Male-Dependent Doubly Uniparental Inheritance of Mitochondrial DNA and Female-Dependent Sex-Ratio in the Mussel *Mytilus galloprovincialis*

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ABSTRACT

We have investigated sex ratio and mitochondrial DNA inheritance in pair-matings involving five female and five male individuals of the Mediterranean mussel *Mytilus galloprovincialis*. The percentage of male progeny varied widely among families and was found to be a characteristic of the female parent and independent of the male to which it was mated. Thus sex-ratio in Mytilus appears to be independent of the nuclear genotype of the sperm. With a few exceptions, doubly uniparental inheritance (DUI) of mtDNA was observed in all families fathered by four of the five males: female and male progeny contained the mother's mtDNA (the F genome), but males contained also the father's paternal mtDNA (the M genome). Two hermaphrodite individuals found among the progeny of these crosses contained the F mitochondrial genome in the female gonad and both the F and M genomes in the male gonad. All four families fathered by the fifth male showed the standard maternal inheritance (SMI) of animal mtDNA: both female and male progeny contained only the maternal mtDNA. These observations illustrate the intimate linkage between sex and mtDNA inheritance in species with DUI and suggest different major roles for each gender. We propose a model according to which development of a male gonad requires the presence in the early germ cells of an agent associated with sperm-derived mitochondria, these mitochondria are endowed with a paternally encoded replicative advantage through which they overcome their original minority in the fertilized egg and this advantage (and, therefore, the chance of an early entrance into the germ line) is countered by a maternally encoded egg factor.

MITOCHONDRIAL DNA (mtDNA) is maternally transmitted in most animal species, although paternal leakage has been reported in some cases (BIRKY 1996). In the mussels of the genus Mytilus, a different pattern of mtDNA transmission, termed "doubly uniparental inheritance" (DUI), has been described (SKIBINSKI *et al.* 1994; ZOUROS *et al.* 1994). In these species there exist two highly diverged mitochondrial genomes, one of which occurs in all individuals and is female-transmitted (F type) and one that occurs in males and is male-transmitted (M type). DUI explains the high levels of heteroplasmy reported in natural populations of Mytilus (FISHER and SKIBINSKI 1990; HOEH *et al.* 1991), the much higher frequency of heteroplasmy among males (FISHER and SKIBINSKI 1990) and the high rate of biparental transmission of mtDNA in laboratory crosses (ZOUROS *et al.* 1992). The recent findings of divergent mitotypes in female and male gonads in the marine mussel *Geukensia demissa* (a close relative of Mytilus) (HOEH *et al.* 1996) and in freshwater mussels of the genera Pyganodon and Fusconaia (separated from Mytilus since >400 mya) (HOEH *et al.* 1996; LIU *et al.* 1996) indicate that DUI is not restricted to Mytilus.

The first genetic study of mtDNA inheritance in mussels involved intraspecific and interspecific crosses of *M. edulis* and *M. trossulus* (ZOUROS *et al.* 1994). In addition to confirming DUI, these crosses revealed large differences in the sex ratio. In some crosses, the sex ratio was biased in favor of males, in others, in favor of females, and in still others, there was no appreciable bias. In two crosses that shared the same female parent, the sex ratio was very similar, but in two other crosses that shared the same male parent, the sex ratio was different. This suggested that sex ratio is under the control of the female parent (ZOUROS *et al.* 1994), but clearly a specially designed set of crosses was necessary to confirm this hypothesis.

Subsequent studies of mtDNA variation in natural populations of Mytilus and the demonstration of DUI in other species have raised a number of questions that have also necessitated a new study of pair matings. Exceptional animals, *i.e.*, animals that do not conform to DUI, have been noted in wild populations and laboratory crosses of Mytilus. They include females that are heteroplasmic for an F and an M type (FISHER and SKIBINSKI 1990; ZOUROS *et al.* 1994; STEWART *et al.* 1995; QUESADA *et al.* 1996), males that are homoplasmic for

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an F type (FISHER and SKIBINSKI 1990; ZOUROS *et al.* 1994; RAWSON and HILBISH 1995; WENNE and SKIBINSKI 1995) and individuals heteroplasmic for more than two genomes (HOEH *et al.* 1991). Some of these exceptions were considered as occasional breakdowns of DUI due to hybridization anomalies (HOEH *et al.* 1991; ZOUROS *et al.* 1994). Systematic search for DUI in other species has been limited as yet, but the phylogenetic distribution of species that are currently known to have DUI suggests either that the phenomenon has had multiple and independent origins or that periodical breakdowns of DUI wipe out the molecular divergence between F and M mitotypes (HOEH *et al.* 1996).

