Male and Female Mitochondrial DNA Lineages in the Blue Mussel (*Mytilus edulis*) Species Group

Donald T. Stewart,* Carlos Saavedra,† Rebecca R. Stanwood,* Amy O. Ball,* and Eleftherios Zouros*‡

*Department of Biology, Dalhousie University; †Departamento de Biologia Fundamental, Universidade de Santiago de Compostela; and ‡Department of Biology, University of Crete and Institute of Marine Biology of Crete, Greece

In blue mussels of the *Mytilus edulis* species complex, mitochondrial DNA (mtDNA) inheritance is coupled with gender. Females receive their mother's mtDNA and pass it on to both their daughters and sons. In addition, males receive mtDNA from their father and transmit this male mtDNA to their sons. If this pattern of "doubly uniparental inheritance" is older than the *M. edulis* species complex, then all members of this group must have two distinct mtDNA lineages: a maternal lineage that is transmitted through females and a paternal lineage that is transmitted through males. To test this hypothesis, we scored mtDNA variation in two taxa in this complex, *M. edulis* and *M. trossulus,* by means of restriction fragment profiles of whole-mtDNA genomes and DNA sequence of a region of the *cytochrome c oxidase subunit* III gene (*CO*III). The various mitotypes present in these mussels were classified as "male" or "female" based on their gender association and as belonging to *M. edulis* or *M. trossulus* based on species-specific allozymes. Both maximum parsimony and neighbor-joining phylogenies based on the *CO*III sequences grouped female and male mtDNAs into two distinct lineages irrespective of specific origin in accordance with the hypothesis that the origin of these lineages predates the divergence of *M. edulis* and *M. trossulus.*

Introduction

In animals, mitochondrial DNA (mtDNA) inheritance is predominantly maternal (Hayashi et al. 1978). However, repeated backcrossing of hybrids to the paternal line combined with sensitive PCR assays succeeded in the detection of low levels of paternal mtDNA contribution in *Drosophila* (Kondo et al. 1990) and mice (Gyllensten et al. 1991). Evidence for paternal transmission has also been obtained from observations of heteroplasmy for divergent mtDNA molecules in *Drosophila* (Satta et al. 1988) and fish (Magoulas and Zouros 1993). These observations suggest that incidental transmission (i.e., leakage) of paternal mtDNA in animals may account for about 10^{-4} to 10^{-3} of an individual's mtDNA (Kondo et al. 1990; Gyllensten et al. 1991). Biparental mtDNA inheritance in the mussel genus *Mytilus* differs from the "leaky" paternal inheritance described for other animals in a number of respects. For example, Fisher and Skibinski (1990) first discovered that specimens of *Mytilus edulis* and *M. galloprovincialis* from Great Britain had a high incidence of heteroplasmy. They also noted that male mussels were more likely to

be heteroplasmic than were females, that certain mitotypes occurred only in males, and that the sequence divergence between mitotypes in heteroplasmic individuals (estimated from restriction profiles) was on the order of 10%–20%. Hoeh et al. (1991) reported similar phenomena in a global survey that included *M. edulis, M. trossulus,* and *M. galloprovincialis* and suggested that interspecific hybridization coupled with biparental inheritance could explain both the high degree of sequence divergence between heteroplasmic mitotypes and the high incidence of heteroplasmy.

Subsequent work by Zouros et al. (1992) showed that biparental inheritance of mtDNA was very common in interspecific and intraspecific pair matings of *M. edulis* and *M. trossulus.* This finding confirmed the suggestion by Hoeh et al. (1991) that heteroplasmy in *Mytilus* is due to biparental inheritance but dispelled the suggestion that in this species group biparental inheritance is an anomaly associated with hybridization. In a second study of these crosses, Zouros et al. (1994a, 1994b) jointly examined gender and mtDNA inheritance and showed that biparental inheritance was completely absent in female progeny of *M. edulis* × *M. edulis* crosses but always present in male progeny. Specifically, female progeny were always homoplasmic for their mother's mtDNA. Male progeny were heteroplasmic for their mother's mtDNA and one of their father's two mtDNA types. This type of mtDNA inheritance was independently dis-

covered by Skibinski et al. (1994a, 1994b), who observed that sperm from heteroplasmic males contain only the mtDNA that the male must have received from his father. This novel pattern of mtDNA transmission has been dubbed "doubly uniparental inheritance" by Zouros et al. (1994a, 1994b).

Doubly uniparental inheritance implies the existence of two mtDNA lineages, the female lineage that is transmitted through the female parent to both sons and daughters and the male lineage that is transmitted through the father to sons only. At present, the occurrence of doubly uniparental inheritance has been firmly established in two taxa of the blue mussel species complex, *M. edulis* and *M. trossulus*, but there is evidence that it is also present in freshwater mussels (Liu et al., in press; and unpublished data). Given the variety of mitotypes present in these two species (Zouros et al. 1992) we wanted to address in this study the following questions: Do the mtDNA polymorphisms within each of the two species, *M. edulis* and *M. trossulus*, sort phylogenetically into two groups consistent with their gender association? If so, do the gender-associated clades of the two species cluster together? If gender-associated mitotypes from the two species turned out to be phylogenetically closer to each other than to their conspecific mitotypes of the opposite gender, this would mean that doubly uniparental inheritance predates the separation of these taxa.

Material and Methods
Specimen Collection, Species Identification, and mtDNA RFLP Characterization

Mussels were collected on April 29 and June 11, 1993, from Lunenburg Bay, Nova Scotia, Canada, where both *Mytilus edulis* and *M. trossulus* are known to occur. The animals were transported alive to the laboratory, where they were sexed by microscopic examination of the gonad. Gonad tissue (which is histologically fused to mantle tissue) was excised for mtDNA analysis and digestive tissue was removed for allozymic analysis. Tissue samples were stored at −80°C until processed.

The taxonomic status of *M. edulis*, *M. trossulus*, and *M. galloprovincialis* is a matter of debate (Gosling 1992a, 1992b) but is not critical to this particular study. Following conventional practice we will refer to each of these taxa as "species" (see McDonald et al. 1991) but refer to them collectively as the *M. edulis* species complex. Assignment to one or the other species was based on the presence of diagnostic alleles at two enzyme loci: mannose phosphate isomerase (*Mpi;* EC 5.3.1.8) and esterase-D (*Est-D;* EC 3.1.1.1). Alleles 90 and 100 at *Mpi* and alleles 100 and 110 at *Est-D* were used as diagnostic alleles for *M. edulis*. Alleles 94 and 104 at *Mpi* and alleles 90 and 95 at *Est-D* were diagnostic for *M.*

trossulus. A review of the use of diagnostic alleles in the classification of these taxa is provided in Gosling (1992b) and in McDonald et al. (1991)

Mitotypes were characterized by their restriction fragment profiles for *Eco*R I and, in the case of size variant individuals, for *Hind* III. For this assay, 2 μg of total DNA was used for digestions. Cut DNA was separated on 0.8% or 1.0% agarose gels and transferred onto nylon membranes. MtDNA fragments were detected by hybridization with a probe consisting of a whole-mtDNA genome of *M. edulis* and a partial-mtDNA genome of *M. trossulus*. The probe was labeled and detected with the digoxigenin-dUTP chemiluminescent assay system (Boehringer-Mannheim).

