

Comparative Studies of American, European and Japanese Forms of *Plumatella emarginata*, a Freshwater Bryozoan

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ABSTRACT Statoblast samples of *Plumatella emarginata* ALLMAN, 1844 were obtained from North America, the Netherlands, and Japan. Colonies derived from these statoblasts were cultured in the laboratory and compared in several features. Fusion readily occurred between ancestrulae of the same geographic type, but never between any of the three different forms. Japanese material produced slightly smaller floatoblasts than the others. Following germination the ancestrulae of the Japanese form tended to be initially smaller than those of the others, but this difference decreased with subsequent growth. The three forms were otherwise similar or identical in the morphology and size of colonies and polypides, and in the electrophoretic mobilities of three enzymes. It is considered appropriate to regard these forms as geographical variants of the same species, *P. emarginata*.

Plumatella emarginata ALLMAN, 1844 is a cosmopolitan species of freshwater bryozoan. Many varieties have been described as separate species, but most of them are now regarded as synonyms of *P. emarginata* (TORIUMI, 1952; LACOURT, 1968).

Using Japanese material, MUKAI *et al.* (1984) found that in the family Plumatellidae intraspecific fusion occurs between the mucous pads of ancestrulae germinated from statoblasts. This fusion has been considered to be species specific. In the present study, we tried fusion experiments between *Plumatella emarginata* populations from North America, the Netherlands, and Japan. We also compared the three forms in the morphology and size of floatoblasts (buoyant statoblasts), colonies and polypides and in the electrophoretic mobilities of three enzymes.

Materials and Methods

Specimens of *Plumatella emarginata* ALLMAN were collected from North America, the Netherlands, and Japan. Viable statoblasts were obtained from the following materials: 1) several colonies collected in 1986 to 1988 at two sites from the Chesapeake and Ohio Canal near the Potomac River, U.S.A.,

2) a single colony collected on 9 July 1986 at Park Randenbroek, Amersfoort, the Netherlands and 3) several colonies collected in 1985 to 1987 at two ponds in Gunma Prefecture, Japan.

Fusibility of *Plumatella emarginata* ancestrulae was tested between two colonies. Several floatoblasts (one to three from each of the two colonies) were placed in close proximity with one another against the inside bottom of an inverted petri dish, 6 cm in diameter (*cf.* MUKAI *et al.*, 1984). The floatoblasts from one colony had been marked previously by cutting the float slightly using a razor's edge.

Plumatella reticulata, which was recently described by WOOD (1988), is very similar at the first glance to *P. emarginata* in the morphology of floatoblasts, although the former can be easily distinguished from the latter by a conspicuous reticulated pattern on the frontal valve of the sessoblast. Interspecific fusion experiments were also tried between *P. reticulata* (collected from the Potomac River) and *P. emarginata* (American and Japanese forms).

Floatoblast-derived colonies of *Plumatella emarginata* were reared at Gunma University from the beginning of April to early May, 1988. The colonies were grown on the bottoms of large petri dishes, 15 cm in diameter, which were held upside down. Water from a pond near the university was used as a culture medium, and the water in the dishes was normally renewed twice a day. At the end of the culture period, the colonies had been producing mature statoblasts.

The mobilities of three enzymes, malate dehydrogenase (MDH), glycerophosphate dehydrogenase (GPDH), and phosphoglucose isomerase (PGI), were examined by starch gel electrophoresis at George Washington University.

Fifty to 100 statoblasts from a single colony were dissected out, moistened with distilled water, and then crushed between two plastic blocks. The resulting exudate was absorbed on a piece of filter paper (approximately 4 × 15 mm) which was then inserted in the gel. Preparations from the different forms of *Plumatella emarginata* were normally placed in side-by-side slots. Comparison was also made with living *P. emarginata* (North American material only). Five to ten polypides from single colonies were dissected out, clumped on a piece of moistened filter paper, and then crushed. The filter paper was then inserted in a gel slot. Other phylactolaemates (including statoblast preparations from *Lophopodella carteri*, *Asajirella gelatinosa*, and *Pectinatella magnifica*, as well as material from living colonies of *Hyalinella punctata* and *Plumatella repens*) were also usually run on the same gels.