Here we report the results from a set of pair matings in the Mediterranean mussel *M. galloprovincialis.* Although the existence of DUI was not explicitly demonstrated in this species, the observation of high levels of male heteroplasmy in natural populations of *M. galloprovincialis* (FISHER and SKIBINSKI 1990; HOEH *et al.* 1991; QUESADA *et al.* 1996) and of gender-associated lineages of mtDNA (RAWSON and HILBISH 1995) suggested that this species shares this mode of mtDNA inheritance with *M. edulis* and *M. trossulus.*

MATERIALS AND METHODS

Crosses: In February, 1994, 100 mussels were taken from a commercial stock near Ría de Arousa (northwest Spain). Mussels were induced to spawn by thermal stimulation in individual containers. Five individuals of each sex were selected for crosses on the basis of large number of eggs spawned (dams) or high frequency of motile sperm (sires). Sperm and eggs from each of the selected individuals were mixed at an egg/sperm ratio of 1:70 in individual plastic trays containing 2 liters of sea water supplied with chloramphenicol at 2.5 mg/liter and forced to pass though a 1-μm filter. After 48 hr larvae were transferred to 8-liter containers where they were fed with a mixture of the microalgae *Isochrysis galbana, Monochrysis lutheri* and *Chaetoceros calcitrans.* The initial density of culture was 8 larvae/ml. The water (same as that used for fertilization but without the antibiotic) was changed every two days. A sample count of larvae at 48 hr after mixing of gametes showed that the combined rate of fertilization, hatching and survival to this age varied from 12 to 52% of eggs. After settlement, the spat was maintained in the containers until the age of 40 days, when it was detached from the walls of the containers and placed in net-bottom cylinders immersed in a 300-liter tank with circulating filtered sea water. During this period the food regime was the same as in larvae, except that *Tetraselmis svecica* was used instead of *C. calcitrans.* At the age of 2 mon, the juvenile mussels were placed in plastic baskets and moved to a raft in Ría de Arousa, where they remained until adulthood. One cross was accidentally lost, so the set of families examined was reduced to twenty four.

Processing of parents and offspring: A sample of sperm or eggs from each parent was taken immediately after spawning. With the exception of female F66 and male M70, which died soon after crossing, parents were sacrificed and digestive gland and gonad stored at −80°. Gonads and gametes were used for mtDNA assays and digestive gland was used for allozyme assays. The sexual development of offspring was monitored during the spring of 1995. In late May 1995, most offspring had developed gonads. A sample of 30–40 progeny were taken from each family and sexed by microscopic examination of a sample of gonadal tissue. Animals in which no gametes could be observed were classified as "not sexed". Digestive gland and gonad from each processed offspring were removed and stored at −80°.

TABLE 1

Parental mitotypes and fragment sizes after digestion of the PCR product of the *COIII* gene from M and F mtDNA types with the restriction enzymes *Bam*HI and *Eco*RI

Mitochondrial genome	*Bam*HI	*Eco*RI
A. Fragment sizes		
M	540 + 320	860
FA	860	860
FB	860	540 + 320
Females		**Males**
B. Parental mitotypes[a]		
F19	FA	M27	M/FA
F20	FB	M28	M/FA
F31	FA	M46	M/FA
F53	FB	M54	M/FB
F66	FB	M70	M/−

[a] Heteroplasmic mitotypes are indicated by a slash. Male 70 died before removal of gonad, so his maternal mitotype could not be determined. His paternal type was determined from sperm.

