (*: $p=0.001$, **: $p=0.0001$).

Egg sizes

Egg sizes are conspicuously different between the small- and large-egg forms (Sato & Tsuchiya, 1991). Egg diameter of the large-egg form was 200–220 μm, while that of the small-egg form was 130–170 μm. No marked geographic variation in size was observed in either species (Figure 8). As in many other species of macrobenthos (Strathmann & Strathmann, 1982), the species with the larger adult body size produces smaller eggs, and the species with the smaller adult body size produces larger eggs. Female body size and egg sizes in the small-egg form collected from the Omoigawa River, southern Japan, however, are not correlated.

Fecundity (clutch size)

Adult females were carefully collected and kept in individual containers. Fecundity (entire number of eggs produced by a female) was calculated by several counts of egg number in diluted egg suspension (Figure 9). Fecundity of the small-egg form was from more than ten thousand to one million eggs. For the large-egg form, it is rather difficult to collect fully mature females without injury. Though only three counts for the large-egg form are available, it is evident that fecundity of the large-egg form was several thousand eggs, i.e. much smaller than that of the small-egg form.

Table 2. Density of microvilli (mv) and total area of the microvillus tips on egg surface in two species of the *Hediste japonica* group. Transmission electron micrographs obtained from 11–16 ultrathin sections of 8–11 eggs were used for measurements and calculations. P. – Prefecture

Species and locality	Density of mv (No./μm^2)	Diameter of mv (μm)	Mean cross section area of a mv (μm^2)	Percent of area occupied by mv on egg surface
Small-egg form				
Hiroshima P.	1.33 ± 0.17	0.156 ± 0.019	0.019	2.6
	(n = 14)	(n = 91)		
Miyagi P.	0.67 ± 0.12	0.188 ± 0.022	0.028	1.9
	(n = 14)	(n = 76)		
Large-egg form				
Aomori P.	0.76 ± 0.10	0.221 ± 0.023	0.038	2.9
	(n = 16)	(n = 108)		

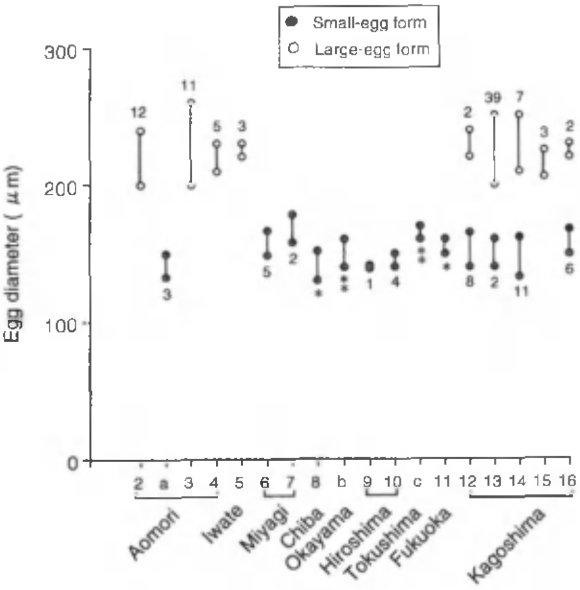

Figure 8. Egg sizes of two species of the '*Hediste japonica*' group. Range of diameters of mature eggs is shown for each population. Living materials measured in media isotonic to the ooplasm (about 15 ‰ salinity in large-egg form, and about 30 ‰ salinity in small-egg form. Sato & Tsuchiya, 1987) except for four localities (*, **). For locality numbers Figure 7, except for additional data of Kominatogawa River (a) (Sato & Tsuchiya, 1991), Kojima Bay (b) (Izuka, 1908) and Shinmachigawa River (c) (Okada, 1960) (Figure 1). Number of females examined shown on each datum. *, eggs which had been shed out from several females and fixed in 80% ethanol were measured; **, sample size not shown in the literature cited.

Early development

Developmental patterns are markedly different between the small- and large-egg forms (Sato & Tsuchiya, 1991). Figure 10 shows a schematic summary of differences in early development between the two forms. In both forms, a jelly substance secreted just after fertilization formed a jelly layer surrounding the developing embryo.