Amplification and Sequencing Protocols

Phylogenetic relationships among the most commonly occurring mitotypes in the two species were based on the nucleotide sequence from a portion of the *cytochrome c oxidase subunit* III (*CO*III) gene. In total, seven female types and five male types (derived from seven female and five male individuals, respectively) were sequenced. Total DNA from female tissues was used directly as a source for PCR amplification (see Zouros et al. 1994b for the amplification protocol) and for sequencing using the following primer pair: forward primer FOR2 5′-GTAACTCAAGCCCATAAGAG-3′ and reverse primer REV1 5′-ATGCTCTTCTTGAATATA-AGCGTACC-3′ (which correspond to nucleotide positions 865–884 and 1326–1301, respectively, of segment 5 of the *M. edulis* FB mitotype; Hoffmann et al. 1992). The amplified product was visualized and excised from a 1% regular agarose gel and cleaned with glass beads. We sequenced 7 μl of the eluted product in both directions using a cycle sequencing protocol (New England Biolabs).

DNA from male tissues could not be directly amplified and sequenced because of the presence of two mtDNA types, one maternally and one paternally derived. To overcome this difficulty we adopted a nested PCR method to sequence the male types. An 860-bp fragment of the *CO*III gene was first amplified from total DNA extracted from male mussels using the following pair of primers: forward primer FOR1 5′-TATGTAC-CAGGTCCAAGTCCGTG-3′ (corresponding to segment 5 positions 460–482; Hoffmann et al. 1992) and the reverse primer, REV1, listed above. This fragment was subsequently digested with an enzyme that did not cut the female mtDNA type but did cut the male type into two fragments of unequal size. This enzyme was *Mbo* I if the source DNA was extracted from an *M. edulis* male and *Ssp* I if extracted from an *M. trossulus* male (see below). The fragments were separated on 1.5% low melting agarose gels and a small plug was taken

from the band corresponding to the largest male fragment. The plug was dissolved in 500 ml of ddH2O at 65°C for 5 min and was used as a source for the amplification of the same fragment amplified directly from female tissues. The primers used for this second amplification and subsequent sequencing of the male types were as follows: forward primer FOR3 5′-CA-AGCCCA(T/C)AAAAGAAT-3′ (corresponding to positions 871–887 of segment 5; Hoffmann et al. 1992) and the reverse primer, REV1.

Digestion of the PCR product from the first amplification from male DNA required prior knowledge of the sequence of the 860-bp fragment from the most common female and male mitotypes in each species. As defined by their *Eco*R I restriction profiles, FB and M, and N and S are the most common female and male types in *M. edulis* and *M. trossulus,* respectively (Fisher and Skibinski 1990; Zouros et al. 1992). The sequences of FB and N were obtained directly by amplification from total DNA extracted from females homoplasmic for these *Eco*R I patterns. To obtain sequences for M and S, we spawned several *M. edulis* and *M. trossulus* males and scored their mtDNA *Eco*R I types from somatic tissues. Sperm from males with the M and S types were used for PCR amplification. Because sperm contains only the male mtDNA type (Skibinski et al. 1994*a*), the problem of multiple PCR products was avoided. By comparing FB and M sequences the restriction enzyme *Mbo* I was chosen for preferential digestion of the male mtDNA type in the mixed product from PCR amplification from *M. edulis* males. Similarly, the enzyme *Ssp* I was used for *M. trossulus* males.

Detection of Female mtDNA in Apparently Homoplasmic Males

The RFLP characterization assay described above is not very sensitive in detecting minority mitotypes in heteroplasmic individuals. This is a common occurrence when the tissue source is male gonad, which is dominated by the paternally derived mtDNA type. To examine the possibility that males whose RFLP profile revealed only the presence of a male type were in fact heteroplasmic for a female type, we designed a PCR assay that made use of a primer (REV2 5′-CACATACACTAAGCAC-CACAATG-3′) that had a greater sequence similarity with the common female types (FB and N) than with the common male types (M and S). Combined with primer FOR2, this primer produced a 350-bp fragment whose presence among the amplification products from male tissue DNA can be detected after restriction with either *Hinf* I (if the source DNA was from a male *M. edulis*) or *Eco*R I (if the source DNA was from a male *M. trossulus*). These enzymes cut the PCR product amplified from these respective female mtDNAs but do not cut the PCR product from the respective male mtDNAs.

Phylogenetic Analysis

Sequences were aligned using the program Clustal (version V). Unrooted and rooted trees were constructed by two methods: neighbor joining (Saitou and Nei 1987) and maximum parsimony. The neighbor-joining trees were generated from a matrix of genetic distances (based on Kimura's two-parameter estimate of nucleotide substitutions per site, K) using the MEGA analysis package (Kumar et al. 1993). Maximum parsimony was implemented using PAUP 3.1.1 (Swofford 1991). Two versions of maximum parsimony were performed. In the first, all transformations at all positions were equally weighted. In the second, we compensated for the greater probability of homoplasy at first- and third-codon positions (Moritz et al. 1987) by giving first, second, and third positions weights of 4, 5, and 1, respectively.

To root the *Mytilus* sequences, an outgroup was needed for which it could be assumed that the phenomenon of doubly uniparental inheritance does not occur. For this we chose two published mollusc sequences: the marine clam *Lasaea australis* (Bivalvia; Ó Foighil and Smith 1995) and the black chiton *Katharina tunicata* (Polyplacophora; Boore and Brown 1994). The fossil record indicates that the *Mytilus* and *Lasaea* lineages (of the bivalve subclasses Pteriomorpha and Heterodonta, respectively) have been separated for approximately 500 million yr (Soot-Ryen 1969). This long period of separation coupled with the lack of evidence for heteroplasmy in *Lasaea* (Ó Foighil, personal communication) makes it unlikely that the exceptional mode of mtDNA transmission observed in *Mytilus* is shared by *Lasaea.* Chitons are thought to be even more basal on the molluscan phylogenetic tree.

Results
Definition and Detection of Male and Female Mitotypes

The pattern of doubly uniparental inheritance of mtDNA in mussels implies that males are heteroplasmic for a female and a male mtDNA type and that females are homoplasmic for a female type. In population surveys, male types can be distinguished from female types by the fact that they do not occur (or occur very rarely) in female individuals. However, detecting male and female types may not be easy for a number of reasons. First, the gender-associated mitotypes may not be sufficiently divergent to be distinguished in a heteroplasmic individual by a simple RFLP assay. Second, different tissues from the same individual may contain very different amounts of the two mitotypes, so that detection

of heteroplasmy may depend critically on the tissue used. This latter point is demonstrated in figure 1. Consistent with the observation of Skibinski et al. (1994a), the spawned sperm samples we examined contained only the male mtDNA type. The two somatic tissues were quite different in this respect. Adductor muscle always contained both types, but mantle contained either the female type only or both types. Our blotting-hybridization assay has a sensitivity of about 10^{-3} (i.e., a minority molecule will be detected if it occurs in a 1:1,000 ratio; Zouros et al. 1992). It is also possible that some mantle preparations inadvertently contained a small amount of sperm (gonad and mantle are histologically fused in *Mytilus*), which could explain the presence of male mtDNA type is some preparations from mantle tissues. Thus, the results of figure 1 establish a quantitative (but not necessarily a qualitative) difference between tissues and confirm the suspicion of Skibinski et al. (1994a) that different tissues from the same animal may contain different proportions of male and female mtDNA types. It also suggests that, in the absence of gametes, a comparative examination of mtDNA profiles from different tissues may be necessary to establish the presence of doubly uniparental inheritance.