Detection of mtDNA types: Total DNA was extracted from gonadal tissue or gametes as previously described (ZOUROS *et al.* 1992). The detection of mitochondrial genomes was done by PCR amplification using the primers 5′-TATGTACCAGGTCCAAGTCCGTG-3′ and 5′-ATGCTCTTCTTGAATATAAGCGTACC-3′ (ZOUROS *et al.* 1994). These primers correspond to the nucleotide positions 460–482 and 1362–1301 of segment #5 of the *M. edulis* F genome in HOFFMANN *et al.* (1992), and amplify an 860-bp fragment of the *COIII* gene from both F and M types of the *M. edulis* species complex (*M. edulis, M. galloprovincialis* and *M. trossulus*). The amplifications were done in 50-μl reactions, with ~0.2 μg of total DNA, 0.25 μM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, and 0.5 units of *Taq* DNA polymerase (USB), in the buffer supplied by the company. The mixture was incubated for 35 cycles at 94° for 1 min, 54° for 30 sec and 72° for 1 min, with an initial denaturation period at 94° for 2 min and a final extension period at 72° for 4 min.

Using the restriction enzymes *Bam*HI and *Eco*RI we recognized one M and two F mtDNA types among the parents (Table 1). All three types correspond to those expected from previously published information (EDWARDS and SKIBINSKI 1987; FISHER and SKIBINSKI 1990; SKIBINSKI *et al.* 1994). In combination, the two enzymes allow the identification of each of the three types. The *Bam*HI site of the M mitotype and the *Eco*RI site of the FB mitotype happen to be in the same position (position 794 of the sequence of HOFFMANN *et al.* 1992).

Allozyme analysis for detection of contaminants: Contamination of cultures of bivalves with foreign individuals either through the sea water or through hatchery practice is a common occurrence (FOLTZ 1986; MALLET *et al.* 1986; ZOUROS *et al.* 1992, 1994; ALLEN and GAFFNEY 1993). Although considerable care was taken during crossing and rearing of the families to avoid this problem, we felt it was necessary to check for contaminants. This was done by scoring the digestive gland tissue of offspring for five allozyme loci: *Lap-1, Lap-2, Odh,*

Pgi and *Pgm*. The electrophoretic techniques used were those of QUESADA *et al.* (1995). In 12 families, all sampled offspring were scored for allozymes. These were families in which exceptions to DUI were observed or families that showed extreme sex ratios. Because the sea water used during the whole period the animals were in the hatchery was filtered for the retention of mussel larvae, the possibility of contamination from the wild was very small. Contamination from the wild when the animals were held at sea can also be excluded on account of the size difference between the progeny of our crosses and "would-be" contaminants. We conclude that contamination could have resulted from accidental transfer of offspring among families during handling of family cages. Because all parents were scored for allozymes, the probability that a progeny of a given family could be a contaminant yet be compatible with the parental genotypes could be estimated assuming contamination was equally possible among crosses. This probability varied among families from 0.032 to 0.290.

Statistical analysis of sex-ratio: Each individual offspring was classified according to sex, female parent and male parent. This led to a three-way contingency table. We tested the effects of male and female parents on the heterogeneity of the sex-ratio by fitting a set of discrete hierarchical log-linear models by means of G-statistics (BISHOP *et al.* 1975; SOKAL and ROHLF 1995). The complete model assumes three main effects, three two-way interactions and one three-way interaction. The logarithm of the expected number of individuals in the cell corresponding to mother i (1 to 5), father j (1 to 5) and sex k (1 or 2) is given by

$$\ln f_{ijk} = m + \alpha_i + \beta_j + \gamma_k + \alpha_i\beta_j + \alpha_i\gamma_k + \beta_j\gamma_k + \alpha_i\beta_j\gamma_k$$

where m is the mean of the logarithms of expected numbers; α_i, β_j and γ_k are the main effects of the female parent, the male parent and sex, respectively; $\alpha_i\beta_j$, $\alpha_i\gamma_k$ and $\beta_j\gamma_k$ are the two-way interactions; and $\alpha_i\beta_j\gamma_k$ is the three-way interaction. Because the expected values for several cells was close to 0, the value 0.5 was added to all cells before analysis (EVERITT and DUNN 1992). Calculations were carried out with the program package SYSTAT (WILKINSON 1987).

RESULTS

Contamination: The number of identified contaminants varied from zero to three in the 12 families that were screened for this purpose, giving a total of 20 contaminants among 418 checked offspring. This represents the minimum number of contaminants. An estimate of the overall rate of contamination was obtained by regressing the frequency of identified contaminants in each family against the prior probability that a contaminant will be detected (see MATERIALS AND METHODS) and forcing the line of regression through the origin. The estimate was 0.06 ± 0.003 (mean ± SE), suggesting that the average number of contaminants per family (mean number of offspring scored per family $n = 36$) was 2.2. All identified contaminants were removed from the data set.