Good results were obtained by running gels for 3.5–4 hr at about 100 volts and 10 milliamperes with 0.135 M Tris-citrate buffer (pH 8.2) in the electrode chambers. Staining was based on the methods of SHAW and PRASAD (1970), with all solutions at pH 8.2. Under the assay conditions GPDH activity was observed only when malate ion was present.

Results

Fusibility of ancestrulae. Fusion invariably occurred between ancestrulae germinated from floatoblasts collected from the same geographic area. But

fusion was never obtained between ancestrulae of different forms, though every combination of the three forms was examined repeatedly (Fig. 1).

No fusion of ancestrulae was observed between *Plumatella reticulata* and *P. emarginata*.

Statoblasts. *Plumatella emarginata* produces both floatoblasts and sessoblasts. The floatoblasts are generally broadly oval. The float, filled with a secreted gas, covers the capsule more widely on the cystigenic (or dorsal) side than on the deutoplasmic (or ventral) side. The fenestra, *i.e.*, central area not covered by the float, is large and nearly circular on the deutoplasmic side, and is small and oval on the cystigenic side.

Photographs of floatoblasts from wild colonies and laboratory-grown colonies of the three forms are shown in Figure 2, and their measurements are listed in Table 1.

The floatoblasts from wild colonies were the largest in the North American form, somewhat smaller in the European form, and smallest in the Japanese form.

The floatoblasts produced by the laboratory colonies were similar in size to those obtained from the wild colonies in the American form. The laboratory floatoblasts of the European form were slightly smaller than the wild floatoblasts of the same form. For the Japanese form, the laboratory floatoblasts were longer than the wild floatoblasts, but were smaller than the laboratory floatoblasts of the American and European forms.

The laboratory colonies of all forms produced some sessoblasts. They were oval with a narrow annular lamella and mammillated on the frontal valve. They were seemingly similar in all forms, although no detailed comparison was made.

Colonies and polypides. The ancestrulae of the Japanese *Plumatella emarginata* were somewhat smaller than those of the other two forms immediately following the germination. However, this difference in size gradually decreased with subsequent development.

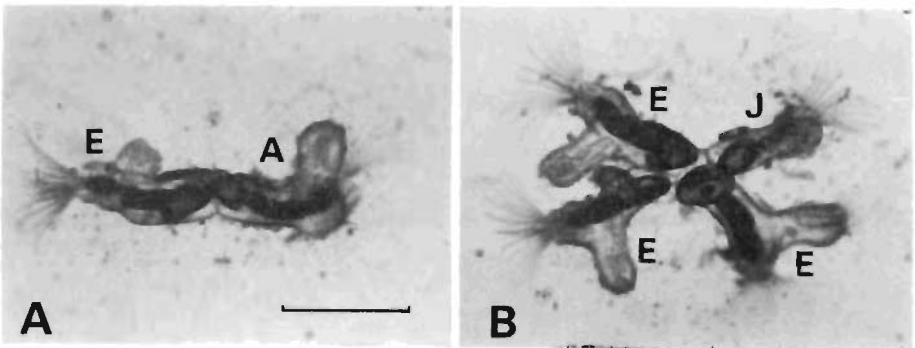


Fig. 1. Failure of fusion between different forms of *Plumatella emarginata* (photographed 4 days after germination). A: Between American (A) and European (E) forms. B: Between European (E) and Japanese (J) forms. Note that the three ancestrulae of the European form have fused. Scale bar in A, applicable to both figures, equals 1 mm.