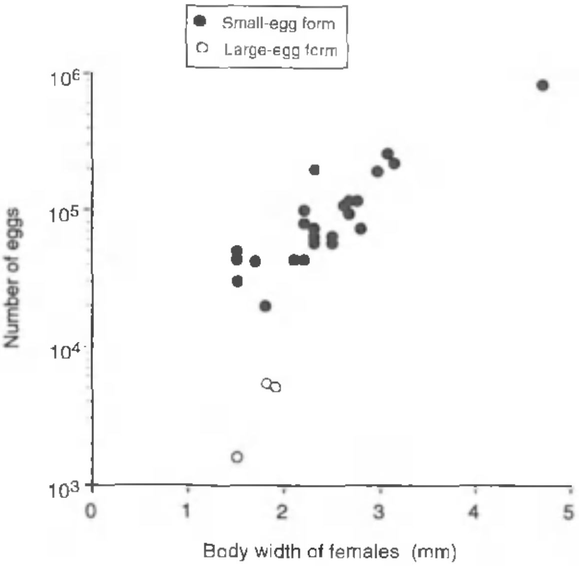

Figure 9. Fecundity of a female in two species of the '*Hediste japonica*' group. Small-egg form collected from Omoigawa River (23 inds) and Nanakitagawa River (1 ind.); large-egg form from Kotsukigawa River (3 ind.).

In the small-egg form, the jelly layer was thick (Table 1), but relatively soft and disappeared gradually, and then the trochophores began to swim freely (Figure 10). During the planktonic phase, the larvae became nectochaetes with long chaetae. It was often observed that the nectochaetes floated motionlessly at the water surface with the chaetae spread out radially. The nectochaete larvae continue the plank-

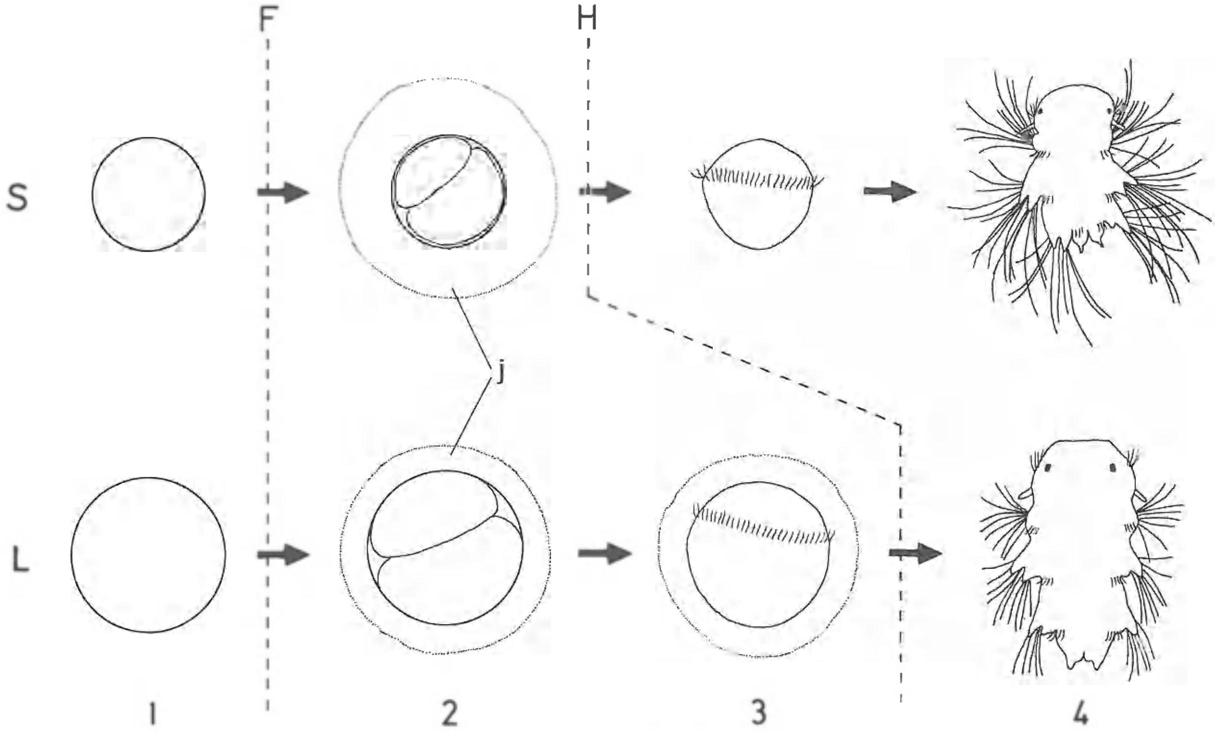

Figure 10. Schematic diagram of early development in two sibling species of the '*Hediste japonica*' group. S – Small-egg form; L – Large-egg form; (1) Unfertilized egg, (2) Two-cell stage, (3) Trochophore, (4) Three-chaetiger nectochaeta. F – Fertilization; H – Hatching from jelly layer; j – Jelly layer.

tonic life until the 6-chaetiger stage, about one month after fertilization, and then settle into brackish waters of the adult habitat, moving upstream on rising tides (Kagawa, 1955; Sato, unpubl.). For the small-egg form, salinity approaching full-strength seawater (at least more than 20 ‰) is essential for the early development, and the 6-chaetiger larvae seem to gain a tolerance to lower salinity (Kagawa, 1955; Sato & Tsuchiya, 1987).