Sex-ratio: Family F20 × M70 was lost accidentally. A total of 854 progeny were examined from the other 24 families (Table 2). Of these, 54 (6.5%) could not be sexed and two (0.2%) were found to be hermaphrodites. Exempting these (as well as the 20 contaminants), the overall number of males and females was 367 and 411. This ratio was not different from 1:1 (chi-square 1.69 on 1 d.f., $P > 0.1$), yet the sex ratio varied widely among families, with one family containing 100% females and another containing 97% males. Visual inspection of Table 2 shows that families that shared the same dam had similar sex ratios, but no such pattern is found when families are grouped according to sire. The results from the discrete log-linear three factor analysis are shown in Table 3. The three way interaction and the sex X sire interaction were not significant, but the sex X dam interaction was highly significant.

The next most important question is how many different sex-ratio levels can be recognized among the five dams. Intradam heterogeneity was significant only for one female (F19), but interdam differences were significant in all pair-wise comparisons except one (F19 and F20) (Table 3). Thus we can recognize four levels of sex-ratio bias among five dams: strong female bias (F31), female bias (F53), no bias (F66), and male bias (F19 and F20). The possibility that F31 produced no sons at all is very unlikely. All five sons found among the 148 offspring of this female were allozymically compatible with parental genotypes and two of them had a *Lap-2* allele that occurred in their mother but was not found in any of the other nine parents.

Doubly uniparental inheritance of mtDNA: As expected (SKIBINSKI *et al* 1994; STEWART *et al.* 1995), the M was the most common mitochondrial genome in the sperm of all male parents. However, presence of some uncut product after digestion with *Bam*HI suggested the presence of a small amount of F genomes in the sperm of males M28, M46, M54 and M70 (Figures 1 and 3). This might, however, have resulted from incomplete digestion of the M molecule. To check this possibility, we digested the same PCR product with *Rsa*I, which was previously found to produce also different RFLP patterns for the F and M genomes. This assay produced evidence for the presence of the F genome only in males M46 and M54. Traces of F type in sperm have been also observed in other Mytilus species (SKIBINSKI *et al.* 1994). It is not certain, however, whether this represents a real "leakage" of F mtDNA into the sperm or it is an artifact owing to a few somatic cells liberated with the sperm during spawning.

The great majority of female progeny (320 out of 322) inherited only the mtDNA of the mother and the majority of male progeny (189 out of 239) inherited the mtDNA of the mother and the M type of the father (Table 2). Our results are, therefore, entirely consistent with the results from a previous analysis of mtDNA inheritance in Mytilus (ZOUROS *et al.* 1994) and provide the second direct confirmation of DUI in this genus.

Occasional and apparently parent-independent breakdowns of DUI: Two female progeny (one from cross F20 × M27 and one from cross F66 × M46) contained the M type in low amounts (Figure 1). They were checked and found to be allozymically compatible with the par-

TABLE 2

Sex distribution and transmission of type M mitochondrial DNA in pair matings of *M. galloprovincialis*

