Marine Biology (1999) 135: 411–423

H. R. Schwaninger

Population structure of the widely dispersing marine bryozoan *Membranipora membranacea* (Cheilostomata): implications for population history, biogeography, and taxonomy

Received: 31 August 1998 / Accepted: 10 August 1999

Abstract Morphologically plastic, cryptic, or geographically widespread species pose similar challenges to the evolutionary biologist: their taxonomic status is often unclear yet must be known to study almost any aspect of their biology, ecology, evolution, or biogeography. The marine bryozoan *Membranipora membranacea* (L.) is morphologically plastic and geographically widespread in temperate oceans of the Northern and Southern Hemispheres, and its taxonomy is unclear. This study examined genetic relationships among allopatric populations and sympatric morphs of this species, or species complex. Colonies were collected from 1992 to 1995. Allozymes were used to elucidate the relationships among four widely separated populations, two in the North Atlantic and two in the North Pacific Ocean. Allozymes and mtDNA sequencing were used to clarify the genetic relationships among three sympatric morphs that might correspond to the species *M. villosa* Hincks and *M. membranacea* in the northeastern Pacific (Washington State). Populations in the North Atlantic and North Pacific had no fixed allelic differences at the loci tested but were separated by an average Nei's genetic distance of 0.581, suggesting their near-sibling species status. Populations from Friday Harbor (Washington) and Catalina Island (California) were not significantly differentiated, which was attributed to high gene flow. Populations on either side of the North Atlantic were genetically indistinguishable, which is most likely due to the recent establishment of the West Atlantic populations from European founders. At Friday Harbor, sympatric morphs varying in their spination and spine inducibility were genetically indistinguishable, supporting the hypothesis that *M. villosa* is an induced phenotype of *M. membranacea* and not a distinct species in the northeastern Pacific. Since such phenotypic plasticity is common in cheilostome bryozoans, the morphospecies concept must be used with caution.

Communicated by J. P. Grassle, New Brunswick

H.R. Schwaninger
Department of Ecology and Evolutionary Biology,
Corson Hall, Cornell University,
Ithaca, New York 14853, USA
e-mail: hs16@cornell.edu,
Fax: +1-607-2558088

Introduction

The fundamental unit in evolutionary biology is the species. Adequately identifying species is often problematic for widely distributed and phenotypically plastic organisms, especially when the plastic characters are used in defining taxonomic affinity. The marine bryozoan *Membranipora membranacea* is widely distributed in temperate oceans of the Northern and Southern hemispheres and is plastic in taxonomically important characters. This raises taxonomic confusion about the relationships of populations and/or species within and between oceans. *M. membranacea* is a colonial invertebrate growing on kelps and has a simple morphology offering few taxonomic characters. Osburn (1950) recognized three species of *Membranipora* along the North American west coast: *M. membranacea* (L.), *M. villosa* Hincks, and *M. serrilamella* Osburn. *M. serrilamella* is distinguished by the presence of a serrated cryptocyst (inner extension of the zooid wall, Fig. 1), *M. villosa* by the presence of a cryptocyst and numerous spines on the frontal membrane and lateral zooid walls, and *M. membranacea* by the lack of both characters (Fig. 1). However, several authors have found variation in these taxonomic characters within single colonies (O'Donoghue 1926; Pinter 1969; Seed 1976). Yoshioka (1982a) found that the *M. villosa* form was temporally and spatially correlated with the presence of nudibranchs in the field and suggested that these predators induced this form. In laboratory experiments, Harvell (1984, 1986, 1991) confirmed that *M. membranacea* exposed to nudibranchs grew *M. villosa*-like spines. In a laboratory common-garden experiment 178

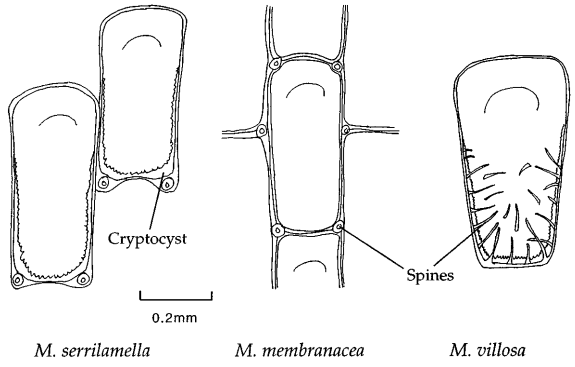

Fig. 1 *Membranipora* spp. Zooids of three species. *M. serrilamella* is characterized by the presence of a crypotcyst, *M. membranacea* by the absence of a cryptocyst and spines on zooid walls, and *M. villosa* by the presence of both characters. Redrawn from Osburn (1950)

colonies were exposed to the nudibranch cue. A total of 80.3% of the colonies produced spines, 13.5% remained unspined, and 6.2% were constitutively spined (spined before exposure to the cue) (Harvell 1998). Thus, at least two of the three species described along the North American west coast might simply be an expression of the polymorphism of one species (*M. membranacea*) induced by exposure to the nudibranch predator and other environmental conditions some time before the type specimens were collected. But since there are three distinct morphotypes there is also the possibility that these are different species. Still other variation in colony form may be associated with substrate type, because *M. membranacea* grows on many species of brown algae. In fact, aspects of colony morphology do seem to vary with substrate. In southern California, few stolons (elongated specialized zooids produced in response to intraspecific competition; Harvell and Padilla 1990) are made on *Macrocystis integrifolia* but many on *Laminaria* spp. (L. Roberson and C. D. Harvell unpublished data). These observations raise the question of whether colonies on different kelps are genetically distinctive or phenotypically plastic in response to different substrates. There is currently no information about plasticity associated with the *M. serrilamella* phenotype.

Similarly, on a global scale the relationship of *Membranipora membranacea* described from various geographic locales is unclear (Osburn 1950; Yoshioka 1982b). Morphological differences between geographic populations of this species have been reported in temperate waters of the Northern and Southern Hemispheres (Osburn 1950) raising the question of whether these allopatric populations belong to the same species. For example, the planktotrophic larvae of *M. membranacea* of the US west coast are smaller and differ in ornamentation from those in European waters (Robertson 1908; Atkins 1955). The populations of *M. membranacea* in the West Atlantic are reported to be recent invaders (Berman et al. 1992; Lambert et al. 1992), but the source population is unknown. In the absence of a solid taxonomic or evolutionary framework we cannot interpret such variation nor can we explain how the global distribution of the species or species complex has come about.

The present study determined the relationships among populations of *Membranipora membranacea* at varying spatial scales and assessed whether different inducible spine morphologies correspond to different species. This study uses allozyme allele frequencies and mitochondrial DNA (mtDNA) sequences to address the following questions: (A) How closely related are populations in the North Atlantic and North Pacific? Did the Northwest Atlantic invaders come from Europe? (B) Are local populations in Friday Harbor, Washington genetically structured? (C) Are populations on different host algae from the same site genetically differentiated? (D) Are the different morphs of *M. membranacea* at Friday Harbor genetically distinct species?

Materials and methods

Sampling and preservation

Genetic structure

Samples of *Membranipora membranacea* (L.) and *M. serrilamella* Hincks were collected from 1993 to 1995 at locations shown on Table 1 and Fig. 2. Relationships among populations in different oceans (Table 1A), in regions within oceans (Table 1A, B), and among local groups growing on different hosts (Table 1C) were determined in different collections. At Friday Harbor (Washington, USA) colonies were collected from kelps, *Nereocystis luetkeana* and *Laminaria groenlandica*, while at Bamfield Marine Station (Vancouver Island, British Columbia, Canada), colonies were collected from *N. luetkeana* and *Macrocystis integrifolia*. This comparison of colonies from different host algae was necessary because *M. membranacea* grows on different hosts worldwide so any geographic analysis could be confounded by host species, i.e. *Macrocystis* spp. at Catalina and Bamfield, *Laminaria* spp. at Friday Harbor, the Isle of Man (UK) and Appledore Island (Maine, USA).

All colonies were obtained by collecting whole blades of kelp with visible colonies growing on them, maintaining them in flowing seawater, and preserving colonies either on site, or after live-shipment to Cornell University, Ithaca, New York, USA. Colonies were identified according to Osburn (1950) and Ryland and Hayward (1977). Individuals were gently scraped off the kelp before freezing in dry ice or liquid nitrogen, and stored at −80 °C. Care was taken to sample each colony (genotype) only once.