In the large-egg form, the jelly layer was relatively solid and did not dissolve and the trochophores never hatched out of the jelly layer. Hatching occurred at the 3-chaetiger nectochaete stage with short chaetae. The 3-chaetiger nectochaetes did not swim and adhered to the bottom, so they were regarded as benthic juveniles. In the large-egg form, the early development can proceed under a wide range of salinity with the optimal salinity around 15 ‰, which is lethal for the development of the small-egg form (Sato & Tsuchiya, 1987).

Thus, the most important difference between the two forms is that the small-egg form has a planktonic and probably planktotrophic development, while the large-egg form has a direct development without a true planktonic phase. The nectochaetes of the small-egg form have longer chaetae than those of the large-egg form.

Advanced juveniles of the large-egg form with 4 or more chaetigers can swim. In the Kotsukigawa River, southern Japan, where the large-egg form is most common, such swimming juveniles were collected by hauling a net at the flood tide. Benthic juveniles of the same stages as the swimming juveniles were found in the sediment at the ebb tide in the same place. Thus, it seems that the juveniles are mainly benthic, and only during flood tide do they float up and swim into the water. By this behaviour, the young produced by the direct development within a female burrow can disperse widely in a brackish-water region without becoming displaced downstream.

Life cycle and interpopulational genetic differences

Life cycles of the small- and large-egg forms are summarized in Figure 11. The life cycle of the small-egg form comprises migrations between brackish waters and the sea, while the life cycle of the large-egg form is usually restricted to brackish waters within a river. Adults of both forms show euryhaline distributions

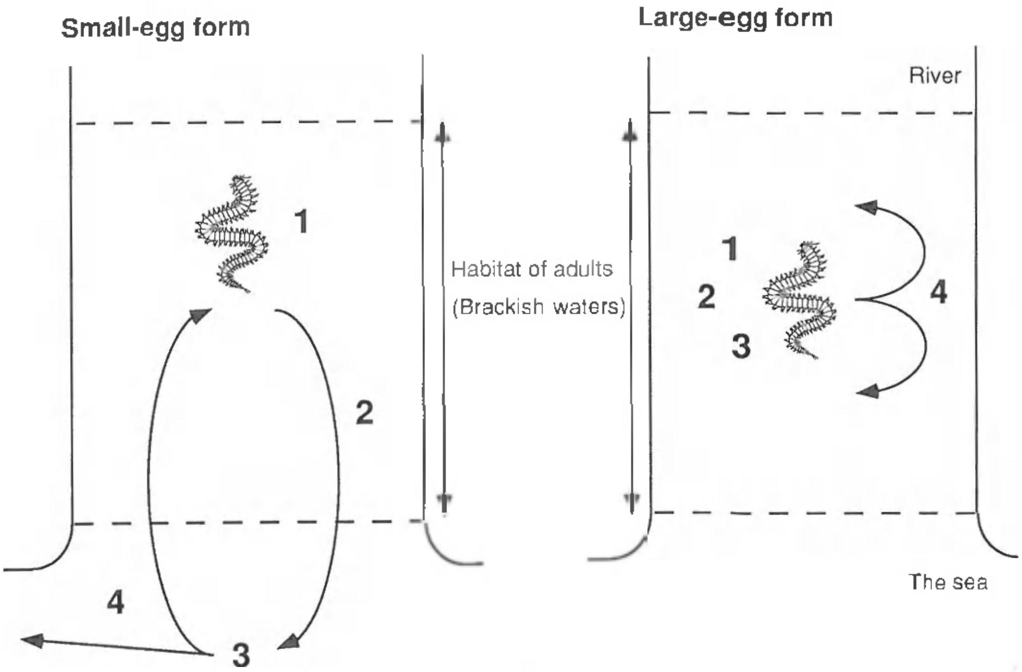

Figure 11. Schematic diagram of life cycles of two species of the '*Hediste japonica*' group, based on Kagawa (1955), Sato & Tsuchiya (1987, 1991). Life cycle of small-egg form (left) comprises migrations between brackish waters and the sea, characterized by (1) benthic life of adults, (2) reproductive swarming of mature adults toward the sea, (3) planktonic life of larvae, and (4) moving upstream and settlement of larvae into brackish waters. Life cycle of large-egg form (right) completed within brackish waters (estuaries), characterized by (1) benthic life of adults, (2) reproduction in or around adult burrows, (3) direct development, and (4) dispersal of juveniles by swimming behaviour.

in estuaries (Kagawa, 1955; Wu et al., 1985; Sato, unpubl.) and sometimes occur sympatrically.