Mitotype Diversity in a Natural Population

The large number of mitotypes in the *Mytilus* species complex requires a system of nomenclature that will recognize their sex and taxon affiliation. We intro-

duce here a system that consists of the letter F or M, for female or male type, two letters denoting the taxon in which the type occurs exclusively or predominantly, and a number according to the order of description. Previously described types FB, M, N, and S (Fisher and Skibinski 1990; Zouros et al. 1992) are renamed F-ed1, M-ed1, F-tr1, and M-tr1. These are the most common female and male types in *Mytilus edulis* and *M. trossulus,* respectively.

Table 1 lists the *Eco*R I mitotype profiles observed among 150 individuals that were classified as pure *M. edulis* or pure *M. trossulus.* The first observation is that individuals assigned to one or the other species on the basis of allozymes have different mitotypes. This allows for the classification of mitotypes into *M. edulis* or *M. trossulus* types. A second observation is that females were homoplasmic and males were heteroplasmic, as expected from doubly uniparental inheritance. However, three *M. edulis* males out of 31 and 26 *M. trossulus* males out of 42 produced a homoplasmic RFLP pattern for a male mtDNA type. Also, one *M. edulis* female produced a heteroplasmic RFLP profile. Because the DNA used in the RFLP assays was extracted from gonad tissue, it was possible that in many males the amount of female mtDNA type in the DNA preparation was too low to be detected by this assay. The PCR assay that was specifically designed to detect female mtDNA in such preparations revealed the presence of female mtDNA in all three apparently homoplasmic male *M. edulis* and in
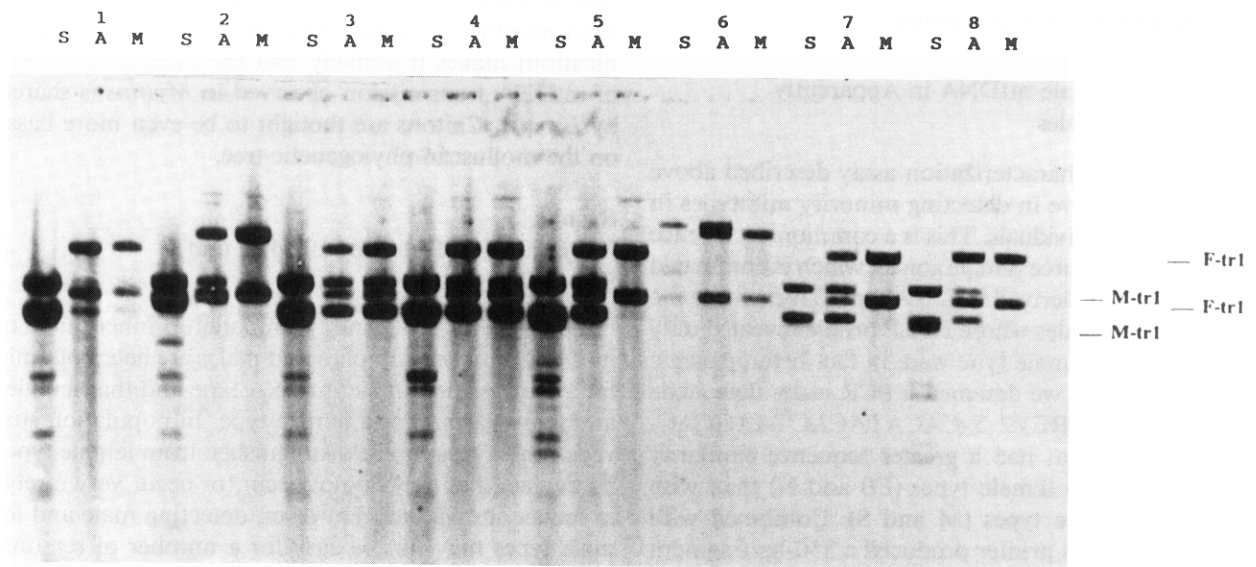

FIG. 1.—Tissue specificity of male and female mitotypes in eight *Mytilus trossulus* males. Individuals 1, 3, 4, 5, 7, and 8 were heteroplasmic for F-tr1 and M-tr1 (the most common *M. trossulus* mitotypes). The bands corresponding to these mitotypes are illustrated in the right margin. Within an individual, mtDNA profiles differed among tissues. Specifically, sperm (S) was always homoplasmic for the male mitotype. Adductor muscle (A) was always heteroplasmic for the male and female types. In contrast, mantle tissue (M) appeared either as homoplasmic for the female type or contained both types in varying proportions.

Table 1
Distribution of mtDNA Types by Sex in *Mytilus edulis* and *Mytilus trossulus* from Lunenburg Bay, Nova Scotia

MITOTYPE	MYTILUS EDULIS		MYTILUS TROSSULUS	
	Male	Female	Male	Female
F-ed1	...	45	...	...
F-ed2	...	1	...	...
F-tr1	...	...	...	17
F-tr2	...	...	...	2
F-tr3[a]	...	...	...	9
F-tr4[a]	...	...	...	2
M-ed1/F-ed1	14	...	...	...
M-ed2/F-ed1	3	...	...	...
M-ed4/F-ed1	2	...	...	...
M-ed5/F-ed1	1	...	...	...
M-ed6/F-ed1	8	1	...	...
M-ed1/F[b]	1	...	...	...
M-ed2/F[b]	1	...	...	...
M-ed3/F[b]	1	...	...	...
M-tr1/F-tr1	...	...	11	...
M-tr2/F-tr1	...	...	2	...
M-tr3/F-tr1	...	...	3	...
M-tr1/F[b]	...	...	12	...
M-tr2/F[b]	...	...	3	...
M-tr3/F[b]	...	...	1	...
M-tr4[a,c]	...	...	10	...

NOTE.—Mitotype designation is based on probing *Eco*R I digests of total DNA with *Mytilus* mtDNA (see text). Heteroplasmic animals are shown by listing the two mitotypes separated by a slash.

[a] Length variable mitotypes.

[b] These animals were provisionally scored as homoplasmic for a male mtDNA type on the basis of their RFLP profile. Presence of female mtDNA was revealed by a preferential PCR assay (see text for details).

[c] M-tr4 was not sequenced and therefore the presence or absence of a female type in these males could not be confirmed using the preferential PCR assay.

16 apparently homoplasmic male *M. trossulus*. Since these female molecules could not be visualized after hybridization of Southern transfers of the whole molecule, their *Eco*R I mitotype could not be determined and were, consequently, designated simply as F (table 1). The 10 *M. trossulus* males whose homoplasmic or heteroplasmic state was not resolved all had the M-tr4 *Eco*R I restriction profile. In this profile the larger *Eco*R I band varies in size, apparently as a result of a repeated sequence whose copy number varies among molecules (Zouros et al. 1992). Because the nucleotide sequence of the *CO*III fragment we used for the preferential PCR assay remains unknown for the M-tr4 profile, this assay could not be applied to these males.

A previous study (Zouros et al. 1994*b*) reported exceptions to the rule of doubly uniparental mtDNA in mussels. These were all found in hybrid crosses between *M. edulis* and *M. trossulus*. In one such cross, males were homoplasmic for the mother's mtDNA and in an-

other cross one female progeny had both the mother's and the father's male mtDNA. Such exceptions are, therefore, expected among animals from a natural population that have a hybrid allozyme genotype, but they may also be found among descendants of hybrids that have acquired an allozymically pure species genotype. The heteroplasmic female and, perhaps, several of the homoplasmic males of type M-tr4 may represent such cases. The exceptions to doubly uniparental inheritance are a matter of special importance that will be addressed by examining the mitotype profiles of animals that were classified as hybrids on the basis of allozymes (unpublished data).