Cross (female × male)	Sampled	Not sexed	Females	Males[a]	Presence of M type mtDNA				
						Females		Males	
					Scored	+	−	+	−
F19 × M27	36	0	6	30 (0.83)	36	0	6	30	0
F19 × M28	29	0	1	28 (0.97)	29	0	1	28	0
F19 × M46	31	0	10	21 (0.68)	31	0	10	21	0
F19 × M54	31	0	6	25 (0.81)	29	0	6	23	0
F19 × M70	29	0	9	20 (0.69)	29	0	9	0	20
F20 × M27	40	1	6	33 (0.85)	10	1	4	5	0
F20 × M28	40	2	8	30 (0.79)	10	0	5	5	0
F20 × M46	37[b]	1	11	24 (0.69)	11[c]	0	5	5	0
F20 × M54	38	1	14	23 (0.62)	37	0	14	23	0
F31 × M27	33	4	28	1 (0.03)	30	0	29	1	0
F31 × M28	35	3	32	0 (0.00)	32	0	32	0	0
F31 × M46	35	6	28	1 (0.03)	29	0	28	0	1
F31 × M54	29	0	27	2 (0.07)	29	0	27	0	2
F31 × M70	32	4	27	1 (0.04)	28	0	27	0	1
F53 × M27	38	3	26	9 (0.26)	10	0	5	5	0
F53 × M28	40	2	28	10 (0.26)	9	0	6	3	0
F53 × M46	30	0	20	10 (0.33)	14	0	5	9	0
F53 × M54	38[b]	0	34	3 (0.08)	38[c]	0	34	2	1
F53 × M70	33	3	22	8 (0.27)	30	0	22	0	8
F66 × M27	36	5	12	19 (0.61)	10	0	5	5	0
F66 × M28	42	6	14	22 (0.61)	10	0	5	5	0
F66 × M46	31	6	9	16 (0.64)	10	1	4	4	1
F66 × M54	35	3	17	15 (0.47)	32	0	17	14	1
F66 × M70	36	4	16	16 (0.50)	32	0	16	1	15
Total	834	54	411	367	565	2	322	189	50

+/− indicate presence or absence, respectively, of M type mtDNA.
[a] Values in parentheses are percentages.
[b] One individual was hermaphrodite.
[c] The hermaphrodite was (+) in the male gonad and (−) in the female gonad.

TABLE 3

Analysis of sex-ratio heterogeneity among crosses of *M. galloprovincialis*

	G statistic	d.f.	*P*
A. Three-factor analysis (dam, sire, sex)			
Three-way interaction	17.91	16	0.329
Dam effect	304.1	20	<0.001
Sire effect	26.5	20	0.150
B. Heterogeneity within dams			
F19 (0.79 ± 0.03)	10.7	4	0.030
F20 (0.74 ± 0.04)	5.8	3	0.120
F66 (0.56 ± 0.04)	2.8	4	0.587
F53 (0.23 ± 0.03)	7.6	4	0.109
F31 (0.03 ± 0.01)	1.7	4	0.784
C. Heterogeneity between dams			
F19 *vs.* F20	1.4	1	0.242
All other comparisons	10.2–211.4	1	<0.001

Parentheses in B give the female-specific rate of sons with its standard error.

ents. A heteroplasmic female was also seen in a cross between *M. edulis* and *M. trossulus* (ZOUROS *et al.* 1994) and was attributed to disruptions of DUI in hybrids. The two M-positive females observed in this study were not hybrids. In all three M-positive females that we have observed, the amount of the M genome was much smaller than that of F. Thus, we cannot exclude the possibility that traces of M mtDNA occur in all females and that the three females that tested positive represent simply cases in which the amount of the M molecule is above the minimum required for detection by our assay. This explanation is consistent with reported low frequencies of heteroplasmic females in natural populations (FISHER and SKIBINSKI 1990; STEWART *et al.* 1995; WENNE and SKIBINSKI 1995; QUESADA *et al.* 1996). Recently, RAWSON, SECOR and HILBISH (1996) reported a high frequency of M-positive females among hybrids of *M. trossulus* and *M. galloprovincialis*. It appears that such females may result from both conspecific and heterospecific mating but are more common among the latter.

The great majority of male progeny without an M

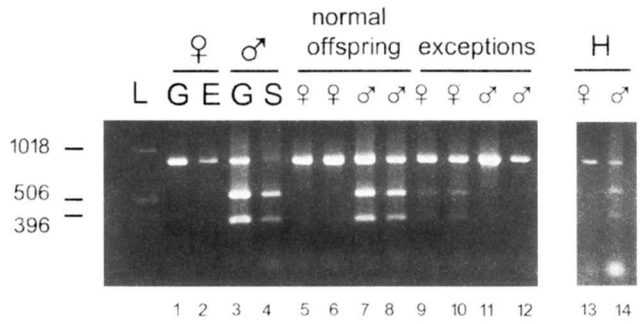

FIGURE 1.—Examples of DUI and of exceptions to DUI in pair matings of mussels. (Lanes 1–8) Typical DUI patterns after digestion with *Bam*HI of the PCR product amplified from DNA extracted from female or male gonad (G), eggs (E) or sperm (S), and female or male offspring. The F genome is represented by the upper (uncut) band, the M genome is represented by the two lower bands (Table 1). The actual cross is F19 × M54. (Lanes 9 and 10) Patterns from the gonads of the two exceptional females encountered in the study, showing presence of both the M and F genomes. (Lanes 11 and 12) Patterns from the gonads of two exceptional males (from the cross F31 × M54), showing absence of the M genome. (Lanes 13 and 14) Patterns from the female and male gonad of one of the two hermaphrodites, showing the "female" and "male" pattern, respectively. L: 1-kb ladder (GIBCO-BRL).