Morphs

Colonies of the three morphs [constitutively spined (CS), constitutively unspined (CU) and inducibly spined (IS)] were obtained as described by Harvell (1998). In summary, Lucite panels (black, 2.5 × 5 cm) were set out in May 1995 at the Friday Harbor Laboratory Dock. Newly settled colonies ($n = 178$) were removed to the laboratory each day to avoid premature exposure to the spine-inducing nudibranch *Doridella steinbergii*. Colony morphologies were determined before the spine-induction experiment as being unspined or constitutively spined (CS). After 1 month in the laboratory, colonies were exposed to the spine inducer using an extract of *D. steinbergii* as stimulus (Harvell 1998). Five days after the exposure, the colonies' morphs were again determined, to yield constitutively unspined (CU; no change) or inducibly spined (IS; no spines before induction, but spined afterwards). After allowing the

Table 1 *Membranipora membranacea*, *M. serrilamella*. Collection sites, sample sizes, loci analyzed, and year collected, organized by question (see "Introduction") (loci abbreviations: *Ada* adenosine deaminase; *Dia* diaphorase; *EstFa-1* esterase; *Gpi* glucose phosphate isomerase; *Idh* isocitrate dehydrogenase; *Me* malic enzyme; *Mdh* malate dehydrogenase; *Np* nucleoside phosphorylase; *Pep-la* peptidase, leucyl-alanine; *Pgd* phosphogluconate dehydrogenase; *Pp* inorganic pyrophosphatase; *Sod* superoxide dismutase; *Tpi* triosephosphate isomerase)

Variation in question, Sample location		Allozymes		mtDNA (*n*)	Year collected
		(*n*)	Loci		
(A)	Inter- vs intra-ocean, North Atlantic, North Pacific				
	Isle of Man, England (IOM)	29	*Pep-la*, *Tpi*, *Gpi*, *Ada*, *Pp*, *Pgd*, *Idh*, *Np* (Monomorphic, $n = 10$ per popul.: *Sod*, *Mdh*, *EstFa-1*, *Dia*)		1992
	Appledore Island, Maine (AI)	29			1992
	Friday Harbor, Wash. (FH)	30			1992
	Catalina Island, Cal. (CAT)	31			1992
(B)	Local populations, Friday Harbor				
	Dock (DO)	27	*Pep-la*, *Tpi*, *Gpi*, *Ada*, *Pp*, *Pgd*, *Idh*, *Np*		1992
	Shady Cove (SC)	28			1992
	West Side (WS)	27			1992
	Turn Island (TI)	28			1992
(C)	Substrates, Bamfield, BC, and Friday Harbor				
	FH *N. luetkeana* (FHN)	27	*Ada*, *Gpi*, *Pgd*		1993
	FH *L. groenlandica* (FHL)	80			1992
	Bamfield *N. luetkeana* (BFN)	26			1993
	Bamfield *M. integrifolia* (BFM)	27			1993
(D)	Morphs, Friday Harbor				
	Constitutively spined (CS)	4	*Pgd*, *Gpi*, *Np*, *Tpi*, *Ada*, *Idh* (Monomorphic: *Mdh*, *Me*)	6	1995
	Constitutively unspined (CU)	11		7	1995
	Inducibly spined (IS)	18		6	1995
	M. serrilamella, Japan (M.ser)	2	–	2	1995

colonies to reach approximately 2 cm in diameter, half of each colony was preserved in 100% ethanol for mtDNA analysis, and the other half was shipped alive to Cornell University, where specimens were frozen at −80 °C for allozyme analysis. In total, four CS, 11 CU, and 18 IS colonies were used for allozyme analysis. Six CS, seven CU and six IS colonies were sequenced. To compare these morphs to known *Membranipora serrilamella* genotypes, two colonies, collected in Japan in 1995 by E. Hunter and preserved in 100% ethanol, were also sequenced.

Molecular methods

Allozymes

Starch gel electrophoresis of allozymes was performed using methods of May (1992). I used 75 µl of extraction buffer for 60 to 120 mm^3 of tissue. To improve resolution, two stain recipes were modified: (1) to stain for *Aat*, the amount of fast garnet GBC salt was doubled and the stain was left on the gel slice rather than poured off, and (2) in the *Pgm* stain 1.0 *M* $MgCl_2$ was used. Gel buffer *C* worked best for loci *Idh*, *Me*, *Mdh*, and *Pgd*, the *R*-buffer for loci *Dia*, *EstFa-1*, *Gpi*, *Pep-la*, and *Sod*, the *4*-buffer for *Ada*, *Np*, and *Tpi*, and the *S-9* buffer for locus *Pp*. An initial survey (27 loci, $n = 10$ representatives each from the four most widely separated populations: Friday Harbor, Catalina Island, Appledore Island, Isle of Man) was performed to identify scorable loci. Of the 12 scorable loci, 11 (7 polymorphic and 4 monomorphic) were used to calculate Nei's *D*. Only polymorphic loci were examined for an additional 18 or 19 colonies from each location to enlarge the sample size (Table 1A). The same polymorphic loci (Table 1B) were used to analyze genetic variation among Friday Harbor populations (Dock, Shady Cove, West Side, Turn Island). The three most informative polymorphic loci in Friday Harbor (*Ada*, *Gpi*, *Pgd*) were used to analyze genetic variation based on algal substrate (Table 1C). Finally, six polymorphic loci and two monomorphic loci were used to analyze the genetic variation among the three morphs of *Membranipora membranacea* (Table 1D). About 10% of the samples on each gel were re-runs, serving as controls to establish allelic identities.

mtDNA

Total DNA was extracted from ethanol-preserved tissue by first letting the ethanol evaporate for several minutes, then grinding the tissue (0.5 to 1 cm^2) mixed with an equal volume of crushed glass in a mortar precooled in liquid nitrogen. The ground tissue/glass mixture was transferred into 700 µl 2× CTAB buffer and incubated with 0.15 µg $µl^{-1}$ Proteinase K for 2 h at 55 °C. DNA was extracted with 650 µl 24:1 chloroform:isoamylalcohol, followed by 650 µl 25:24:1 phenol:chloroform:isoamylalcohol, followed by 650 µl 24:1 chloroform:isoamylalcohol. DNA was precipitated using 1 ml of 100% cold ethanol and 50 µl 3 *M* sodium acetate, and washed twice with 1 ml 80% cold ethanol (Danforth et al. 1996).

A 710-basepair fragment of the mitochondrial cytochrome oxidase 1 (COI) gene was amplified using published PCR primers (Folmer et al. 1994). The primer sequences were LCO1490: 5′-ggt caa caa atc ata aag ata ttg g-3′ and HCO2198: 5′-taa act tca ggg tga cca aaa aat ca-3′. Fifty-microliter reactions were first denatured for 3 min at 96 °C immediately followed by 35 cycles of 1 min at 95 °C, 30 s at 55 °C, and 1.5 min at 72 °C, with additional 5 min of

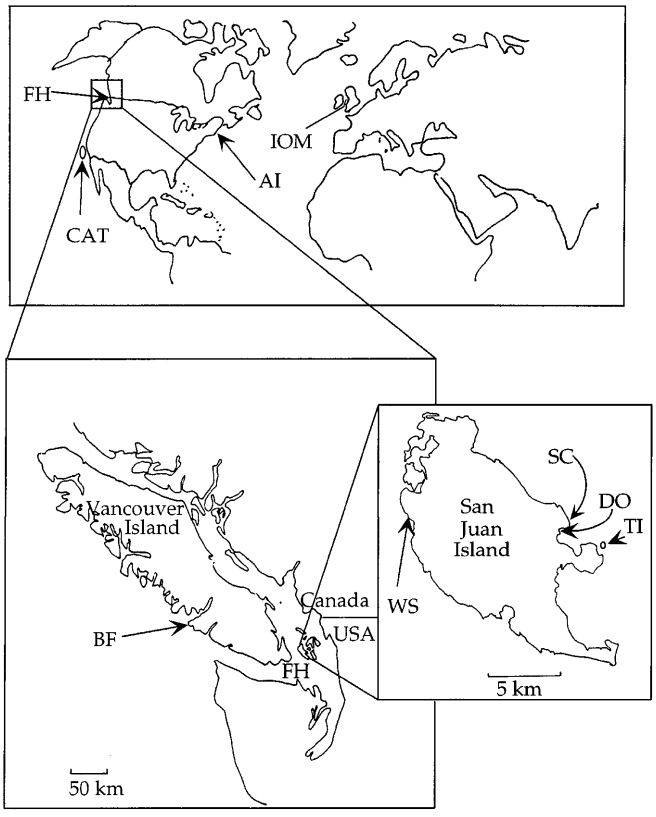

Fig. 2 *Membranipora membranacea*. Locations referred to in Table 1 and in text. Abbreviations are: *FH* Friday Harbor, Washington, which includes *WS* West Side, *DO* Dock, *SC* Shady Cove, *TI* Turn Island; *CAT* Catalina Island, California; *AI* Appledore Island, Maine; *IOM* Isle Of Man, UK; *BF* Bamfield, Vancouver Island, Canada

extension at 72 °C at the end. $MgCl_2$ concentration was 3.0 m*M*. PCR bands were visualized on 1% agarose gels stained with ethidium bromide. At least one negative control without any template DNA was included in each PCR run and these controls never produced bands.