Phylogenetic Relationships of Mussel Mitotypes

We obtained 321 bp of *CO*III sequences from 12 individuals chosen because they had different *Eco*R I mitotypes (App. A). Two *M. edulis* females (F-ed1 and F-ed1′) had the same restriction fragment pattern and were sequenced to gauge the level of divergence among molecules of the same profile compared to molecules of different profiles. Similarly, two *M. trossulus* females (F-tr3 and F-tr3′) with the same *Eco*R I profile were sequenced. In Appendix A, 261 bp of the homologous *CO*III sequence from *Lasaea* (Ó Foighil and Smith 1995) and *Katharina* (Boore and Brown 1994) is also presented. Because the *CO*III gene of *Mytilus* appears to be longer than the *CO*III gene in all other animals examined to date (Hoffmann et al. 1992) including *Lasaea* (Ó Foighil and Smith 1995) and *Katharina* (Boore and Brown 1994), only 261 bp of the *Lasaea* and *Katharina* sequences could be aligned against the 321 bp of *Mytilus* sequence. The full 321 bp of *Mytilus* sequence was used in both the unrooted and rooted analyses.

Unrooted parsimony analysis of 104 informative sites for the *Mytilus* sequences resulted in 12 equally parsimonious trees for both the unweighted and weighted analyses. The strict consensus of these trees is presented in figure 2. The consensus tree and 99% of 1,000 bootstrapped trees indicated a primary division of these sequences into male and female types. Within each of the gender-associated clades, the sequences formed monophyletic groups according to their species' affiliation. Bootstrapping is a conservative measure for assessing confidence in phylogenies (Hillis and Bull 1993); therefore, these values provide strong support for the groupings. In addition, the unrooted neighbor-joining tree (not shown) generated from the matrix of genetic distances (table 2) produced the same gender-associated and species-affiliated clades as the unrooted maximum parsimony tree presented in figure 2.

The rooted neighbor-joining analysis also supported the gender-associated clades as it rooted the *Mytilus* sequences along a branch separating the male and female
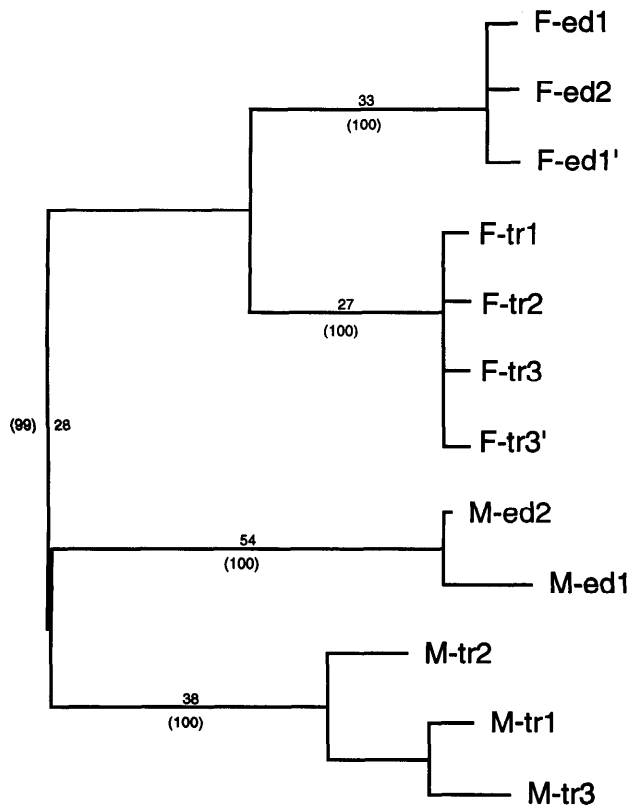
FIG. 2.—Unrooted weighted parsimony network of male and female *M. edulis* and *M. trossulus* mitotypes based on *cytochrome c oxidase subunit* III (App. A). Tree presented is a strict concensus of 12 equally parsimonious trees. Numbers indicate branch lengths or (in brackets) the percent frequency of occurrence of the given branch in 1,000 bootstrap replicates.

sequences (fig. 3). Rooted parsimony analysis produced, however, different results depending on whether weighting was or was not used. The weighted parsimony tree was identical in its main characteristics to the unrooted trees and to the neighbor-joining tree (fig. 4A). The unweighted parsimony tree grouped all male sequences together and recognized species affiliation as a secondary division within the male lineage in agreement with the other trees (fig. 4B). Female sequences were also grouped according to species affiliation, but the *M. trossulus* female lineage appeared as a sister group to the male lineage rather than as a sister group to the female *M. edulis* lineage. This discrepancy is most likely an artifact of the long evolutionary distance between *Mytilus* and each of the two outgroups. The time of divergence of *Mytilus* from either *Lasaea* or *Katharina* is long enough for substitutions at third positions to reach saturation so that homoplasies could distort true phylogenetic relationships between the compared sequences.

Discussion

Mitochondrial DNA has been considered an excellent molecule for phylogenetic reconstruction in animals because of its maternal inheritance and lack of recombination (Avise 1994). For the purposes of phylogenetic reconstruction, it is generally assumed that mtDNA polymorphisms are neutral (Moritz et al. 1987). However, recent reports of selective sweeps (Ballard and Kreitman 1994; Nachman et al. 1994; Rand et al. 1994) suggest that the assumption of strictly neutral behavior may not apply to mtDNA variation. In addition, in contrast to the norm in animals, male inheritance is commonplace in *Mytilus* (Skibinski et al. 1994a; Zouros et al. 1994a, 1994b) and occurs alongside maternal inheritance. The transmission of mtDNA from fathers to sons and from mothers to daughters implies that there are distinct female and male lineages. As Hurst and Hoekstra (1994) suggested, distinct female and male lineages may be a defense against selfish cytoplasmic elements as long as the lineages remain intact and the mitotypes do not recombine.

Our analysis of 12 *Mytilus* sequences, five of which were drawn from *M. edulis* and seven from *M. trossulus*, provides firm support for the existence of two gender-associated mtDNA lineages in these mussels (figs. 2, 3, and 4A). Furthermore, our results suggest that the origin of these lineages is older than the *Mytilus edulis*/*M. trossulus* split. This conclusion is supported by all methods of phylogenetic analysis used except the unweighted, rooted maximum-parsimony method (fig. 4B). A requirement of rooted analyses is that the outgroup taxon (or taxa) not be part of the group of interest yet be as closely related as possible. Rooting the *Mytilus* sequences is therefore problematic. Since we do not yet know how widespread the phenomenon of doubly uniparental inheritance of mtDNA is throughout the bivalvia, we were forced to use as outgroups taxa that are distantly related to *Mytilus*. Both *Lasaea* and *Katharina* have diverged from *Mytilus* perhaps more than 500 million yr ago (Mya). The danger of a false phylogeny resulting from a distant outgroup is larger when no weighting is used because of the increased number of homoplasies at third- and, to a lesser extent, first-codon positions. We are therefore less inclined to accept the unweighted rooted parsimony tree compared with the weighted rooted parsimony tree, which places less weight on rapidly evolving sites and more weight on relatively conserved sites (Moritz et al. 1987; Hillis et al. 1994). Indeed, the strong similarity of the weighted rooted parsimony tree with the unrooted parsimony and neighbor-joining trees adds credence to the weighted rooted analysis.