mtDNA molecule (44 out of 50) were fathered by one individual (M70) and will be examined below. The remaining six were fathered by M46 and M54. These happened to be the same male parents for which the evidence for presence of the F genome in the sperm was more definitive (see above). It is possible that a small fraction of sperm cells from these parents carried no M mtDNA type and that the M-negative sons were produced by such sperm. Alternatively, it is possible that both the F and M type molecules entered the egg and that the latter were lost during development. The presence of M-negative sons does not necessarily differentiate M46 and M54 from the other males in the population, if presence of F mtDNA in the sperm is a "leaky" phenomenon with a low probability of occurrence. With regard to all sons produced by M46 and M54, the exceptional sons represent a small minority (2/39 for M46 and 4/62 for M54). No exceptional males were produced by the other two male parents (0/46 for M27 and 0/41 for M28, respectively). The heterogeneity of these four ratios is on the borderline of significance (chi-square 7.713, d.f. = 3, $P = 0.052$), thus the null hypothesis that there is no intrinsic difference between the two sires that produced a few M-negative sons and the two that produced no such sons cannot be rejected. At present, these four male parents should be viewed as representing a homogeneous and typical sample of the majority of males in the population, for which the stochastic probability of producing a son with no M type mtDNA is 0.025.

If M-negative males were produced by sperm which contained mainly or exclusively the maternal mtDNA

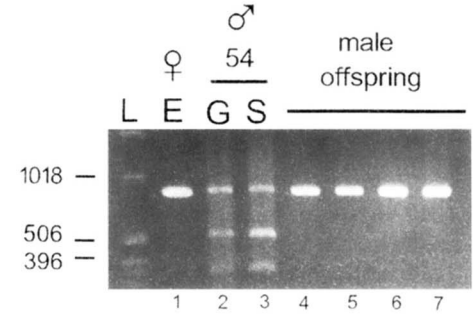

FIGURE 2.—*Eco*RI patterns among typical and exceptional sons of M54. E: eggs from F31 (type FA). G and S: gonad and sperm from M54 (type FB). The upper band is the M type (indistinguishable from the FA type) and the two lower bands correspond to the FB type (Table 1). Lanes 4–7 are gonads from four sons. No son contains the father's F mtDNA. Upon digestion with *Bam*HI, the sons in lanes 4 and 5 produced the pattern shown in lanes 7 and 8 of Figure 1, thus they were normal heteroplasmic (for FA and M). Upon digestion with *Bam*HI, the sons in lanes 6 and 7 produced the pattern shown in lanes 11 and 12 of Figure 1, thus they were homoplasmic (FA) having inherited neither of their father's mtDNA. L: 1-kb ladder.

of the father, it would be possible to detect this molecule in the adult sons provided that the father's maternal type was different from that of their mother. This was the case for crosses F31 × M54 and F19 × M54, where the female parents had type FA and the maternal mtDNA of M54 was of type FB. The gonads of two M-negative male offspring from cross F31 × M54, examined for this purpose, produced no evidence for presence of the father's maternal mtDNA (Figure 2).