The different life histories seem to result in the different levels of genetic differentiation among geographically separated populations. It seems that the planktonic larvae of the small-egg form easily migrate from river to river through the sea, resulting in frequent gene flow among populations. In contrast, the gene flow seems to be limited among populations of the large-egg form, which lacks a planktonic larval stage, though dispersal from river to river is exceptionally probable by swimming juveniles, which may flow downstream by accidents such as a flood, and may come back to another brackish-water area. Thus, higher interpopulational genetic differentiation may be expected in the large-egg form.

This idea was tested by electrophoretic studies (Sato & Masuda, 1997). Genetic differentiation among five populations of the small-egg form and five populations of the large-egg form was investigated on 14 isozyme loci by electrophoretic analysis (both forms were obtained from three rivers). As was expected, the genetic differentiation among populations was higher in the large-egg form (Figure 12). In the small-egg form, genetic structure was rather homogeneous even between populations from northern and southern Japan.

Comparison of life histories in *Hediste*

Table 3 compares life-history traits of five *Hediste* species. In contrast to similarity in adult morphology and ecology among these species, the modes of their life history are remarkably variable. The small-egg form is considered to be the true *H. japonica*, because the developmental and reproductive features are identical with those in the original description of *H. japonica* (Izuka, 1908).

The life history of *H. diversicolor* (Dales, 1950; Smith, 1964; Bartels-Hardege & Zeeck, 1990) resembles that of the large-egg form in, e.g. egg size, reproductive behaviour and early development, but differs in having demersal development with hatching out at the trochophore stage. Moreover, an aberrant sex ratio with few males was reported in *H. diversicolor* (Dales, 1950; Smith, 1964). High levels of genetic differentiation between European populations of this species (Hateley et al., 1992; Fong & Garthwaite, 1994; Abbiati & Maltagliati, 1996) are comparable to

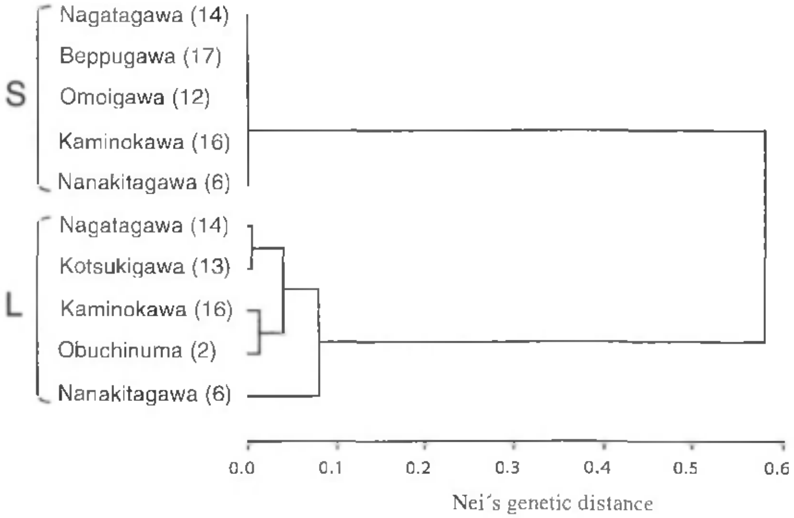

Figure 12. Dendrogram showing UPGMA clustering by Nei's genetic distances among populations of the small-egg form (S) and large-egg form (L). Numbers in parentheses correspond to sites in Figure 1. After Sato & Masuda (1997), partially modified.

Table 3. Life-history traits of five species of *Hediste*

Species	Distribution	Egg diameter (μm) (development)	Remarks on reproduction	References
H. japonica (small-egg form)	Japan China	130–170 (planktonic)	swarming towards the sea	Sato & Tsuchiya, 1987, 1991; Wu et al., 1985
Hediste sp. 1 (large-egg form)	Japan	200–250 (direct)	female spawning within burrows	Sato & Tsuchiya, 1987, 1991
Hediste sp. 2 (Ariake form)	Japan (Ariake Sea)	200–210 (?)	?	Sato & Nakashima, unpubl.
H. diversicolor	Europe North American Atlantic coast	195–275 (demersal)	female spawning within burrows, few males (1–30%)	Dales, 1950; Smith, 1964; Bartels-Hardege & Zeeck, 1990
H. limnicola	North American Pacific coast	120–170, 210 (viviparous)	hermaphrodite, self-fertilization	Smith, 1950 Fong & Pearse, 1992a

those of the large-egg form in Japan (Sato & Masuda, 1997).