The PCR products were manually sequenced after labeling using a cycle sequencing protocol modified from Stratagene's Cyclist Taq DNA Sequencing Kit. To prepare the templates, the PCR reactions were precipitated with 50 µl fresh 5 *M* ammonium acetate and 1 ml cold 100% isopropanol and resuspended in half the original PCR volume of water. The primer (either one of the end primers or a specifically designed sequencing primer) was labeled with ^{33}P ATP. To label the templates, four separate cycling reactions were set up per template, one for each ddNTP and using ordinary *Taq* polymerase (Gibco). Templates were again denatured at 95 °C for 3 min, then run through 38 cycles of 30 s at 95 °C, 30 s at 55 °C and 45 s at 72 °C followed by a final 5 min of extension at 72 °C. Reaction products were separated on vertical 6% acrylamide sequencing gels. Autoradiographs were produced using Kodak BioMax X-ray film and exposures of 2 to 8 d. Of the 600 bp used for this analysis, the first 350 nt came from the light strand (primers LCO), the remaining 250 nt from the heavy strand (primers HCO).

Analysis

Allozyme data

Genotype frequency data obtained from the gels were analyzed using the computer programs Genes In Populations (designed by B. May and C. C. Krueger, written by W. Eng) and FSTAT (Goudet 1998). Allele frequencies (Table 2), heterozygosity (not reported), a test for Hardy–Weinberg equilibrium, and Nei's standard genetic distance *D* (Table 4) were obtained using Genes In Populations (May and Krueger 1990). The Hardy–Weinberg test determines whether the observed frequency of genotypes fits what one would expect based on allelic frequencies of the sample, and random mating. The program computes a *G*-test statistic that is distributed as a X^2 variable. This program pools rare genotypic classes to reduce the problem of low cell counts. Genes In Populations computes the statistics F_{is} and F_{st} (reviewed by Nei 1987; Hartl and Clark 1989) based on expected (under Hardy–Weinberg equilibrium conditions) and observed average heterozygosities within and between populations. These parameter estimates are not reported here because the program offers no test of significance for F_{is} and F_{st}. Weir and Cockerham's (1984) *f* (unbiased estimator of F_{is}, Table 3) and θ (unbiased estimator of F_{st}, Table 4) were obtained from FSTAT (Version 2.3), which offers permutations to test the significance of *f* and bootstrapping, jackknifing, and permutations to test the significance of θ. Weir and Cockerham's (1984) hierarchial *F*-statistics are based on variance components of gene frequencies (Nei 1987; Hartl and Clark 1989; Weir 1996). F_{is} calculates deviations from Hardy–Weinberg expectations within populations due to non-random mating. F_{is} values can range from −1 to 1, with 1 indicating 100% selfing (heterozygote deficit), 0 indicating random mating, and −1 indicating extreme outcrossing or negative assortative mating (heterozygote excess). F_{st} is a measure of differentiation (inbreeding) due to structure among such populations and may provide an index of average historical gene flow. Assuming Wright's island model of migration, no selection and negligible mutation (Nei 1987), F_{st} is mathematically related to N_em, the number of migrants exchanged per generation between populations, by the equation $F_{st} = 1/(4N_em + 1)$ (Wright 1951, Table 4). Values of F_{st} near zero indicate no sub-structuring and high levels of migration while values near 1 indicate very low levels of migration. Estimates of the parameter may be negative because: (1) The estimate may be below or above the true value; thus a negative θ may occur if the true value is positive but close to zero (Weir 1996). (2) The parameter may be negative which would indicate that there is a negative intra-class correlation, i.e. the alleles are more related between than within populations (Weir 1996). When θ is negative, migration rates calculated from the formula above are negative. In these cases, migration between populations is infinite if the island model is a suitable representation of the populations (J. Goudet personal communication). Confidence intervals for θ were obtained by 1000 bootstrap replicates over loci. Bootstrapping over loci, suggested by Weir and Cockerham (1984), obtains an empirical distribution of the *F*-statistic using many subsamples of the complete data set. Because the confidence intervals are empirical, the presence of linkage disequilibrium between loci does not violate implicit assumptions of the model (Hartl and Clark 1989). Significance of θ was tested by 1000 permutations of alleles among samples as well as permuting (1000) multi-locus genotypes among samples. Genotypes were permuted in cases where F_{is} was not zero, in which case the alleles within an individual are no longer independent (Goudet 1998, manual for version 1.2). A sequential Bonferroni correction was used to reduce the type-1 error rate when pairwise F_{st} were calculated from one data set (Rice 1989, Table 4).

DNA data

DNA data were analyzed using LaserGene (DNA Star, Madison, Wisconsin) and the test version 4.0d64 of PAUP* (written by D. L. Swofford). Sequences were aligned in DNA Star's Megalign using the Clustal option and the default parameters (gap penalty = 10, gap length penalty = 10) and by eye. A neighbor-joining tree using Kimura two-parameter distance was obtained in PAUP* (Fig. 3). A cladogram based on maximum parsimony was also obtained in PAUP*. Two congeneric bryozoans, *Membranipora chesapeakensis* and *M. arborescens*, were sequenced and used as outgroups. Be-

Table 2 *Membranipora membranacea*. Allele frequencies at eight polymorphic loci (*n* number of diploid individuals per sample per locus; *NR* not run; other abbreviations see Table 1)

Locus	(A) Inter- vs intra-ocean				(B) Local populations				(C) Substrate				(D) Morphs		
	IOM	AI	FH	CAT	DO	SC	WS	TI	BFM	BFN	FHN	FHL	IS	CU	CS
Ada															
1	0.019	–	0.867	0.759	0.796	0.804	0.917	0.821	0.840	0.778	0.958	0.810	0.882	0.955	0.625
2	0.442	0.517	0.117	0.204	0.185	0.161	0.056	0.161	0.120	0.130	0.042	0.162	0.088	0.045	0.375
3	0.481	0.483	–	0.019	–	0.018	–	–	–	0.018	–	0.007	0.029	–	–
4	0.038	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	0.019	–	0.017	0.019	0.019	0.018	0.028	0.018	0.040	0.074	–	0.021	–	–	–
(*n*)	26	29	30	27	27	28	18	28	25	27	24	71	17	11	4
Gpi															
1	0.946	0.983	0.019	–	–	0.045	0.068	0.048	0.040	0.025	0.064	0.062	0.083	0.045	–
2	0.054	0.017	0.185	0.071	0.154	0.091	0.136	0.286	0.160	0.275	0.109	0.169	0.167	0.182	0.125
3	–	–	0.444	0.536	0.500	0.432	0.386	0.286	0.440	0.325	0.500	0.315	0.333	0.364	0.250
4	–	–	0.296	0.286	0.269	0.386	0.409	0.333	0.285	0.300	0.283	0.400	0.306	0.409	0.625
5	–	–	0.019	0.036	0.038	0.023	–	0.024	0.040	0.075	0.022	0.039	0.111	–	–
6	–	–	–	0.071	–	–	–	–	–	–	–	–	–	–	–
7	–	–	0.019	–	–	–	–	0.024	–	–	–	–	–	–	–
8	–	–	0.019	–	0.038	0.023	–	–	0.040	–	–	0.015	–	–	–
9	–	–	–	–	–	–	–	–	–	0.022	–	–	–	–	–
(*n*)	28	29	27	14	13	22	22	21	25	20	23	65	18	11	4
Idh									NR	NR	NR	NR			
1	0.184	0.083	1.000	1.000	1.000	1.000	1.000	0.958					1.000	1.000	1.000
2	0.816	0.917	–	–	–	–	–	0.042					–	–	–
(*n*)	19	24	23	23	24	21	21	26					18	11	4
Np									NR	NR	NR	NR			
1	0.019	–	1.000	0.941	1.000	1.000	1.000	1.000					1.000	0.955	0.875
2	0.926	0.948	–	–	–	–	–	–					–	–	0.125
3	0.037	–	–	–	–	–	–	–					–	0.045	–
4	0.019	–	–	0.029	–	–	–	–					–	–	–
5	–	0.052	–	–	–	–	–	–					–	–	–
6	–	–	–	0.029	–	–	–	–					–	–	–
(*n*)	27	29	25	17	16	19	7	23					15	11	4
Pgd															
1	–	–	–	–	–	–	–	–	–	–	–	0.006	–	–	–
2	1.000	0.982	0.538	0.567	0.500	0.563	0.650	0.611	0.540	0.500	0.538	0.590	0.472	0.545	0.500
3	–	0.018	0.442	0.433	0.435	0.417	0.325	0.352	0.420	0.444	0.365	0.366	0.500	0.364	0.250
4	–	–	0.019	–	0.022	0.021	0.025	0.019	0.040	–	0.039	0.019	0.028	0.045	0.250
5	–	–	–	–	0.043	–	–	–	–	0.037	0.039	0.013	–	–	–
6	–	–	–	–	–	–	–	0.019	–	–	–	0.006	–	–	–
7	–	–	–	–	–	–	–	–	–	0.019	0.019	–	–	0.045	–
(*n*)	29	28	26	15	23	24	20	27	25	27	26	78	18	11	4
Pep-la									NR	NR	NR	NR	NR	NR	NR
1	0.948	0.931	0.929	1.000	0.935	0.972	1.000	0.925							
2	0.034	0.069	0.018	–	–	–	–	0.025							
3	0.017	–	0.054	–	0.065	0.028	–	0.050							
(*n*)	29	29	28	27	23	18	27	20							