Systematic and parent-dependent breakdowns of DUI: Only one of the 45 sons of male M70 contained the M mtDNA genome (cross F66 × M70). The allozyme genotypes of this offspring were compatible with paternal genotypes, thus the probability it was a contaminant is 0.06 × 0.29 (average rate of contamination and probability of detection of contamination in this particular cross, respectively), or 0.017. The 44 M-negative sons of M70 were produced in all four crosses in which M70 was involved (Table 4a and Figure 3), so

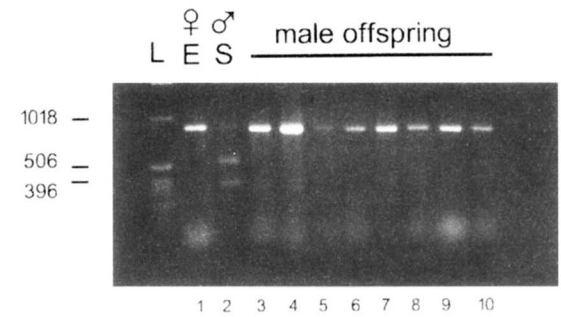

FIGURE 3.—*Bam*HI patterns of male offspring of M70. E: eggs from F19. S: sperm from M70. Lanes 3 and 4 gonads from male offspring from cross F19 × M70, lanes 5 and 6 from cross F53 × M70 and lanes 7 to 9 from cross F66 × M70. Lane 10 sperm from individual in lane 9. L: 1-kb ladder.

the production of M-negative sons is a property of the male parent itself and not of the females to which it was crossed. The possibility that the sperm of M70 did not carry an M genome was excluded by direct examination (Figure 3). Also, no obvious difference was detected in the mtDNA profile of sperm from M70 and the other male parents. Because only sperm of M70 was saved before this animal was lost, the type of its maternal mtDNA could not be determined. However, gonads from sons from crosses F19 × M70 and F31 × M70 showed the presence of only FA and from sons from crosses F53 × M70 and F66 × M70 showed the presence of only FB, which correspond to the mtDNA of the respective mothers. This means that the sons of M70 did not receive (or did not retain) the F mtDNA molecule of the father. In addition, because their gonads contained the F genome of their mother, the possibility exists that if they produce sons, these sons would inherit F molecules from both parents.

M-negative sons are more common among female-biased mothers: Table 4B gives the distribution among the five mothers of M-negative and M-positive sons produced by males other than M70 (sires with DUI). Three of the six M-negative sons were produced by the highly female-biased dam F31, which, in total, produced only four sons with these sires. No M-negative son was produced by the male-biased dams F19 and F20, which collectively account for 140 of the 194 sons sired by these males. The other two dams produced three M-negative sons among 50. These results are strongly heterogeneous, whether considered collectively or after exempting either the female- or male-biased females (Table 4), and suggest that an M-negative son sired by an DUI male is more likely to appear in female-biased broods than in male-biased broods.

Hermaphrodites: Both hermaphrodite offspring had mature gametes. In both cases, it was possible to recognize a female side and a male side of the gonad by visual inspection and also examination for presence of eggs or sperm under the microscope. When the two kinds of gonad were assayed for the kind of mtDNA they contained, the female gonad was found to contain only the mother's F type and the male gonad to contain both the mother's F type and the father's M type (Figure 1). Thus, the hermaphrodites were mosaics both with respect to sex and to mtDNA content.

TABLE 4

The distribution of M-negative and M-positive sons among dams sired by the SMI male (M70) or by the DUI males (M27, M28, M46 and M54)

	A. M70		B. M27 + M28 + M46 + M54		
Female	M^-	M^+	M^-	M^+	Total
F31 (0.03)	1	0	3	1	4
F53 (0.23)	8	0	1	19	20
F66 (0.56)	15	1	2	28	30
F19 + F20 (0.77)	20	0	0	140	140
Total	44	1	6	188	194

Parentheses give the dam's percentage of sons. Dams F19 and F20 were pooled because their sex ratio was not different (Table 3). Results of exact tests of heterogeneity (RAYMOND and ROUSSET 1995) for part B: All dams: $P < 0.001$; excluding F19 + F20: $P = 0.003$; excluding F31: $P = 0.019$.