The taxonomy of marine blue mussels has been a matter of extensive study and controversy. At least five or six taxa are currently listed under the genus name *Mytilus*: *M. californianus*, *M. edulis*, *M. trossulus*, *M. galloprovincialis*, *M. desolationis*, and *M. planulatus*. Of

Table 2
Matrix of Genetic Distances Based on Kimura's Two-Parameter Estimate for all Pairwise Combinations of Male and Female Mitotypes of *Mytilus edulis* and *Mytilus trossulus*, *Lasaea australis*, and *Katharina tunicata*

OTU	COIII MITOTYPE												
	F-ed1	F-ed1'	F-ed2	F-tr1	F-tr2	F-tr3	F-tr3'	M-ed1	M-ed2	M-tr1	M-tr2	M-tr3	Lasaea
F-ed1'	0.013												
F-ed2	0.029	0.036											
F-tr1	0.185	0.177	0.199										
F-tr2	0.190	0.182	0.195	0.016									
F-tr3	0.190	0.182	0.204	0.006	0.009								
F-tr3'	0.190	0.182	0.204	0.013	0.016	0.006							
M-ed1	0.241	0.250	0.241	0.273	0.262	0.267	0.267						
M-ed2	0.238	0.248	0.247	0.261	0.260	0.255	0.255	0.026					
M-tr1	0.257	0.267	0.277	0.260	0.265	0.260	0.250	0.264	0.243				
M-tr2	0.272	0.282	0.292	0.246	0.240	0.245	0.235	0.212	0.217	0.063			
M-tr3	0.250	0.259	0.269	0.243	0.257	0.252	0.243	0.257	0.243	0.022	0.059		
Lasaea	0.522	0.546	0.530	0.546	0.571	0.562	0.562	0.631	0.622	0.548	0.600	0.516	
Katharina	0.622	0.649	0.622	0.640	0.649	0.640	0.640	0.604	0.630	0.587	0.660	0.587	0.416

these, the first is a distinct species (in that it does not hybridize with any of the other taxa) and the last two have been considered by some to be forms of *M. edulis* (see Gosling 1992*b*). The difficulty in resolving the *edulis/trossulus/galloprovincialis* complex stems from the fact that their geographical distributions overlap and that hybridization is known to occur in areas of sympatry (McDonald and Koehn 1988; Varvio et al. 1988; McDonald et al. 1991). Furthermore, all three forms have possibly been spread in bilge water to other parts of the globe, making it difficult to separate "true" historical distributions from recent introductions (McDonald et al. 1991).

Basing his opinions on fossil material, Kafacov proposed that the common ancestor to the *M. edulis* species complex arose in the North Pacific during the early Eocene (i.e., 40–50 Mya) and subsequently dispersed into the Atlantic via the Bering Sea (Kafacov 1987 cited in Vermeij 1992). Electrophoretic data indicate that *M. edulis*, *M. trossulus,* and *M. galloprovincialis* are genetically distinct, but because each has diverged by similar amounts, they essentially form an unresolved trichotomy (Varvio et al. 1988; McDonald et al. 1991; reviewed in Gosling 1992*a*). E. Kenchington (personal communication) has also come to the same conclusion based on sequence data for the nuclear gene 18S rRNA. Assuming a molecular clock, Varvio et al. (1988) concluded that these three taxa originated during a brief period of time roughly 1–3 Mya. An observation from our mitotype data (table 1) also supports the recognition of *M. edulis* and *M. trossulus* as separate species. For the "pure" specimens examined here, the *Eco*R I mitotypes were correlated with the multilocus allozyme genotypes (i.e., pure male *M. edulis* mitotypes were never

found in pure male *M. trossulus* and vice versa). While this still does not provide a completely diagnostic marker for these species, it implies that there is minimal introgression of the female and male mitotypes from one taxon to the other, despite allozymic evidence for hybridization (unpublished data). The exceptions to doubly uniparental inheritance observed in hybrid laboratory crosses (Zouros et al. 1994*b*) are of special importance because they may provide clues about how this exceptional mode of organelle DNA works. However, it is clear from the results of this study that these hybridization anomalies have prevented neither the evolution of two distinct gender-associated mtDNA lineages nor the formation of species-specific lineages within each gender.
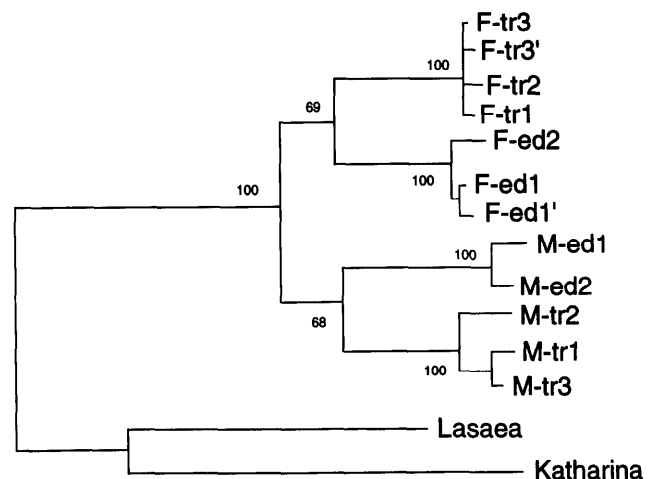

FIG. 3.—Neighbor-joining tree of male and female *M. edulis* and *M. trossulus* COIII sequences rooted with *Lasaea australis* and *Katharina tunicata* as outgroup taxa. Numbers indicate the support for each of the clades out of 1,000 bootstrap replicates.
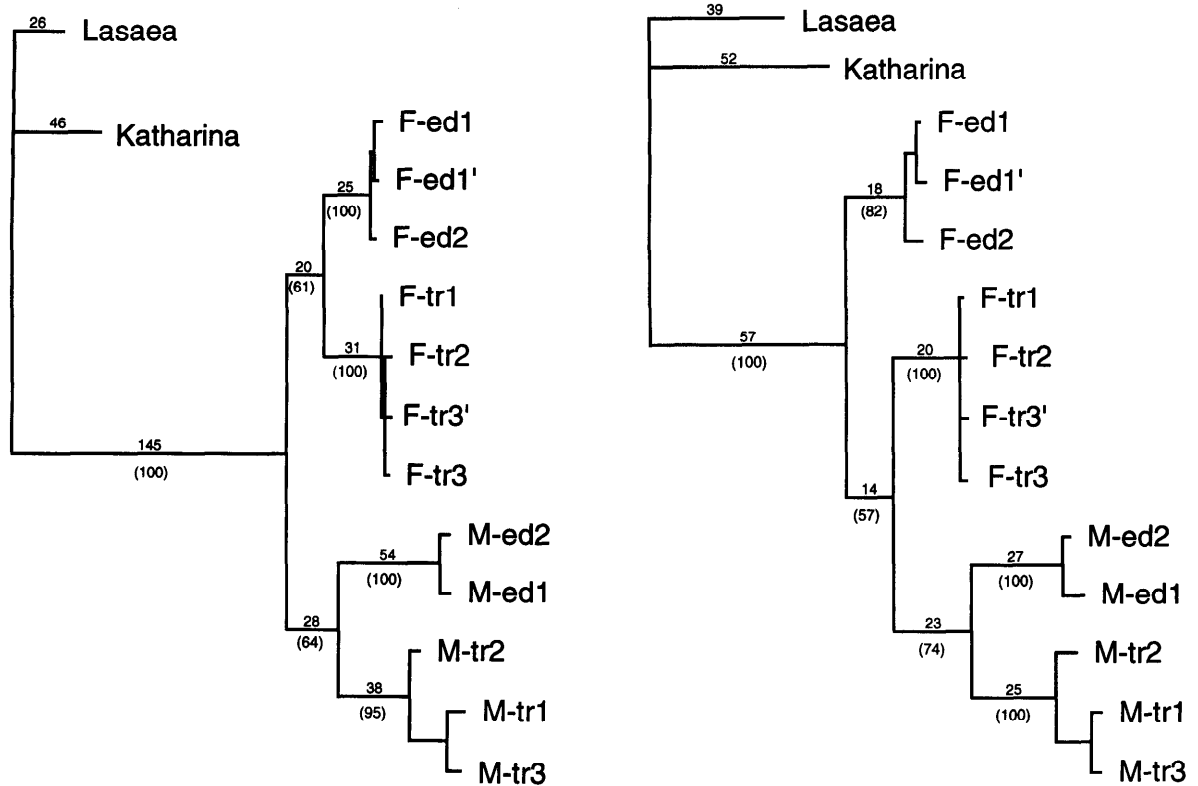
FIG. 4.—Maximum parsimony trees of male and female *M. edulis* and *M. trossulus CO*III sequences rooted with *Lasaea australis* and *Katharina tunicata* as outgroup taxa. *A*, Strict concensus of two trees generated from differential weighting of first-, second-, and third-codon positions (weighted 4, 5, and 1, respectively). *B*, Strict concensus of five trees generated from equal weighting of all positions. Numbers indicate branch lengths or (in brackets) the percent frequency of occurrence of the given branch in 1,000 bootstrap replicates.