(contd. overleaf)

Table 2 Continued

Locus	(A) Inter- vs intra-ocean				(B) Local populations				(C) Substrate				(D) Morphs		
	IOM	AI	FH	CAT	DO	SC	WS	TI	BFM	BFN	FHN	FHL	IS	CU	CS
Pp									NR	NR	NR	NR	NR	NR	NR
1	0.940	0.946	0.020	–	–	–	–	0.071							
2	0.060	0.054	–	–	–	–	–	–							
3	–	–	0.980	1.000	1.000	1.000	1.000	0.929							
(*n*)	25	28	25	27	4	8	6	7							
Tpi									NR	NR	NR	NR			
1	0.759	0.948	0.789	0.967	0.850	0.904	0.760	0.917					0.917	1.000	0.750
2	0.019	0.017	0.105	–	0.150	0.096	0.160	0.021					0.083	–	0.125
3	0.222	0.034	0.088	0.017	–	–	0.080	0.021					–	–	–
4	–	–	–	0.017	–	–	–	0.021					–	–	0.125
5	–	–	0.018	–	–	–	–	0.021					–	–	–
(*n*)	27	29	28	30	20	26	25	24					18	11	4

Table 3 *Membranipora membranacea*. Weir and Cockerham's (1984) unbiased estimator of F_{is}, *f*. *P*-value of Hardy–Weinberg test: $*P < 0.05$, $**P < 0.005$. *P f* not >0: probability of F_{is} being not bigger than zero (a very small number means that there is a significant probability that F_{is} is positive) (*N*/*A* monomorphic locus)

Locus	Population			
(A) Inter- vs intra-ocean	FH	CAT	AI	IOM
Pep-la	−0.043	N/A	−0.057	−0.024
Tpi	0.425	−0.009	−0.024	0.127
Ada	0.165	0.337	−0.087	0.277
Pp	−0.000	N/A	−0.038	−0.043
Pgd	−0.254	−0.189	−0.000	N/A
Idh	N/A	N/A	−0.070	0.151
Np	N/A	0.500	−0.037	−0.035
All loci	0.045	0.088	−0.064	0.150
P f not >0	0.314	0.276	0.846	0.030
(B) Local populations	DO	SC	WS	TI
Pep-la	−0.048	−0.000	N/A	−0.036
Tpi	−0.152	0.353	0.603**	−0.034
Ada	−0.212	0.146	−0.041	0.300
Pp	N/A[a]	N/A	N/A	−0.000
Pgd	0.047	0.203	0.281	−0.014
Idh	N/A	N/A	N/A	1.000
Np	N/A	N/A	N/A	N/A
Average	−0.068	0.200	0.356	0.117
P f not >0	0.744	0.048	0.005	0.072
(C) Substrate	FHN	FHL	BFN	BFM
Ada	−0.022	0.031	−0.075	0.015
Pgd	0.147	0.190*	−0.125	0.115
Average	0.126	0.129	−0.105	0.081
P f not >0	0.129	0.048	0.836	0.334
(D) Morphs	IS	CU	CS	
Pgd	0.078	0.084	0.333	
Idh	N/A	N/A	N/A	
Np	N/A	0.000	0.000	
Ada	−0.076	0.000	−0.500	
Tpi	−0.063	N/A	−0.091	
Gpi	−0.011	−0.184	0.200	
Average	0.005	−0.053	0.032	
P f not >0	0.459	0.732	0.455	

cause of the large number of taxa, a heuristic search was performed. The characters were treated as unordered and carried equal weights. Starting trees were obtained by stepwise (random) addition, and five trees were held at each step. The branch-swapping algorithm used was tree–bisection–reconnection (TBR). No topological constraints were enforced. A total of 100 bootstrap replicates were performed to estimate the support for each node of the cladogram (Fig. 3).

Results

Intraspecific genetic structure

Global

The genetic differences between populations of the two oceans were large, while populations within each ocean were less differentiated. This pattern was reflected by allele frequency distributions, θ (F_{st}), and Nei's genetic distances. Table 2A shows the allele distributions for the polymorphic allozyme loci scored. Allele frequencies at the most informative polymorphic loci (*Ada*, *Idh*, *Np*,

Table 4 *Membranipora membranacea*. *Below diagonal*: Weir and Cockerham's (1984) unbiased estimator of F_{st}, θ (calculated from sample), with 99% confidence intervals (from 1000 bootstrap replicates over loci, *in parentheses*) and N_em (average number of successful migrants per generation). *Above diagonal*: Nei's *D* (only in A and D, where monomorphic loci were included in the analysis). N_em was calculated from θ assuming an island model for gene exchange; negative θ interpreted as infinite (*inf*) gene flow. In C the *P*-value of θ was obtained by permuting genotypes among the total; permuting alleles among the total gave similar *P*-values. Locus *Gpi* was excluded in A, B, and C

(A) Inter- vs intra-ocean	FH	CAT	AI	IOM
FH	–	0.008[e]	0.597	0.606
CAT	0.014[a] (−0.017, 0.079)[b] N_em = 17.453[c]	–	0.555	0.567
AI	0.740* (0.369, 0.912) N_em = 0.088	0.760* (0.402, 0.915) N_em = 0.088	–	0.005
IOM	0.691* (0.304, 0.889) N_em = 0.112	0.711* (0.366, 0.884) N_em = 0.102	0.019 (−0.013, 0.086) N_em = 13.111	
Average inter-ocean θ = 0.726, N_em = 0.098, Nei's *D* = 0.581				
(B) Local populations, Friday Harbor	DO	SC	WS	
Shady Cove (SC)	−0.014[a] (−0.016, −0.009)[b] N_em = inf[d]			
West Side (WS)	0.015 (−0.009, 0.052) N_em = 16.637[c]	0.006 (−0.013, 0.024) N_em = 42.958		
Turn Island (TI)	−0.002 (−0.032, 0.021) N_em = inf	−0.010 (−0.020, 0.002) N_em = inf	0.008 (−0.021, 0.049) N_em = 30.260	
Average θ (99% CI) = −0.001(−0.009, 0.015)				
(C) Substrate	FHN	FHL	BFN	BF (combined hosts)
Friday Harbor *L. groenlandica* (FHL)	0.011[a] *P* = 0.157 N_em = 22.477[c]			
Bamfield *N. luetkeana* (BFN)	0.015 *P* = 0.148 N_em = 16.417	−0.001 *P* = 0.386 N_em = inf[d]		
Bamfield *M. integrifolia* (BFM)	−0.004 *P* = 0.433 N_em = inf	−0.009 *P* = 0.764 N_em = inf	−0.013 *P* = 0.794 N_em = inf	
Friday Harbor (combined hosts)				−0.001 *P* = 0.433 N_em = inf
(D) Morphs	IS	CU	CS	
Inducibly spined (IS)	–	0.006[e]	0.036	
Constitutively unspined (CU)	−0.012[a] (−0.017, 0.033)[b] N_em = inf[d]	–	0.036	
Constitutively spined (CS)	0.048 (0.010, 0.185) N_em = 4.976[c]	0.047 (−0.050, 0.320) N_em = 5.083		
Average *D* of morphs = 0.026				