The life history of *H. limnicola*, which inhabits fresh waters, is very specialized in that the species is hermaphroditic, self fertilizing and viviparous (Smith, 1950). Within the coelom of a parent, the young develop to juveniles. The juveniles emerge by rupturing of the body wall of the parent when they become 4–8 mm long with 20–30 segments. Cross-fertilization has never been observed in the field, and isolated individuals produced viable offspring in laboratory cultures (Fong & Pearse, 1992a,b). However, the genetic evidence from allozyme electrophoretic analysis suggests that *H. limnicola* is not entirely self-fertilizing but outcrosses frequently enough to maintain relatively high levels of heterozygosity (Fong & Garthwaite, 1994).

Since the Ariake form has just recently been discovered, we have only little information about its life-history traits. Mature eggs of the Ariake form were

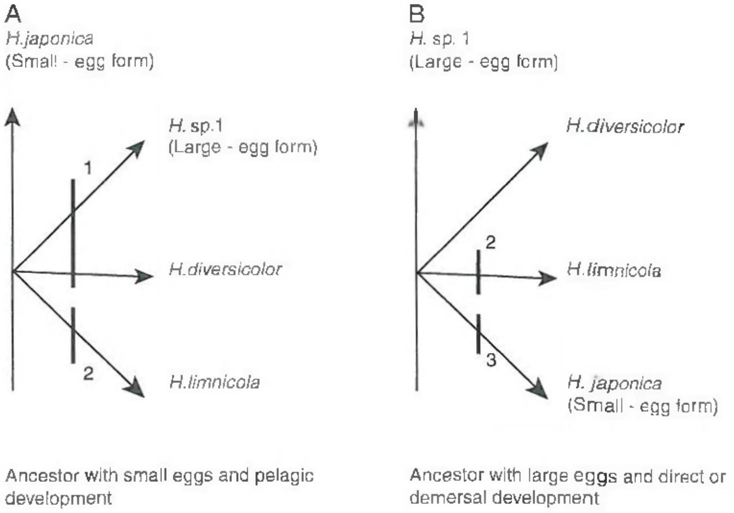

Figure 13. Two hypotheses of speciation in *Hediste* species group. (A) Hypothesis that the ancestral form had a life history with small egg size and planktonic development. (B) Hypothesis that the ancestral form had a life history with large egg size and direct or demersal development. (1) Large egg size and direct or demersal development. (2) Hermaphrodite, self-fertilization and viviparity. (3) Diadromous life history with swarming and planktonic development.

obtained from only one laboratory-cultured female. The relatively large egg size (average egg diameter: 205 μm) suggests that the Ariake form may have the direct development like the large-egg form.

Conclusion

Smith (1958) first discussed the direction of speciation in *Hediste*. He suggested that life history of the small-egg form with reproductive swarming might be the plesiomorphic situation. According to his hypothesis, species with direct or demersal development should be derived from an ancestor with planktonic development (Figure 13A).

Indeed, many marine nereidid species show reproductive swarming accompanied by epitokous metamorphosis of adults to the heteronereid form (Schroeder & Hermans, 1975). However, mature adults of the small-egg form do not metamorphose to the typical heteronereid form: they lack the characteristic paddle-shaped epitokous chaetae, though many homogomph spinigers are added (Smith, 1958; Sato, unpubl.).

Large egg size and direct development are not necessarily derivative characteristics for adaptation in brackish waters of rivers, but commonly occur also in marine species of polychaetes and other macrobenthos (Thorson, 1950; Mileikovsky, 1971; Schroeder & Hermans, 1975; M. E. Petersen, personal commmunication). Contrasting life histories with direct or planktonic development are known in marine polychaetes such as Nereididae (Hauenschild, 1951; Reish, 1957; Clark, 1977), Hesionidae (Westheide, 1967), Capitellidae (Grassle & Grassle, 1976), Sabellidae (McEuen et al., 1983) and Lumbrineridae (Sato et al., 1982). In many polychaete taxa, it appears to be the rule rather than the exception that reproduction and developmental patterns are variable. Therefore, either direct or planktonic development could be ancestral for the life history of a brackish-water species.