Two observations from our data suggest that the male lineage evolves faster than the female. First, branches in the male lineage are generally longer than the corresponding branches in the female lineage (figs. 2, 3, and 4). Second, there is a greater diversity of male types ($n = 10$) than female types ($n = 6$) in the natural population we have studied. From table 2, the average rate of substitution, K, among *M. edulis* and *M. trossulus* F molecules is 0.193 ± 0.029 and among M molecules is 0.237 ± 0.032. These estimates are not significantly different at the 5% level. However, estimates of substitution rates for synonymous, K_S, and nonsynonymous, K_A, sites produce a different result. The mean K_S value for F *M. edulis* versus F *M. trossulus* types is 0.665 ± 0.025 and that for the M types is 0.710 ± 0.048, again not significantly different. In contrast, the corresponding K_A values are 0.006 ± 0.002 and 0.042 ± 0.006, which are different at the 0.001 level.

The unique mode of doubly uniparental inheritance may offer an explanation for this difference. Imagine that a deleterious mutation arises in a female type molecule. This molecule would be selected against in female individuals if its frequency, relative to a wild female type, became high through stochastic assortment during de-

velopment. Such purifying selection could even operate at the cellular level by causing death to cells that become highly homogeneous for the mutant mtDNA. A similar mutation in a male type molecule may escape selection because this molecule will occur in heteroplasmy with a female type (there is, at present, no documented case of a somatic tissue, in either males or females, that does not contain female mtDNA). The intensity with which the mutated female lineage will be removed from the population would be inversely related to the mutation's recessivity, but the preferential removal of female lineages relative to male lineages must hold even for completely recessive mutations.

Although male types may face relaxed selection compared with female types and be able to hitchhike on the back of female types, their exclusive presence in sperm (Skibinski et al. 1994*a*; fig. 1) implies that male types may be only partially relieved of selective constraints. This may explain why there are no nonsense mutations in the portion of the male *CO*III gene sequence described here. Alternatively, mitochondria in males may undergo more replication events during spermatogenesis than do female mitochondria during oogenesis. This explanation is analogous to the hypoth-

esis of male-driven molecular evolution (Miyata et al. 1987; Chang et al. 1994), which postulates a higher rate of mutation for male gametes than female gametes because of the greater number of germ cell divisions per generation in the male line than in the female line. Prior to meiosis in *Mytilus,* as in other molluscs, there are seven mitotic divisions leading up to the production of mature spermatids, whereas mature eggs develop after only four mitotic divisions (Selwood 1968; Zwaan and Mathieu 1992). If mitochondrial replications occur with approximately the same frequency as the mitotic divisions, this difference between males and females could account for the different rate of substitution between male and female lineages but would not account for the observation that the difference appears to be restricted to nonsynonymous sites.

It is tempting to use our molecular data to date the origin of doubly uniparental inheritance. We feel, however, that the use of a molecular clock in this context is presently unwarranted. First, rates of sequence divergence for *CO*III have not been calibrated for bivalves in general or mussels in particular and rates of molecular evolution are known to differ considerably among taxa (Martin and Palumbi 1993; Stewart and Baker 1994). Second, a molecular clock assumes rate constancy, but as discussed above, the two lineages appear to evolve at different rates. Third, determination of the age of doubly uniparental inheritance cannot be divorced from its distribution among bivalves. Highly divergent mitotypes have been isolated from the sperm and eggs of *Mytilus californianus* and from the gonads of male and female *Geukensia demissa* (Mytilidae: Modiolinae; D. Stewart, W. R. Hoeh, B. Sutherland, and E. Zouros, unpublished data). The Modiolinae are believed to have diverged from the lineage leading to *Mytilus* approximately 150 Mya (Soot-Ryen 1969). In addition, gender-associated mitotypes have been observed in freshwater mussels of the genus *Pyganodon* (formerly *Anodonta*) by Liu et al. (in press) and by W. R. Hoeh, B. Sutherland, D. Stewart, and E. Zouros (unpublished data). While it is probable that the phenomenon existed in the ancestor of *M. edulis, M. trossulus,* and *M. californianus,* and possibly *G. demissa,* this appears to be unlikely for the common ancestor of *Mytilus* and *Pyganodon.* The freshwater mussels (Paleoheterodonta:Unionoida) have been a distinct group for at least 350 million yr (McCormick and Moore 1969). If the gender-associated lineages found in *Mytilus* and *Pyganodon* had their origin in a common ancestor to these two taxa, then the male and female types observed in *Mytilus* would presumably have diverged more than the 25% observed in this study (table 2). The presence of gender-associated mitotypes in *Pyganodon* may, therefore, represent an independent acquisition of doubly uniparental inheritance. However, in the absence of a phylogenetic

comparison of male and female mtDNA types from distantly related species with doubly uniparental inheritance, such as *Mytilus* and *Pyganodon,* it would be impossible to decide between a theory of independent and relatively recent origins of the phenomenon and a theory of a single and very old origin for all bivalves.