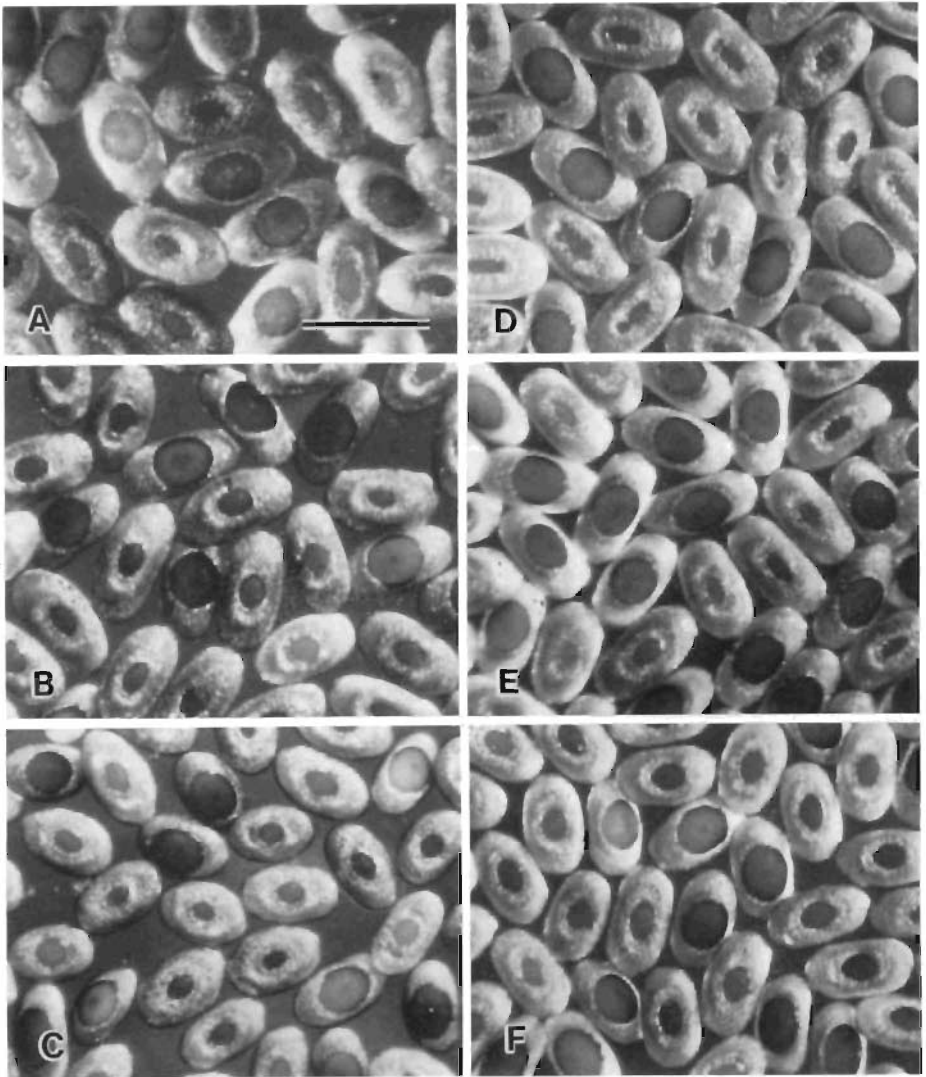


Fig. 2. Floatoblasts of *Plumatella emarginata*. A–C: From wild colonies. D–F: From laboratory-grown colonies. A, D: American form; B, E: European form; C, F: Japanese form. Scale bar in A, applicable to all figures, equals 0.5 mm.

The growth pattern of colonies was essentially the same in the three forms (Fig. 3A–C). Young colonies were moderately branched and entirely reptent. Most ancestrulae produced 5–6 daughter zooids, and septa between zooids were common. The ectocyst was colorless in young parts of the colony, but was

Table 1. Measurements in micrometers of floatoblasts of *Plumatella emarginata*.