In *Hediste*, another hypothesis of speciation is proposed here: Life history with direct or demersal development may be primitive, and life history with the reproductive swarming and planktonic development may be derived (Figure 13B). Electrophoretic studies (Fong & Garthwaite, 1994; Abbiati & Maltagliati, 1996; Sato & Masuda, 1997) suggested that greater genetic differentiation between populations can occur in species with direct or demersal development, as generally expected (Scheltema, 1971; Burton & Feldman, 1982; Hoskin, 1997). If such a form is ancestral, allopatric speciation and establishment of sibling-species groups seem to be easy.

In the genus *Hediste*, only *H. limnicola* inhabits freshwaters. Its specialized life-history traits such as hermaphroditism, self-fertilization and viviparity may be considered as adaptation to the freshwater habitats.

The other *Hediste* species inhabit brackish waters in North Temperate Zone. Establishment of the diadromous life-history of the small-egg form may be related to the higher primary productivity in temperate latitudes. In general, oceans are more productive than freshwaters in temperate latitudes, while freshwaters are much more productive than oceans in tropical latitudes (Gross et al., 1988). One of the most important benefits of planktonic development for benthic animals is that their larvae can use the phytoplankton for food. In a temperate region with high oceanic productivity, rich resources of phytoplankton in seawater may cause the evolution even for a brackish-water or freshwater species to yield a life history with planktonic larval life in the sea. This idea can illustrate establishment of the diadromy in fishes (Gross, 1987). Thus, it is probable that the small-egg form with planktonic development evolved from an ancestral form like the large-egg form with direct development.

According to this hypothesis, polychaetes with planktonic development and a diadromous life history should be rare in tropical brackish waters or freshwaters. The following facts seem to support this prediction, though life-history studies of tropical brackish-water or freshwater species are not enough: 1. There is no report of planktonic development, reproductive swarming and any other characteristic of the diadromy in brackish-water or freshwater polychaetes inhabiting tropical latitudes. 2. Nondiadromous life history with direct development was found in a subtropical brackish-water nereidid, *Neanthes glandicincta*, which is morphologically very similar to *Hediste* spp. (Sato, unpubl.). 3. In temperate latitudes of Japan, a diadromous life history with reproductive swarming and planktonic development, as in the *Hediste* small-egg form, is known in another brackish-water nereidid, *Tylorrhynchus heterochaetus* (Izuka, 1903; Osanai, 1978), which is not closely related to *Hediste* spp.

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References

Abbiati, M. & F. Maltagliati, 1996. Allozyme evidence of genetic differentiation between populations of *Hediste diversicolor* (Polychaeta: Nereididae) from the western Mediterranean. J. mar. biol. Ass. U.K. 76: 637–647.

Åkesson, B., 1973. Reproduction and larval morphology of five *Ophryotrocha* species (Polychaeta, Dorvilleidae). Zool. Scr. 2: 145–155.

Bartels-Hardege, H. & D. E. Zeeck, 1990. Reproductive behavior of *Nereis diversicolor* (Annelida: Polychaeta). Mar. Biol. 106: 409–412.

Bertout, M., 1976. Spermatogenèse de *Nereis diversicolor* O. F. Müller (Annélide Polychète) 1. Évolution du cytoplasme et élaboration de l'acrosome. J. Micros. Biol. Cell. 25: 87–94.

Burton, R. S. & M. W. Feldman, 1982. Population genetics of coastal and estuarine invertebrates: Does larval behavior influence population structure? In Kennedy, V. S. (ed.), Estuarine Comparisons. Academic Press, New York: 537–551.

Christie, G., 1985. A comparative study of the reproductive cycles of three Northumberland populations of *Chaetozone setosa* (Polychaeta: Cirratulidae). J. mar. biol. Ass. U.K. 65: 239–254.

Clark, R. B., 1977. Reproduction, speciation and polychaete taxonomy. In Reish, D. J. & K. Fauchald (eds), Essays on Polychaetous Annelids in Memory of Dr Olga Hartman. Allan Hancock Foundation, Univ. Southern California, Los Angeles: 477–501.

Dales, R. P., 1950. The reproduction and larval development of *Nereis diversicolor* O. F. Müller. J. mar. biol. Ass. U.K. 29: 321–360.

Eckelbarger, K. J. & J. P. Grassle, 1987a. Spermatogenesis, sperm storage and comparative sperm morphology in nine species of *Capitella*, *Capitomastus* and *Capitellides* (Polychaeta: Capitellidae). Mar. Biol. 95: 415–429.

Eckelbarger, K. J. & J. P. Grassle, 1987b. Interspecific variation in genital spine, sperm and larval morphology in six sibling species of *Capitella*. Bull. biol. Soc. Wash. 7: 62–76.