The presence of doubly uniparental inheritance generates a complication in the use of mtDNA as a tool for phylogenetic and population studies in *Mytilus.* Comparisons of conspecific populations may produce erroneous results if only female types are scored in one population, only male types in another, and a combination of types in still another population. This problem is more serious in interspecific studies, where rather different results can be obtained depending on whether the comparison involves representatives from the same or different gender-associated lineages. Blot et al. (1990) observed little sequence divergence among mitotypes of *M. desolationis,* but this is because they used exclusively mature female gonad as a source of mtDNA. By doing so, they restricted their study to the female lineage, which is evolving more slowly than the male lineage. On the other hand, Geller et al. (1993) observed a considerably higher level of mtDNA divergence among *M. trossulus* types than among mitotypes from *M. edulis* and *M. galloprovincialis.* This apparently contradictory result can be understood if the *M. trossulus* types they sequenced belonged to both gender-associated lineages, whereas the *M. edulis* and *M. galloprovincialis* types were from the female lineage. Recognition of the two mtDNA types and strict adherence to the use of one or the other type is required in future uses of mtDNA for population studies of *Mytilus.*

Acknowledgments

APPENDIX A

Table A1

Sequence for a 321 bp Segment of the *Cytochrome oxidase c subunit* III Gene for 12 *Mytilus edulis* and *Mytilus trossulus* Individuals

	GTA	CAA	CTG	CGG	GAA	TAT	TAT	TGA	AAC	TCC	TAT	ACT	ATT	GCA	GAT	AGG	GTT	TAT
F-ed1	GTA	CAA	CTG	CGG	GAA	TAT	TAT	TGA	AAC	TCC	TAT	ACT	ATT	GCA	GAT	AGG	GTT	TAT
F-ed′	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
F-ed2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
F-tr1	..G	...	..T	..A	...	..C	..C	...	...	..G	..C	...	...	...	...	...	..G	...
F-tr2	..G	...	..T	..A	...	..C	..C	...	...	..A	..C	...	...	...	...	...	..G	...
F-tr3	..G	...	..T	..A	...	..C	..C	...	...	..G	..C	...	...	...	...	...	..G	...
F-tr3′	...	...	..T	..A	...	..C	..C	...	...	..G	..C	...	...	...	...	...	..C	...
M-ed1	...	...	G..	..A	..G	...	.G.	...	...	..T	..C	.GG	...	...	..C	..T	...	...
M-ed2	...	...	G..	..A	..G	...	.G.	...	...	..T	..C	.GG	...	...	..C	..T	..C	...
M-tr1	...	...	G.C	..A	..G	...	..C	...	...	..G	..C	...	..C	T..	...	..C	..G	..C
M-tr2	...	.G.	G.C	..A	..G	...	..C	...	...	..G	..C	...	..C	T..	...	..C	..G	..C
M-tr3	...	...	G.C	..A	..G	...	..C	...	...	..G	..C	...	..C	T..	...	..C	..G	..C
Lasaea	C.G	...	GCT	GAA	..G	...	C.C	.CT	GCT	..T	.T.	...	..C	T.T	...	G..	AG.	...
Katharina	T..	..G	GCT	G.A	...	...	.TA	GA.	.CT	AG.	.T.	...	...	..C	..C	..T	TG.	...

	GGT	AGG	GTT	TTT	TAC	TTA	TTA	ACT	GGA	TTC	CAT	GGG	ATA	CAT	GTT	GTC	GTA	GGG
F-ed1	GGT	AGG	GTT	TTT	TAC	TTA	TTA	ACT	GGA	TTC	CAT	GGG	ATA	CAT	GTT	GTC	GTA	GGG
F-ed1′	...	...	...	...	...	...	C..	...	...	...	..C	...	...	..C	...	...	...	...
F-ed2	C.C	...	...	..C	...	...	C..	...	...	...	...	..A	...	...	...	..T	...	...
F-tr1	..A	..A	..G	...	..T	...	C..	...	..C	..T	...	..A	..G	..C	..A	...	..G	...
F-tr2	..A	..A	..G	...	..T	...	C..	...	..G	...	...	..A	..G	..C	..A	..T	..G	...
F-tr3	..A	..A	..G	...	..T	...	C..	...	..G	..T	...	..A	..G	..C	..A	...	..G	...
F-tr3′	..A	..A	..G	...	..T	...	C..	...	..G	..T	...	..A	..G	..C	..A	...	..G	...
M-ed1	..C	..A	...	...	..T	C..	C..	...	..G	...	...	...	GC.	...	..A	..A	...	..T
M-ed2	..C	..A	...	...	..T	...	C..	...	..G	..T	...	...	GC.	...	..A	..A	...	..T
M-tr1	..A	..A	..G	...	..T	...	..G	..C	..G	..T	...	...	GC.	..C	..A	..T	..T	...
M-tr2	..A	..A	..A	...	..T	C..	..G	..C	..G	...	...	...	GC.	..C	..A	..T	...	...
M-tr3	..A	..A	..G	...	..T	C..	..G	..C	..G	..T	...	...	G..	..C	..A	..T	..T	...
Lasaea	..G	TCT	T.A	...	.TT	G.T	A..	..A	..T	..T	...	..A	...	...	...	T.A	A.T	...
Katharina	..A	TCA	ACA	...	.TT	G..	GCT	..A	..G	..T	...	..C	T.T	...	..G	T.A	...	..T

	ACT	ATT	TCG	CTA	ATG	GTA	AGG	TTA	GTT	CGA	CTA	TGA	CGC	GGG	GAG	TTT	TCT	---
F-ed1	ACT	ATT	TCG	CTA	ATG	GTA	AGG	TTA	GTT	CGA	CTA	TGA	CGC	GGG	GAG	TTT	TCT	---
F-ed1′	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
F-ed2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
F-tr1	...	C..	..A	...	...	..G	...	...	...	...	...	...	..G	..T	...	...	..C	...
F-tr2	...	C..	..A	...	...	..G	...	...	..C	...	...	...	..G	..T	...	...	..C	...
F-tr3	...	C..	..A	...	...	..C	...	...	..C	...	...	...	..G	..T	...	...	..C	...
F-tr3′	...	C..	..A	...	...	..G	...	...	..C	...	...	...	..G	..T	...	...	..C	...
M-ed1	...	..C	...	...	..A	...	..T	C..	..C	..C	T..	..G	..T	..A	...	..C	...	...
M-ed2	...	..C	...	...	..A	...	...	C..	..C	..C	T..	..G	..T	..A	...	..C	...	...
M-tr1	..C	G..	..A	T.G	T.A	...	...	..G	.GC	..G	...	...	..T	..T	...	...	...	...
M-tr2	..C	..C	..A	T.G	..A	...	...	..G	..C	..G	...	..G	..T	..T	...	..C	...	...
M-tr3	..C	...	..A	T.G	T.A	...	...	..G	...	..G	...	...	..T	..T	...	...	...	...
Lasaea	...	T.A	.TT	T..	T..	...	G..	...	...	...	ACT	ATT	..G	TAT	C.T	...	...	GTT
Katharina	.GA	C..	.TT	T..	T.A	..T	.CT	..G	TGA	...	AAT	.TT	A.T	T.T	C.T	...	...	...

744

NOTE.—The *Lasaea australis* sequence is from Ô Foighal and Smith (1995), and the *Katharina tunicata* sequence is from Boore and Brown (1994). See text for an explanation of the naming system.

LITERATURE CITED

AVISE, J. C. 1994. Molecular markers, natural history and evolution. Chapman & Hall, New York.

BALLARD, J. W., and M. KREITMAN. 1994. Unraveling selection in the mitochondrial genome of *Drosophila*. Genetics **138**:757–772.

BLOT, M., B. LEGENDRE, and P. ALBERT. 1990. Restriction fragment length polymorphism of mitochondrial DNA in subantarctic mussels. J. Exp. Mar. Biol. Ecol. **141**:79–86.

BOORE, J. L., and W. M. BROWN. 1994. Complete DNA sequence of the mitochondrial genome of the black chiton, *Katharina tunicata*. Genetics **138**:423–443.

CHANG, B. H.-J., L. C. SHIMMIN, S.-K. SHYUE, D. HEWITT-EMMETT, and W.-H. LI. 1994. Weak male-driven molecular evolution in rodents. Proc. Natl. Acad. Sci. USA **91**:827–831.