[a] θ calculated from sample
[b] 99% confidence interval from 1000 bootstrap replicates over loci
[c] N_em, the average number of successful migrants per generation
[d] negative θ interpreted as infinite gene flow
[e] Nei's genetic distance *D*
* P < 0.01 Bonferroni corrected

and *Pp*) showed clear inter-ocean differences and within-ocean similarities. Table 3A shows the *f* (F_{is})-values calculated from these allele frequencies. *Gpi* was excluded from Table 3A–C because of scoring problems and was not used to calculate θ (F_{st}) in Table 4A–C nor Nei's *D* in Table 4A. *Gpi* allele frequencies are reported in Table 2 because the information is qualitatively useful by showing a large number of alleles in the North Pacific compared to the North Atlantic (Table 2A). Further, GPI was unambiguously scorable for the morphs and was used in calculations in Tables 3D and 4D. To test the null hypothesis of no population structure (F_{st} not significantly different from zero) pairwise θ were calculated based on about 30 colonies per population and seven polymorphic loci (excluding *Gpi*). The average θ between the oceans was 0.726, between Friday Harbor and Catalina (within the Pacific) θ was 0.014, and between the Isle of Man and Appledore Island (within the North Atlantic) it was 0.019 (Table 4A). All pairwise inter-ocean θs (AI–FH, AI–CAT, IOM–FH, IOM–

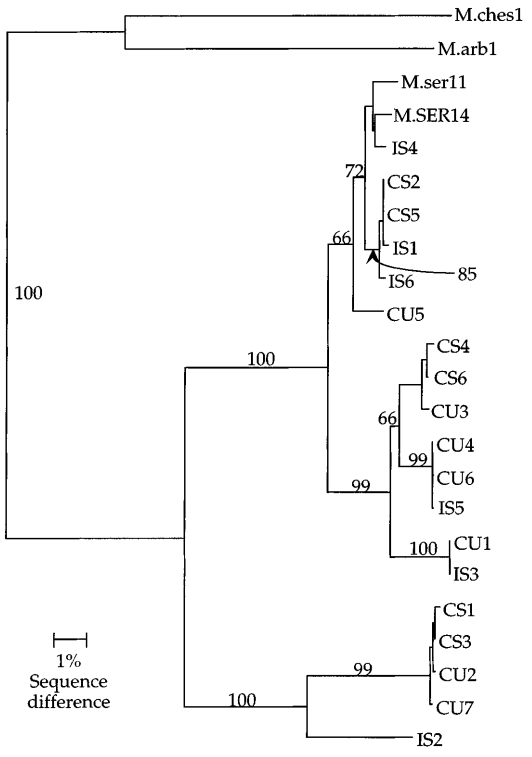

Fig. 3 *Membranipora* spp. Neighbor-joining tree showing phylogenetic relationships among individuals of three morphs of *M. membranacea* (*IS* inducibly spined; *CU* constitutively unspined; *CS* constitutively spined), two members of *M. serrilamella* (M.ser), and the two congeneric outgroups *M. chesapeakensis* (M.ches) and *M. arborescens* (M.arb). The tree was obtained in PAUP* (test version 4.0d64) based on 600 bp of COI sequence and Kimura two-parameter distance. The strict consensus of 50 most parsimonious trees was based on 141 parsimony-informative characters and had a length of 333 steps. The strict consensus tree and the bootstrap tree had a topology identical to the neighbor-joining tree. Bootstrap values are shown above branches

CAT) were significant at $\alpha = 0.01$ (Bonferroni corrected), while both intra-ocean θs (CAT–FH, IOM–AI) were not significant. Similarly, Table 4A shows that, based on seven polymorphic and four monomorphic loci, and ten colonies per population, Nei's *D* is 0.581 between oceans, 0.008 between Friday Harbor and Catalina Island, and 0.005 between Isle of Man and Appledore Island. Average gene flow between the oceans was estimated from θ to be 0.098 migrants per generation, while within the oceans it is 17 migrants between Catalina and Friday Harbor and 13 between the Isle of Man and Appledore Island (Table 4A).

Local populations and substrate

Local populations in Friday Harbor and colonies growing on different hosts belong to one panmictic population. Similarly, colonies on different algal host species belong to the same panmictic population (Table 4C). All θ-values are small and insignificant (Table 4B,C) and gene flow is at least 16 successful migrants per generation among the local populations in Friday Harbor (Table 4B), as well as between Friday Harbor and Bamfield, and among populations on different substrates (Table 4C). This indicates that "host" is not a confounding variable in the analysis of population structure or cannot be detected with this method. Note that calculations for Table 4C use only a subset of the loci used for those for Table 4A and B because, partly due to tissue limitations, only the most polymorphic loci were analyzed to address the question of differentiation based on substrate. In retrospect, it appears that inclusion of *Tpi* and *Pep-la* would have allowed stronger inference in this question. Also, to test for significance of θ, *P*-values from permuting genotypes within the total are given because there were too few loci to generate confidence intervals by bootstrapping over loci. Similar *P*-values were obtained when permuting alleles among the total (Table 4C).

Morphs

Both allozyme and mtDNA data (GenBank Accession Numbers AF147957 to AF147977) showed that the three morphs were not genetically distinguishable from each other. There were no fixed allelic differences at allozymes between the morphs (Table 2D). The θ-values were insignificant and ranged from −0.012 to 0.048 (Table 4D). As expected, genetic distances were also small, ranging from 0.006 to 0.036 (Table 4D; note: different loci from calculations in Table 4A). The 99% confidence interval of θ between IS and CS (Table 4D) indicates (by not including zero) the only departure from this general pattern of insignificance of the genetic differences between the morphs. One needs to consider that these results contain a considerable amount of error due to the small number of loci and small sample sizes especially for CS colonies (Nei 1978; Rosenblatt and Waples 1986). When alleles or genotypes were permuted this θ (IS–CS) was not significantly different from zero. All loci were in Hardy–Weinberg equilibrium.

The neighbor-joining tree (Fig. 3) shows the relationship among the morphs based on 600 bp of the mitochondrial COI gene. Among the ingroup (all sequences from the three morphs and two sequences from *Membranipora serrilamella*), the sequences show Kimura two-parameter distances of 0 to 16.938%. The three major mitochondrial lineages do not correspond to the morphological (spines) characteristics of the colonies analyzed, nor do the morphs cluster together in monophyletic clades as would be expected of well-defined phylogenetic species or even races. The *M. serrilamella* genotypes from Japan cluster well within the ranges of the three morphs of *M. membranacea* from Friday Harbor.

Maximum parsimony analysis yielded 50 most parsimonious trees with a length of 333 steps. These trees were based on 141 parsimony-informative characters. The strict consensus tree was identical to the bootstrap

50% majority-rule tree that had high bootstrap values (Fig. 3) and was identical to the neighbor-joining tree. The trees obtained by maximum parsimony are not shown here because they are identical to the neighbor-joining tree in Fig. 3.

Discussion

Intraspecific genetic structure

The allozyme data from populations of *Membranipora membranacea* from the North Atlantic and the North Pacific Oceans show clear geographic patterns. First, there was strong population genetic differentiation between populations in the Atlantic compared to those in the Pacific. Second, populations within the same ocean (both Atlantic and Pacific), but separated by hundreds to thousands of miles, were only slightly differentiated. Third, populations from the same locale but living on different algal substrates were not genetically distinct.

Structure between oceans

The populations of *Membranipora membranacea* were highly differentiated between oceans, with a Nei's D of 0.581 (but no fixed allelic differences). Strong differentiation was also present at the mtDNA sequence level, where the average divergence between 33 North Pacific and 25 North Atlantic sequences was 13% at the COI gene (Schwaninger 1999 and in preparation). There were no obvious differences in adult morphology between these populations. Similarly, there seem to be no great ecological differences, except perhaps that the kelp beds of the east coast of the United States may be damaged more severely (Lambert et al. 1992) by the epiphytic activity of *M. membranacea* than those elsewhere. Thus, the genetic results of this study raise the question of whether the populations in the Atlantic and the Pacific are allopatric populations of the same species or whether they are sibling species, i.e. true species that are difficult to distinguish morphologically (Mayr and Ashlock 1991; Knowlton 1993). More generally, these results raise the question of why the populations are so differentiated; are there intrinsic or only extrinsic barriers to gene exchange?