Fong, P. P. & R. L. Garthwaite, 1994. Allozyme electrophoretic analysis of the *Hediste limnicola* – *H. diversicolor* – *H. japonica* species complex (Polychaeta: Nereididae). Mar. Biol. 118: 463–470.

Fong, P. P. & J. S. Pearse, 1992a. Photoperiodic regulation of parturition in the self-fertilizing viviparous polychaete *Neanthes limnicola* from central California. Mar. Biol. 112: 81–89.

Fong, P. P. & J. S. Pearse, 1992b. Evidence for a programmed circannual life cycle modulated by increasing daylengths in *Neanthes limnicola* (Polychaeta: Nereidae) from central California. Biol. Bull. 182: 289–297.

Grassle, J. P. & J. F. Grassle, 1976. Sibling species in the marine pollution indicator *Capitella* (Polychaeta). Science 192: 567–569.

Gross, M. R., 1987. Evolution of diadromy in fishes. American Fisheries Society Symposium 1: 14–25.

Gross, M. R., R. M. Coleman & R. M. McDowall, 1988. Aquatic productivity and the evolution of diadromous fish migration. Science 239: 1291–1293.

Hateley, J. G., A. Grant, S. M. Taylor & N. V. Jones, 1992. Morphological and other evidence on the degree of genetic differentiation between populations of *Nereis diversicolor*. J. mar. biol. Ass. U.K. 72: 365–381.

Hauenschild, C., 1951. Nachweis der sogenannten atoken Geschlechtsform des Polychaeten *Platynereis dumerilii* Aud. et M. Edw. als eigene Art auf Grund von Zuchtversuchen. Zool. Jb. Allg. Zool. Physiol. 63: 107–127.

Hoagland, K. E. & R. Robertson, 1988. An assessment of poecilogony in marine invertebrates: Phenomenon or fantasy? Biol. Bull. 174: 109–125.

Hoskin, M. G., 1997. Effects of contrasting modes of larval development on the genetic structures of populations of three species of prosobranch gastropods. Mar. Biol. 127: 647–656.

Imajima, M., 1972. Review of the annelid worms of the family Nereidae of Japan, with description of five new species or subspecies. Bull. natn. Sci. Mus., Tokyo 15: 37–153.

Izuka, A., 1903. Observations on the Japanese Palolo, *Ceratocephale osawai*, n. sp. J. Coll. Sci. imp. Univ. Tokyo 17: 1–37.

Izuka, A., 1908. On the breeding habit and development of *Nereis japonica* n. sp. Annot. Zool. Jap. 6: 295–305.

Kagawa, Y., 1955. Note on the optimum salinities, studied in the adult and larva of the brackish-water polychaete worm, *Nereis japonica*. J. Gakugei Coll. Tokushima Univ. nat. Sci. 6: 11–16 (in Japanese, English summary).

Kirkegaard, J. B., 1995. Bathyal and abyssal polychaetes (errant species). Galathea Rep. 17: 7–56.

Levin, L. A. & T. S Bridges, 1995. Pattern and diversity in reproduction and development. In McEdward, L. (ed.), Ecology of Marine Invertebrate Larvae. CRC Press, Boca Raton: 79–122.

Levin, L. A. & E. L. Creed, 1986. Effect of temperature and food availability on reproductive responses of *Streblospio benedicti* (Polychaeta: Spionidae) with planktotrophic or lecithotrophic development. Mar. Biol. 92: 103–113.

Levin, L. A., J. Zhu & E. Creed, 1991. The genetic basis of life-history characters in a polychaete exhibiting planktotrophy and lecithotrophy. Evolution 45: 380–397.

McEuen, F. S., B. L. Wu & F. S. Chia, 1983. Reproduction and development of *Sabella media*, a sabellid polychaete with extratubular brooding. Mar. Biol. 76: 301–309.

Mileikovsky, S. A., 1971. Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Mar. Biol. 10: 193–213.

Okada, K., 1960. Photomicrographs of fertilization-reactions on the *Nereis* eggs, taken by means of the phase-contrast microscope. J. Gakugei Coll. Tokushima Univ. nat. Sci. 10: 63–71 (in Japanese).

Osanai, K., 1978. Early development of the Japanese Palolo, *Tylorrhynchus heterochaetus*. Bull. mar. biol. Stn Asamushi, Tohoku Univ. 16: 59–69.

Pasteels, J. J., 1966. La réaction corticale de fécondation de l'oeuf de *Nereis diversicolor*, etudiée au microscope électronique. Acta Embryologiae et Morphologiae Experimentalis 6: 155–163.