FISHER, C., and D. O. F. SKIBINSKI. 1990. Sex-biased mitochondrial DNA heteroplasmy in the marine mussel *Mytilus*. Proc. Roy. Soc. Lond **242**:149–156.

GELLER, J. B., J. T. CARLTON, and D. A. POWERS. 1993. Interspecific and intrapopulation variation in mitochondrial DNA sequences of *Mytilus* spp. (Bivalvia: Mollusca). Mol. Mar. Biol. Biotech. **2**:44–50.

GOSLING, E. M. 1992a. Systematics and geographic distribution of *Mytilus*. Pp. 1–20 in E. M. Gosling, ed. The mussel *Mytilus*: ecology, physiology, genetics and aquaculture. Elsevier, Amsterdam.

———. 1992b. Genetics of *Mytilus*. Pp. 309–382 in E. M. Gosling, ed. The mussel *Mytilus*: ecology, physiology, genetics and aquaculture. Elsevier, Amsterdam.

GYLLENSTEN, U., D. WHARTON, A. JOSEFSSON, and A. C. WILSON. 1991. Paternal inheritance of mitochondrial DNA in mice. Nature **352**:255–257.

HAYASHI, J.-I., H. YONEKAWA, O. GOTOH, J. WATANABE, and Y. TAGASHIRA. 1978. Strictly maternal inheritance of rat mitochondrial DNA. Biochem. Biophys. Res. Commun. **83**:1032–1038.

HILLIS, D. M., and J. J. BULL. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. **42**:182–192.

HILLIS, D. M., J. P. HUELSENBECK, and D. L. SWOFFORD. 1994. Hobgoblin of phylogenetics? **369**:363–364.

HOEH, W. R. 1990. Phylogenetic relationships among eastern North American *Anodonta* (Bivalvia: Unionidae). Malocol. Rev. **23**:63–82.

HOEH, W. R., K. H. BLAKLEY, and W. M. BROWN. 1991. Heteroplasmy suggests limited biparental inheritance of *Mytilus* mitochondrial DNA. Science **251**:1488–1498.

HOFFMANN, R. J., J. L. BOORE, and W. M. BROWN. 1992. A novel mitochondrial genome organization for the blue mussel, *Mytilus edulis*. Genetics **131**:397–412.

HURST, L. D., and R. F. HOEKSTRA. 1994. Shellfish genes kept in line. Nature **368**:811–812.

KONDO, R., Y. SATTA, E. T. MATSUURA, H. ISHIWA, N. TAKAHATA, and S. I. CHIGUSA. 1990. Incomplete maternal transmission of mitochondrial DNA in *Drosophila*. Genetics **126**:657–663.

KUMAR, K. T., K. TAMURA, and M. NEI. 1993. MEGA: molecular evolutionary genetics analysis, version 1.0. Pennsylvania State University, University Park.

LIU, H.-P., J. B. MITTON, and S.-K. WU. Gender-specific mitochondrial DNA within and among populations of the freshwater mussel, *Anodonta grandis grandis*. Evolution (in press).

MAGOULAS, A., and E. ZOUROS. 1993. Restriction-site heteroplasmy in anchovy (*Engraulis encrasicolus*) indicates incidental biparental inheritance of mitochondrial DNA. Mol. Biol. Evol. **10**:319–325.

MARTIN, A. P., and S. R. PALUMBI. 1993. Body size, metabolic rate, generation time, and the molecular clock. Proc. Natl. Acad. Sci. USA **90**:4087–4091.

MCCORMICK, L., and R. C. MOORE. 1969. Outline of classification. Pp. 218–224 in R. C. MOORE, ed. Treatise on invertebrate paleontology. Pt. N, Vol. **1**. Mollusca 6: Bivalvia. University of Kansas and Geological Society of America, Lawrence.

MCDONALD, J. H., and R. K. KOEHN. 1988. The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. Mar. Biol. **99**:111–118.

MCDONALD, J. H., R. SEED, and R. K. KOEHN. 1991. Allozyme and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Mar. Biol. **111**:323–335.

MIYATA, T., H. HAYASHIDA, K. KUMA, K. MITSUYASU, and T. YASUNAGA. 1987. Male-driven molecular evolution: a model and nucleotide sequence analysis. Cold Spring Harbor Symp. Quant. Biol. **52**:863–867.

MORITZ, C., T. E. DOWLING, and W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annu. Rev. Ecol. Syst. **18**:269–292.

NACHMAN, M. W., S. N. BOYER, and C. F. AQUADRO. 1994. Nonneutral evolution at the mitochondrial NADH dehydrogenase subunit 3 gene in mice. Proc. Natl. Acad. Sci. USA **91**:6364–6368.

Ó FOIGHIL, D., and M. J. SMITH. 1995. Evolution of asexuality in the marine clam, *Lasaea*. Evolution **49**:140–150.

RAND, D. M., M. DORFSMAN, and L. M. KANN. 1994. Neutral and non-neutral evolution of *Drosophila* mitochondrial DNA. Genetics **138**:741–756.

SAITOU, N., and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.

SATTA, Y., N. TOYOHARA, C. OHTAKA, Y. TATSUNO, T. K. WATANABE, E. T. MATSUURA, S. I. CHIGUSA, and N. TAKAHATA. 1988. Dubious maternal inheritance of mitochondrial DNA in *D. simulans* and evolution of *D. mauritiana*. Genet. Res. **52**:1–6.

SELWOOD, L. 1968. Interrelationships between the developing oocytes and ovarian tissues in the chiton, *Sypharochiton septentriones* (Ashby) (Mollusca, Polyplacophora). J. Morphol. **125**:71–104.

SKIBINSKI, D. O. F., C. GALLAGHER, and C. M. BEYNON. 1994a. Mitochondrial DNA inheritance. Nature **368**:817–818.

————. 1994b. Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis*. Genetics **138**:801–809.

SOOT-RYEN, T. 1969. Superfamily Mytilacea Rafinesque, 1815. Pp. 271–280 *in* R. C. MOORE, ed. Treatise on invertebrate paleontology. Pt. N, Vol. **1**. Mollusca **6**: Bivalvia. University of Kansas and Geological Society of America, Lawrence.

STEWART, D. T., and A. J. BAKER. 1994. Patterns of sequence variation in the mitochondrial D-loop region of shrews. Mol. Biol. Evol. **11**:9–21.

SWOFFORD, D. L. 1991. PAUP: phylogenetic analysis using parsimony, version 3.1.1. Illinois Natural History Survey, Champaign.

VARVIO, S.-L., R. K. KOEHN, and R. VÄINÖLÄ. 1988. Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. Mar. Biol. **98**:51–60.

VERMEIJ, G. J. 1992. Trans-equatorial connections between biotas in the temperate eastern Atlantic. Mar. Biol. **112**:343–348.

ZOUROS, E., A. O. BALL, C. SAAVEDRA, and K. R. FREEMAN. 1994a. Mitochondrial DNA inheritance. Nature **368**:818.

————. 1994b. An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. Proc. Natl. Acad. Sci. USA **91**:7463–7467.

ZOUROS, E., K. R. FREEMAN, A. O. BALL, and G. H. POGSON. 1992. Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel *Mytilus*. Nature **359**:412–414.

ZWAAN, A., and M. MATHIEU. 1992. Cellular biochemistry and endocrinology. Pp. 223–307 *in* E. M. GOSLING, ed. The mussel *Mytilus*: ecology, physiology, genetics and aquaculture. Elsevier, Amsterdam.