Because the species status of the populations in the different oceans is unresolvable by other criteria, it is useful to compare the genetic distance obtained in this study with that of other studies where genetic distances of known species are compared. Two difficulties associated with such a comparison are the problem of associating a certain value of D with reproductive isolation (Avise and Aquadro 1982; Harrison 1991), and the dependence of D on loci chosen and laboratory procedures (Thorpe 1982; Nei 1987). Ayala et al. (1973) found that in the *Drosophila willistoni* group local populations were separated by $D = 0.031$, subspecies by $D = 0.23$, semi-species by $D = 0.226$, sibling species by $D = 0.581$, and non-sibling species by $D = 1.056$. Knowlton (1993) found that many marine sibling species have a Nei's D of 0.5 or greater. Thus, using genetic distance as a proxy for (unknown) reproductive compatibility, it appears that *Membranipora membranacea* populations in the North Atlantic and North Pacific Oceans with a $D = 0.581$ are as differentiated or more differentiated than many pairs of sibling species. The findings presented here contrast with those for sea urchins where mitochondrial DNA revealed little differentiation between Atlantic and Pacific populations (Palumbi and Wilson 1990; Palumbi and Kessing 1992), although these sea urchins have potentially widely dispersing larvae like those of *M. membranacea*. A consideration of how *M. membranacea* and the sea urchins are affected by barriers to gene flow might explain the difference.

The extrinsic barriers to gene flow in *Membranipora membranacea* are obvious: current climatic conditions are not conducive to migration across the Arctic Ocean because it is too cold and algal hosts are lacking. The route across the tropics is also unavailable because of unfavorable currents (Tchernia 1980) and too high temperatures (Yoshioka 1973, 1982a). Genetic distances of the magnitude calculated between North Pacific and North Atlantic populations of *M. membranacea* are thought to take millions of years to develop (Nei 1987). Suitably ancient opportunities for gene flow between the North Atlantic and the North Pacific for temperate marine organisms occurred during the trans-Arctic Interchange about 3.5 to 3.1 million years ago (e.g. Vermeij 1991) or perhaps as early as 7 million years ago (Marincovich and Gladenkov 1999) when the Bering Strait was opened during a warm period. The fossil record indicates that during that time a large number of cold-water marine species dispersed predominantly from the Pacific to the Atlantic (Durham and MacNeil 1967; Vermeij 1991). The Northern Hemisphere glaciations from 3.1 to 2.5 million years ago made the Arctic much less hospitable to migrants, although Pleistocene interglacial periods may have offered windows for dispersal via the trans-Arctic route (Shackleton et al. 1984). The cold-tolerant sea urchins, whose North Atlantic and North Pacific populations are genetically very similar, may have used this passage during Pleistocene interglacials (Palumbi and Kessing 1991), or may have been reintroduced anthropogenically even more recently. As for *M. membranacea*, a Nei's D of 0.581 (or a 13% divergence at the COI gene) suggests that this species did migrate during the trans-Arctic Interchange (either from the Atlantic to the west or from the Pacific to the east, but alternative routes through the Southern Hemisphere are possible) but did not experience gene flow as recent as the Pleistocene interglacial periods. Similar arguments have been made for cod ($D = 0.415$, Grant and Ståhl 1988) and halibut ($D = 0.162$, Grant et al. 1984). Intrinsic barriers to gene flow initiating reproductive isolation and speciation could have developed during this long time of separation, but there is no evidence of such

barriers. Although the present study did not examine intrinsic barriers, laboratory studies of reproductive compatibility using colonies from the Atlantic and Pacific Oceans would constitute a first step. Since colonies are capable of selfing (Temkin 1994) the use of a genetic marker would be required to determine if the offspring resulted from inbreeding or outbreeding.

Structure within oceans

The Pacific populations (Friday Harbor, Catalina) as well as the Atlantic populations (Isle of Man, Appledore Island) are characterized by relatively small F_{st}-values and small genetic distances (Table 4A), indicating that the two samples from their respective oceans represent freely interbreeding populations, or have only recently been separated. Considering that the larval stage of *Membranipora membranacea* is planktonic for about 4 weeks (Yoshioka 1982a), but may last up to 2 months (Reed 1987, causes not detailed) it is not surprising that the two populations from the US west coast are not genetically subdivided: the California current sweeps down that coast from the north (Tchernia 1980) and offers efficient transport for larvae to other populations in the south.

In the Atlantic, the close relationship between populations from England and from the US east coast is more easily explained by recent history, than by current-induced patterns of dispersal. *Membranipora membranacea* is an invader, first observed on the east coast at the Isles of Shoals, off the coast of New Hampshire and southern Maine in 1987, which has since been spreading both northward and southward (Berman et al. 1992). Thus, the close genetic relationship between the two Atlantic populations is consistent with European populations being the source of the invaders. It is not known how the founding individuals came across the Atlantic, but the surface currents across the Atlantic are not conducive to the transport of propagules to the coasts of New Hampshire or southern Maine (Tchernia 1980) where *M. membranacea* was first seen in the eastern USA. A more likely site of first habitation would be near the southern limit of the laminarian kelps, approximately halfway down Long Island, New York if the invasion were natural and not human-mediated. However, the triangular cyphonautes larvae characteristic of the bryozoans *Membranipora* spp., *Electra* spp. and *Alcyonidium* spp. have been observed in ballast water (Carlton 1985). Since these larvae feed on phytoplankton, they might survive a trip across the Atlantic. Thus, human introduction of *M. membranacea* to the US east coast is more likely. It would be interesting to know where the founders came from exactly and whether the east coast was colonized in a single or multiple event(s). One way to address both questions is to analyze more European and US east coast populations, preferably at the DNA level, to infer recent history from the pattern of DNA variation. Such studies are currently in progress (Schwaninger 1999 and in preparation).

Even though the F_{st} is not significant, the populations on either side of the North Atlantic seem to differ genetically. It is possible that the US east coast population experienced a genetic bottleneck when (probably few) founders first established it. One signature of the founder effect is a loss of rare alleles. The present data-set does contain such evidence: the recently established Appledore Island (AI) population lacks several rare alleles present in the population at Isle of Man (IOM) at the loci *Ada*, *Np*, and *Pep-la* (Table 2A). On the other hand, the Appledore Island population is the only population in this study whose F_{is} is slightly negative at all loci analyzed (Table 3A), indicating strong outbreeding. In a population just recently established from a few individuals one would expect strong inbreeding, which would translate into positive F_{is}-values. In *Membranipora membranacea* inbreeding can take the form of mating among close relatives, as well as selfing, since each colony is a single genotype and hermaphroditic, and fertilization occurs internally before the fertilized eggs are released into the water (Ryland and Bishop 1993; Temkin 1994). Thus, the data presented in Table 3 indicate that this species is largely outbreeding. These findings are consistent with those of other authors (Thorpe and Beardmore 1981). It is most likely that the reduction in number of rare alleles in the AI sample is a chance result of small sample size – both in number of individuals and number of loci tested.

Local populations and substrate

As indicated by small and insignificant F_{st}-values, this study shows that there is unrestricted gene flow among local populations at Friday Harbor (Table 4B) as well as between Friday Harbor and Bamfield (Table 4C). Thus, propagules of outer-coast populations (represented by Bamfield) are not restricted from interbreeding with sheltered, inner-coast populations (represented by Friday Harbor). Similarly, there is no barrier to gene flow among colonies on different algal hosts (Table 4C). While this inference is based on only two loci, there is no compelling reason to think that colonies mate assortatively based on algal substrate rather than, for example, based on proximity. Even if there were assortative mating for any reason, the long planktonic larval stage ensures thorough mixing of zygotes before the larvae settle. Thus, unless there is a genetic tendency to settle on the same species of host alga where the parent colony came from, or the host alga acts as a disruptive selective agent on the bryozoans, substrate will not cause population subdivision. The small and insignificant F_{st}-values in Table 4C suggest that these possible mechanisms are not in effect in this system. It is expected that additional loci, had they been analyzed, would concur with this conclusion. Thus, host algae are not a confounding variable in this analysis of population structure in *Membranipora membranacea*.