Source of statoblasts*	Length				Width			
	Maximum	Minimum	Mean \pm s.d.	No. measurements	Maximum	Minimum	Mean \pm s.d.	No. measurements
Floatoblast:								
American form (w)	531	438	485 \pm 25	49	281	250	264 \pm 8	49
European form (w)	521	427	473 \pm 23	40	260	240	247 \pm 6	40
Japanese form (w)	417	345	380 \pm 21	40	255	219	237 \pm 10	40
American form (l)	521	448	483 \pm 20	49	271	240	253 \pm 8	49
European form (l)	490	417	458 \pm 22	40	250	224	236 \pm 8	40
Japanese form (l)	448	396	419 \pm 15	46	240	219	233 \pm 7	46
Deutoplasmic side fenestra:								
American form (w)	229	198	216 \pm 10	25	203	177	189 \pm 6	25
European form (w)	229	208	216 \pm 6	21	203	182	193 \pm 6	21
Japanese form (w)	219	188	203 \pm 10	22	177	156	166 \pm 4	22
American form (l)	234	208	221 \pm 6	21	193	167	183 \pm 7	21
European form (l)	224	188	206 \pm 11	21	188	167	172 \pm 7	21
Japanese form (l)	229	208	217 \pm 7	25	182	167	171 \pm 5	25
Cystigenic side fenestra:								
American form (w)	156	104	131 \pm 15	30	94	62	80 \pm 8	30
European form (w)	146	104	130 \pm 9	34	104	73	91 \pm 10	34
Japanese form (w)	135	89	110 \pm 14	39	94	63	80 \pm 9	39
American form (l)	151	109	130 \pm 13	36	83	52	61 \pm 9	36
European form (l)	135	94	114 \pm 14	30	83	52	65 \pm 9	30
Japanese form (l)	151	125	136 \pm 8	39	104	78	95 \pm 6	39

* (w): wild colony; (l): laboratory-grown colony.

yellowish-brown to brown in older regions. No difference was found among the three forms in either the appearances or sizes of growing branches or polypides (Fig. 3D-F).

The three forms were also similar in the number of tentacles. Nine days after germination, the ancestrulae had about 24-26 tentacles, and other polypides around the ancestrulae had about 37-41 tentacles in all forms. In older colonies (16 days after germination), most polypides on actively growing branches possessed 40-44 tentacles.

Gel electrophoresis. Relative mobilities of the three enzymes are diagrammatically shown in Figure 4. Statoblast preparations from the three forms of *Plumatella emarginata* showed no differences with respect to the band positions of the three enzymes. Polypides of North American *P. emarginata* yielded the same bands observed in the statoblast preparations.

Other phylactolaemate species consistently yielded band patterns different from those of *Plumatella emarginata* involving at least two (and frequently all three) enzymes.