Reish, D., 1957. The life history of the polychaetous annelid *Neanthes caudata* (delle Chiaje), including a summary of development in the family Nereidae. Pac. Sci. 11: 216–228.

Sato, M., 1992. Differences in paragnath number between two sibling species in the brackish-water polychaete, *Neanthes japonica*. Zool. Sci. 9: 1298.

Sato, M. & M. Ikeda, 1992. Chromosome complements of two forms of *Neanthes japonica* (Polychaeta, Nereididae) with evidence of male-heterogametic sex chromosomes. Mar. Biol. 112: 299–307.

Sato, M. & Y. Masuda, 1997. Genetic differentiation in two sibling species of the brackish-water polychaete *Hediste japonica* complex (Nereididae). Mar. Biol. 130: 163–170.

Sato, M. & K. Osanai, 1986. Morphological identification of sperm receptors above egg microvilli in the polychaete, *Neanthes japonica*. Dev. Biol. 113: 263–270.

Sato, M. & M. Tsuchiya, 1987. Reproductive behavior and salinity favorable for early development in two types of the brackish-water polychaete *Neanthes japonica* (Izuka). Benthos Research, Japan 31: 29–42.

Sato, M. & M. Tsuchiya, 1991. Two patterns of early development in nereidid polychaetes keying out to *Neanthes japonica* (Izuka). Ophelia, Suppl. 5: 371–382.

Sato, M., M. Tsuchiya & M. Nishihira, 1982. Ecological aspect of the development of the polychaete, *Lumbrineris latreilli* (Audouin et Milne-Edwards): Signification of direct development and non-simultaneous emergence of the young from the jelly mass. Bull. mar. biol. Stn Asamushi, Tohoku Univ. 17: 71–85.

Scheltema, R. S., 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. Biol. Bull. 140: 284–322.

Schneider, S., A. Fischer & A. W. C. Dorresteijn, 1992. A morphometric comparison of dissimilar early development in sibling species of *Platynereis* (Annelida, Polychaeta). Roux's Arch. dev. Biol. 201: 243–256.

Schroeder, P. C. & C. O. Hermans, 1975. Annelida: Polychaeta. In Giese, A. C. & J. S Pearse (eds), Reproduction of Marine Invertebrates. Vol. III. Academic Press, New York: 1–213.

Sella, G., R. Vitturi, L. Ramella & M. S. Colomba, 1995. Chromosomal nucleolar organizer region (NOR) phenotypes in nine species of the genus *Ophryotrocha* (Polychaeta: Dorvilleidae). Mar. Biol. 124: 425–433.

Smith, R. I., 1950. Embryonic development in the viviparous nereid polychaete, *Neanthes lighti* Hartman. J. Morph. 87: 417–466.

Smith, R. I., 1958. On reproductive pattern as a specific characteristic among nereid polychaetes. Syst. Zool. 7: 60–73.

Smith, R. I., 1964. On the early development of *Nereis diversicolor* in different salinities. J. Morph. 114: 437–464.

Smith, R. I., 1977. Physiological and reproductive adaptations of *Nereis diversicolor* to life in the Baltic Sea and adjacent waters. In Reish, D. J. & K. Fauchald (eds), Essays on Polychaetous Annelids in Memory of Dr Olga Hartman. Allan Hancock Foundation, Univ. Southern California, Los Angeles: 373–390.

Sun, R., B. Wu & D. Yang, 1980. Study of the morphology and larval development of the *Neanthes japonica* (Izuka) from the China Sea. J. Shandong Coll. Ocean. 10: 100–110 (in Chinese, English summary).

Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25: 1–45.

Ushakov, P. V., 1955 (English translation, 1965). Polychaete worms of the far-eastern seas of the USSR. Israel Program for Scientific Translations, Jerusalem. 419 pp.

Westheide, W., 1967. Monographie der Gattungen *Hesionides* Friedrich und *Microphthalmus* Mecznikow (Polychaeta, Hesionidae). Ein Beitrag zur Organisation und Biologie psammobionter Polychaeten. Z. Morph. Tiere 61: 1–159.

Westheide, W. & R. M. Rieger, 1987. Systematics of the amphiatlantic *Microphthalmus-listensis*-species-group (Polychaeta: Hesionidae): Facts and concepts for reconstruction of phylogeny and speciation. Z. zool. Syst. u. Evolut.-forsch. 25: 12–39.

Wu, B. L., R. Sun & D. Yang, 1985. The Nereidae (Polychaetous Annelids) of the Chinese Coast. China Ocean Press, Beijing, 234 pp.