Morphs

This study clearly shows that the three morphs of *Membranipora membranacea* growing around Friday Harbor, Washington belong to the same species. The neighbor-joining tree (Fig. 3) is especially convincing in that the morphs do not cluster together in monophyletic clades as would be expected of a well-defined phylogenetic species ["…an irreducible (basal) cluster of organisms, diagnosably distinct from other such clusters, and within which there is a parental pattern of ancestry and descent" (Cracraft 1989)]. On the other hand, it is possible that both the allozyme markers and the mitochondrial COI gene are evolving too slowly and that effective population sizes are too large to show genetic separation among the morphs if reproductive isolation has originated very recently. However, COI is sufficiently variable to differentiate populations of *M. membranacea* in different oceans (Schwaninger 1999 and in preparation). Thus one would expect it to separate the morphs if they were different species. I extrapolate these results to the three species described for the west coast of North America and conclude that *M. villosa* is an alternative morphology of the polymorphic species *M. membranacea*. This view is supported by the fact that several researchers have found characteristics of *M. villosa* in a single clonal colony of *M. membranacea* (O'Donoghue 1926; Seed 1976; Yoshioka 1982b) and that the "villosa" morph can be induced on a *M. membranacea* colony by exposure to nudibranchs (Harvell 1984). The fact that the known *M. serrilamella* colonies from Japan fall squarely within the range of mtDNA variation observed for the three morphs of *M. membranacea* (Fig. 3) suggests that *M. serrilamella* is also a synonym of *M. membranacea* (Schwaninger 1999 and in preparation) although clustering due to insufficient time to sort ancestral polymorphisms cannot be excluded. The findings reported in this paper have implications for the concept of morphospecies in cheilostome bryozoans. A morphospecies is a species that is defined exclusively based on its morphology (Mayr and Ashlock 1991), usually skeletal characters in bryozoans, and is the species concept most frequently used for fossil taxa. Cheilostome bryozoans have an excellent fossil record, thus the correspondence of biological species with morphospecies is of great interest to paleontologists. Jackson and Cheetham (1990) tested this correspondence with thorough breeding studies using two to four species each of three distantly related Panamanian genera of bryozoans. Their statistical and genetic (allozyme) analysis showed that in their common garden experiment morphology was sufficiently heritable to reliably assign F_2 colonies to species based on morphology. The present study found no genetic separation between morphs differing in taxonomically important characters (spines) and indicates that Jackson and Cheetham's (1990) conclusions are not applicable to all cheilostome bryozoans. If they were applied to *M. membranacea*, at least two of the morphs would be assigned to different species depending on whether or not they had encountered certain predators. Thus, skeletal morphology alone is not adequate to distinguish highly variable species (Levinton et al. 1991; Lidgard and Buckley 1994). Detailed quantitative genetic analysis indicates that the taxa tested by Jackson and Cheetham do show environmental variation among zooids within colonies, but have highly heritable (additive genetic) variation among colonies in local populations (Cheetham et al. 1993, 1994, 1995). In these cases morphology appears a reliable indicator of species identity.

Discovery of morphologically cryptic species by genetic methods in many phyla also cautions against exclusive use of morphology to describe species. Species in the very common bryozoan genus *Alcyonidium* have a gelatinous skeleton, thus depriving the taxonomist of most characters usually applied in bryozoan taxonomy. Genetic examination of three intertidal (*A. hirsutum*, *A. polyoum, A. mytili*) and one subtidal (*A. gelatinosum*) nominal species revealed that what had been regarded as only four (or even three) species include at least 12 genetically distinct entities. Some of these sympatric species are morphologically indistinguishable but show fixed allelic differences at multiple loci (Thorpe et al. 1978a, b; Thorpe and Ryland 1979). The geographic scale of sampling was quite limited in these studies while the four nominal species were generally thought to occur over thousands of miles or worldwide (Thorpe and Ryland 1979). Thus, one wonders how many cryptic species will be discovered when populations from the whole geographic ranges of these nominal species are examined. The results are bound to dramatically change our thinking about evolution and speciation in these and possibly other widely distributed species. Similarly, one of the most common and important reef-building corals, *Montastraea annularis*, was thought to be broadly distributed with respect to depth and location (Goreau 1959, cited by Knowlton and Jackson 1994). By applying molecular techniques, measurements of aggressive behavior and corralite morphology, *M. annularis* was found to comprise at least three different species which are differentiated with respect to their depth range (Knowlton et al. 1992; Weil and Knowlton 1994). Knowlton (1993 and references therein) and Knowlton and Jackson (1994) review many other examples of marine sibling species and discuss the importance of accurate taxonomy. It is obvious that good taxonomy is essential for studies of the biology, ecology and evolution of present or fossil taxa.

Conclusions

In this study I have used molecular methods to understand genetic structuring of the widely dispersing and morphologically plastic bryozoan *Membranipora membranacea*. These data represent a first step, yet have already given a more complete view of the genetic connectedness of distant allopatric populations and

sympatric morphs than has previously been possible using morphological information. The results of the genetic analysis of *M. membranacea*'s allopatric populations is consistent with the idea that populations within an ocean represent a shared gene pool, while the North Pacific and the North Atlantic harbor highly differentiated populations that may no longer belong to the same species. The genetic distances between Atlantic and Pacific populations indicate that these gene pools have been isolated for millions of years, and suggest that *M. membranacea* participated in the trans-Arctic Interchange, although migration through the Southern Hemisphere cannot be excluded in the absence of Southern Hemisphere populations in this study. In the analysis of sympatric morphs, allozymes and mtDNA data concur that these morphs (CS, CU, IS) are conspecifics, confirming that morphology is not always sufficient to identify a cheilostome bryozoan. Specifically, this congruence of independent molecular data with experimental and field data is powerful evidence that spines are not a reliable taxonomic character in this species and implies that the species *M. villosa* in the eastern Pacific is an induced morph of *M. membranacea*.

Acknowledgements The completion of this project was made possible with the help of many people, and I am grateful to all of them. C. D. Harvell, C. McFadden, S. Eisner, E. Hunter, and J. Winston contributed samples to this study. B. May, M. Peterson, R. Sawyer, S. Bogdanowicz, L. Katz, and B. Danforth helped by teaching molecular techniques; J. Goudet clarified many points about FSTAT. This paper was based on material submitted in partial fulfillment of a Ph.D. at Cornell University, Ithaca, New York. This paper was improved by C. D. Harvell, R. Harrison, and members of their laboratories, especially K. Kim, R. Broughton, S. Stanley, C. Willett, and C. Ross. Financial support was provided by Sigma Xi, The Theodore Roosevelt Memorial Fund of the American Museum of Natural History, The Graduate School, the Andrew W. Mellon Memorial Fund of Cornell University, the Clare Booth Luce Fellowship, and National Science Foundation grants IBN-9408228 (to C. D. Harvell) and dissertation improvement grant DEB-9423868 (to C. D. Harvell and H. R. Schwaninger). The experiments and collections reported in this paper comply with the current laws of the United States where the experiments were conducted and with the laws of the specific countries where samples were collected.

References

Atkins J (1955) The cyphonautes larvae of the Plymouth area and the metamorphosis of *Membranipora membranacea* (L.). J mar biol Ass UK 34: 442–449

Avise JC, Aquadro CF (1982) A comparative summary of genetic distances in vertebrates. Evolutionary Biol 15: 151–185

Ayala FJ, Tracey ML, Hedgecock D, Richmond RC (1973) Genetic differentiation during the speciation process in *Drosophila*. Evolution 28: 576–592

Berman J, Harris L, Lambert W, Buttrick M, Dufresne M (1992) Recent invasions of the Gulf of Maine: three contrasting ecological histories. Conserv Biol 6: 435–441

Carlton JT (1985) Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. Oceanogr mar Biol A Rev 23: 313–371

Cheetham AH, Jackson JBC, Hayeck LC (1993) Quantitative genetics of bryozoan phenotypic evolution. I. Rate tests for random change versus selection in differentiation of living species. Evolution 47: 1526–1538

Cheetham AH, Jackson JBC, Hayeck LC (1994) Quantitative genetics of bryozoan phenotypic evolution. II. Analysis of selection and random change in fossil species using reconstructed genetic parameters. Evolution 48: 360–375

Cheetham AH, Jackson JBC, Hayeck LC (1995) Quantitative genetics of bryozoan phenotypic evolution. III. Phenotypic plasticity and the maintenance of genetic variation. Evolution 49: 290–296

Cracraft J (1989) Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and process of differentiation. In: Otte D, Endler JA (eds) Speciation and its consequences. Sinauer, Sunderland, Massachusetts, pp 28–59

Danforth BN, Neff JL, Baretto-Ko P (1996) Nestmate relatedness in a communal bee, *Perdita texana* (Hymenoptera: Andrenidae), based on DNA fingerprinting. Evolution 50: 276–284

Durham JW, MacNeil FS (1967) Cenozoic migrations of marine invertebrates through the Bering Strait region. In: Hopkins DM (ed) The Bering land bridge. Stanford University Press, Stanford, California, pp 326–349

Folmer O, Black M, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molec mar Biol Biotechnol 3: 294–299