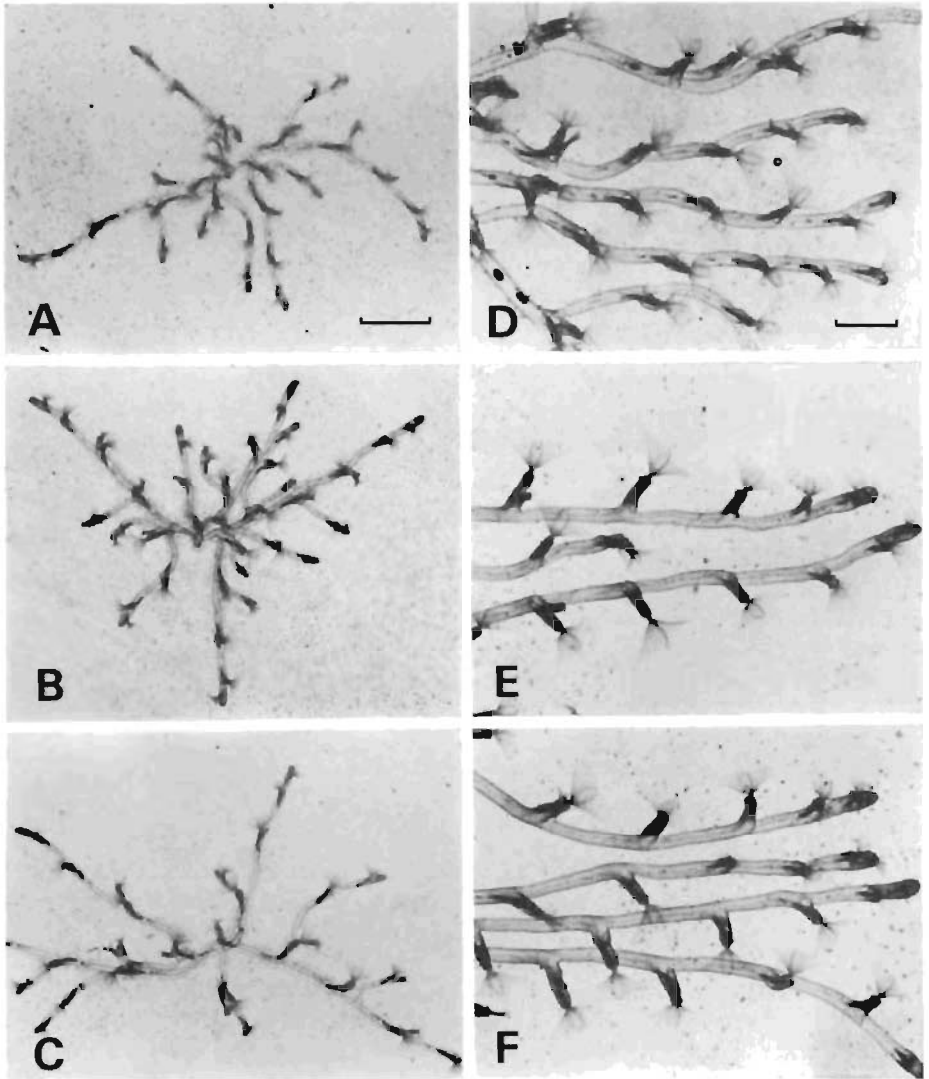


Fig. 3. Colonies of *Plumatella emarginata* cultured in the laboratory. A-C: Young colonies, photographed 12 days after the germination of floatoblasts. D-F: Growing branches, photographed 18 days after the germination. A, D: American form; B, E: European form; C, F: Japanese form. Scale bar in A, applicable to A-C, equals 5 mm; scale bar in D, applicable to D-F, equals 2 mm.