Goreau TF (1959) The ecology of Jamaican coral reefs. I. Species composition and zonation. Ecology 40: 67–90

Goudet J (1998) FSTAT, a program for IBM PC compatibles to calculate Weir and Cockerham's (1984) estimators of *F*-statistics (Version 2.3, in beta testing stage). Available from the author, Institut de Zoologie et d'Ecologie Animale, B'timent de Biologie, Universitè de Lausanne, CH-1015 Dorigny, Switzerland

Grant WS, Ståhl G (1988) Evolution of Atlantic and Pacific cod: loss of genetic variation and gene expression in Pacific cod. Evolution 42: 138–146

Grant WS, Teel DJ, Kobayashi T, Schmitt C (1984) Biochemical population genetics of Pacific halibut (*Hippoglossus stenolepis*) and comparison with Atlantic halibut (*H. hippoglossus*). Can J Fish aquat Sciences 44: 490–498

Harrison RG (1991) Molecular changes at speciation. A Rev Ecol Syst 22: 281–308

Hartl DL, Clark AG (1989) Principles of population genetics, 2nd edn. Sinauer, Sunderland, Massachusetts

Harvell CD (1984) Predator-induced defense in a marine bryozoan. Science 224: 1357–1359

Harvell CD (1986) The ecology and evolution of inducible defenses in a marine bryozoan: cues, costs and consequences. Am Nat 128: 810–823

Harvell CD (1991) Coloniality and inducible polymorphism. Am Nat 138: 1–14

Harvell CD (1998) Genetic variation and polymorphism in the inducible spines of a marine bryozoan. Evolution 52: 80–86

Harvell CD, Padilla DK (1990) Inducible morphology, heterochrony, and size hierarchies in a colonial monoculture. Proc natn Acad Sci USA 87: 508–512

Jackson JBC, Cheetham AH (1990) Evolutionary significance of morphospecies: a test with cheilostome Bryozoa. Science 248: 579–583

Knowlton N (1993) Sibling species in the sea. A Rev Ecol Syst 24: 189–216

Knowlton N, Jackson JBC (1994) New taxonomy and niche partitioning on coral reefs: jack of all trades or master of some? Trends Ecol Evolut 9: 7–9

Knowlton N, Weil E, Weight LA, Guzman HM (1992) Sibling species in *Montastraea annularis*, coral bleaching, and the coral climate record. Science 255: 330–333

Lambert WJ, Levin PS, Berman J (1992) Changes in the structure of a New England (USA) kelp bed: the effects of an introduced species? Mar Ecol Prog Ser 88: 303–307

Levinton J, Jackson JBC, Cheetham AH (1991) Bryozoan morphological and genetic correspondence: what does it prove? Science 251: 318–319
Lidgard S, Buckley GA (1994) Toward a morphological species concept in cheilostomates: phenotypic variation in *Adeonellopsis yarraenisis* (Waters). In: Hayward PJ, Ryland JS, Taylor PD (eds) Biology and paleobiology of bryozoans. Olsen and Olsen, Fredensborg, pp 101–105
Marincovich L Jr, Gladenkov AY (1999) Evidence for an early opening of the Bering Strait. Nature 397: 149–151
May B (1992) Starch gel electrophoresis of allozymes. In: Hoelzel AR (ed) Molecular genetic analysis of populations: a practical approach. Oxford University Press, New York, pp 1–27 and 271–280
May B, Krueger CC (1990) Use of allozyme data for population analysis. In: Whitmore DH (ed) Electrophoretic and isoelectric focusing techniques in fisheries management. CRC Press, Boca Raton, Florida, pp 157–171
Mayr E, Ashlock PD (1991) Principles of systematic zoology, 2nd edn. McGraw-Hill, New York
Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590
Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York
O'Donoghue CH (1926) Observations on the early development of *Membranipora villosa*. Contrib Can Biol Fish, NS 3: 249–263
Osburn RC (1950) Bryozoa of the Pacific coast of America. Part 1, Cheilostomata-Anasca. Allan Hancock Foundation Publication 14, University of Southern California Press, Los Angeles
Palumbi SR, Kessing BD (1991) Population biology of the trans-Arctic exchange: mitochondrial DNA sequence similarity between Pacific and Atlantic sea urchins. Evolution 45: 1790–1805
Palumbi SR, Wilson AC (1990) Mitochondrial DNA diversity in sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. Evolution 44: 403–415
Pinter P (1969) Bryozoan-algal associations in southern California waters. Bull south Calif Acad Sci 68: 199–218
Reed CG (1987) Phylum Bryozoa. In: Strathman MF (ed) Reproduction and development of marine invertebrates of the northern Pacific coast. University of Washington Press, Seattle, pp 497–510
Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223–225
Robertson A (1908) The encrusting cheilostomatous Bryozoa of the west coast of North America. Univ Calif Publs Zool 4: 253–344
Rosenblatt RH, Waples RS (1986) A genetic comparison of allopatric populations of shore fish species from the eastern and central Pacific Ocean: dispersal or vicariance? Copeia 1986: 275–284
Ryland JS, Bishop JDD (1993) Internal fertilization in hermaphroditic colonial invertebrates. Oceanogr mar Biol A Rev 31: 445–477
Ryland JS, Hayward PJ (1977) British anascan bryozoans. The Linnean Society, London and Academic Press, New York
Schwaninger HR (1999) Evolutionary genetics and biogeography of the marine bryozoan *Membranipora membranacea* (Cheilostomata): implications for population history, dispersal routes, and taxonomy. Dissertation, Cornell University, Ithaca, New York
Shackleton NJ, Backman J, Zimmerman H, Kent DV, Hall MA, Roberts DG, Schnitker D, Baldauf JG, Desprairies A, Homrighausen R, Huddleston P, Keene JB, Kaltenback AH, Krumsiek KAO, Morton AC, Murray JW, Westberg-Smith J (1984) Oxygen isotope calibration of the onset of ice-rafting and the history of glaciation in the North Atlantic region. Nature 307: 620–623
Seed R (1976) Observations on the ecology of *Membranipora* (Bryozoa) and a major predator *Doridella steinbergae* (Nudibranchiata) along the fronds of *Laminaria saccharina* at Friday Harbor, Washington. J exp mar Biol Ecol 24: 1–17
Tchernia P (1980) Descriptive regional oceanography. Pergamon Press, New York
Temkin MH (1994) Gamete spawning and fertilization in the gymnolaemate bryozoan *Membranipora membranacea*. Biol Bull mar biol Lab, Woods Hole 187: 143–155
Thorpe JP (1982) The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. A Rev Ecol Syst 13: 139–168
Thorpe JP, Beardmore JA (1981) Genetic variation in natural populations of marine bryozoans. In: Larwood GP, Nielsen C (eds) Recent and fossil Bryozoa: proceedings of the 5th conference of the International Bryozoology Association. Olsen and Olsen, Fredensborg, pp 263–272
Thorpe JP, Beardmore JA, Ryland JS (1978a) Genetic evidence for cryptic speciation in the marine bryozoan *Alcyonidium gelatinosum*. Mar Biol 49: 27–32
Thorpe JP, Ryland JS (1979) Cryptic speciation detected by biochemical genetics in three ecologically important intertidal bryozoans. Estuar cstl mar Sci 8: 395–398
Thorpe JP, Ryland JS, Beardmore JA (1978b) Genetic variation and biochemical systematics in the marine bryozoan *Alcyonidium mytili*. Mar Biol 49: 343–350
Vermeij GJ (1991) Anatomy of an invasion: the trans-Arctic Interchange. Paleobiology 17: 281–307
Weil E, Knowlton N (1994) A multi-character analysis of the Caribbean coral *Montastraea annularis* (Ellis and Solander, 1786) and its two sibling species, *M. faveolata* (Ellis and Solander, 1786) and *M. franksi* (Egregory, 1895). Bull mar Sci 55: 151–175
Weir BS (1996) Genetic data analysis. II. Sinauer Associates, Sunderland, Massachusetts
Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358–1370
Wright S (1951) The genetical structure of populations. Ann Eugen 15: 323–354
Yoshioka PM (1973) The population dynamics and ecology of the encrusting ectoproct *Membranipora membranacea*. Dissertation, University of California, San Diego, California
Yoshioka PM (1982a) Role of planktonic and benthic factors in the population dynamics of the bryozoan *Membranipora membranacea*. Ecology 63: 457–468
Yoshioka PM (1982b) Predator-induced polymorphism in the bryozoan *Membranipora membranacea* (L). J exp mar Biol Ecol 61: 233–242