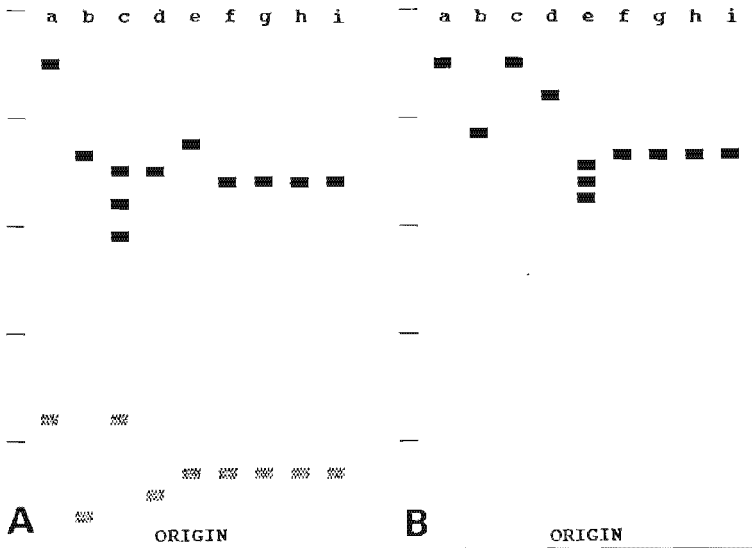


Fig. 4. Relative enzyme mobilities. A: Malate dehydrogenase (MDH), shown in solid, and glycerophosphate dehydrogenase (GPDH), shown in cross-hatching. B: Phosphoglucose isomerase (PGI), shown in solid. a: *Lophopodella carteri* statoblasts. The same results were obtained from both Japanese and North American samples. b: *Pectinatella magnifica* statoblasts. c: *Asajirella gelatinosa* statoblasts from Lake Tataranuma, Japan. All 12 samples analyzed were heterozygous at the MDH locus. d: *Hyalinella punctata* polypides. e: *Plumatella repens* polypides and statoblasts from a number of sources. Heterozygosity at the PGI locus is shown, although some samples were homozygous for either the faster or slower allele. f-i: *Plumatella emarginata*. f: North American polypides. g: North American statoblasts. h: European statoblasts. i: Japanese statoblasts.

Discussion

The three forms of *Plumatella emarginata* used in the present study can be clearly distinguished by fusibility. Fusion never occurred between different forms, although within each form ancestrulae were invariably fusible.

The size (especially the length) of floatoblasts was largest in the North American form, slightly smaller in the European form, and much smaller in the Japanese form. This was the case both in the floatoblasts of wild colonies and in those of laboratory-grown colonies. The measurements for the wild floatoblasts of the Japanese form agree well with those given by TORIUMI (1952) for other Japanese materials. Thus, the relative smallness of the floatoblasts may be a general feature of Japanese *Plumatella emarginata*. However, before a definite statement can be made concerning the taxonomic value of the differences in size

of floatoblasts observed, it is necessary to compare collections from more localities. Our culture experiments showed that the size of floatoblasts was more or less variable between wild colonies and laboratory colonies in each form, suggesting some influence of external conditions on the size of floatoblasts. The measurements of *P. emarginata* floatoblasts by previous workers have been listed in BUSHNELL (1965).

Other features examined in the present study, such as the appearance of colonies and polypides, the number of tentacles and the electrophoretic mobilities of three enzymes, were similar in all three forms. The ancestrulae of the Japanese form were somewhat smaller than those of the others when evaginated, probably correlated with the smaller floatoblasts, but this difference disappeared as they grew following germination.

There is no doubt that the failure of fusion in ancestrulae among the three geographical forms reflects some genetic difference. However, assigning these forms to distinctive species or subspecies does not appear appropriate from the viewpoint of practical taxonomy, because no well-defined morphological differences seem to exist. Consequently, at the present time, it appears best to regard the three forms as geographical variants of the same species.

摘 要

向井秀夫(群馬大学教育学部)・B. T. BACKUS (George Washington Univ., U.S.A.)・T. S. Wood (Wright State Univ., U.S.A.)——北米, ヨーロッパおよび日本産ヤハズハネコケムシ *Plumatella emarginata* の比較研究.

北米, ヨーロッパ(オランダ) および日本からヤハズハネコケムシ *Plumatella emarginata* ALLMAN, 1884 と同定された群体を採集した. それらの休芽に由来する群体を室内で飼育し, いくつかの形質について比較した. 同一地域に由来する初虫同士は癒合したが, 異地域由来の初虫間には癒合はおこらなかった. 日本産の群体(野生のものおよび室内飼育のものとも)が形成した浮遊性休芽は, 他の2地域のものより小型であった. また, 日本産の浮遊性休芽に由来する初虫は他の2地域のものより小さかったが, 大きさの違いは出芽後の成長にともなって減少した. 群体および虫体の形状と大きさ, 触手数, 3種の酵素(リンゴ酸デヒドロゲナーゼ, グリセロリン酸デヒドロゲナーゼおよびグルコースリン酸イソメラーゼ)の電気泳動移動度については, 3地域間で違いは見られなかった. 現時点では, これら3地域に由来する標本を同一種 *P. emarginata* の地理的変異体と見なしておくのが妥当と思われる.

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