DISCUSSION

Female-dependent sex ratio: Sex determination in Mytilus remains unknown, except that most individuals are gonochoristic with hermaphrodites occurring rarely in nature (SASTRY 1979). No sex-specific differences in karyotype have been identified (reviewed in GOSLING 1992). The crosses described here represent the second set of Mytilus pair-matings in which sex and mtDNA inheritance was studied jointly. The first set (ZOUROS *et al.* 1992, 1994) was designed to study mainly mtDNA transmission and involved a smaller number of crosses with only a few parents used in multiple matings. These crosses also involved two taxa, *M. edulis* and *M. trossulus*, which created the complication that exceptions to DUI could be interpreted as hybridization anomalies. The present study was designed to address these limitations. Based on the observation that two crosses that shared the same male parent had different sex ratios, but two crosses that shared the same female parent had similar sex ratios, ZOUROS *et al.* (1994) suggested that the sex ratio in pair matings of Mytilus might be controlled by the mother. The present study provides clear evidence for this hypothesis. The nuclear genome of the sperm has no bearing on the sex ratio of the offspring, which is predominantly but not exclusively determined by the mother. The possibility that external to the mother factors may play some minor role is suggested by two observations. First, among the five females that we have employed, the degree of sex bias varied significantly in four (which suggests that sex bias may not have a simple genetic basis) and, second, for one dam the intradam sex-ratio variance was significantly larger than 0 (even though much smaller than the among-dam variance).

Of special interest is the existence of females that are nearly sonless. In the previous study (ZOUROS *et al.* 1994), one of the six females produced no son among the 20 offspring examined. In this study, female F31 produced four sons (fathered by three different males) out of 147 progeny scored. The sex ratio between these two females is not significantly different ($P = 0.6$ by Fisher's exact test), which gives an overall rate of 2% for sons produced by mothers with the most extreme sex ratio. The other extreme of sex bias is represented by F19, which produced daughters at a rate of 20%.

Several processes (or combinations of them) may bring about sex-ratio bias. The distinction between primary and secondary sex ratio (the first appearing at the moment

of zygote formation, the second afterward) is not possible in mussels where sexing requires presence of a mature gonad. A primary bias may, in principle, be under the control of the nuclear or the mitochondrial genotype of the mother (or an interaction of the two). A secondary bias can be caused by sex-dependent mortality or by sex reversal. These possibilities are discussed below in conjunction with the control of mtDNA transmission.

Male-dependent transmission of the M type mtDNA genome: Our results demonstrate that with regard to transmission of paternal mtDNA, mussel males belong to two distinct categories: those that do not transmit it and those that do. For convenience, we will call the former SMI males (males that follow the standard maternal inheritance) and the latter DUI males (males that follow doubly uniparental inheritance). At present, DUI males were found in the genera Mytilus and Geukensia, both of the family of Mytilidae, and in the genera Pyganodon and Fusconaia of the distantly related family Unionidae (Liu *et al.* 1996; Hoeh *et al.* 1996). One SMI male was observed in our first study (Zouros *et al.* 1994), but because it was crossed only to one female, it could not be deduced whether the absence of the M genome among its sons was an attribute of the male or the female parent. Also, because this was an *M. edulis* × *M. trossulus* cross, it was not possible to exclude the possibility that the anomalous behavior of the family was due to hybridization. The discovery of a male (M70) in this study that failed to produce sons carrying an M genome when crossed to four different conspecific females provides clear evidence that the phenomenon is father-specific and not related to hybridization. In combination, we have observed two such males among 12, thus the frequency of SMI males in natural populations of mussels can be fairly high. It is important to know if these males always fail to pass their M mtDNA to offspring. No son with the M molecule was detected among 13 sons produced by the SMI male of our first study, and one such son was produced among the 45 produced by the SMI male of this study. Because there is a small probability that this exceptional son is a contaminant, the question cannot be answered in a definitive way. The question can, however, be answered for DUI males. In these males, transmission of the M genome to sons is not strict, failing with a probability of 0.03. This is a higher rate of breakdown than that seen in SMI mussels and certainly much higher than the rate of failure of SMI in other animals, where leaky paternal inheritance [so far reported in Drosophila (Kondo *et al.* 1990), mice (Gyllensten *et al.* 1991) and anchovy (Magoulas and Zouros 1993)], is of the order of 10^{-4}.

A model to explain the link between sex and mtDNA inheritance in mussels: It is obvious that sex determination and DUI are related in a complex way. The observations we have at present allow only a preliminary discussion of the basic features of the underlying mechanism.

In principle, the mother-controlled sex ratio can be viewed in two different ways. One is that the sex of any particular egg is determined before fertilization, so that mothers differ with respect to the percentage of female and male eggs they produce. The other is that the sex of an egg is not determined upon spawning, but only the probability it would become of one or the other sex. In the latter case, mothers differ with respect to this probability. In either case, the question, how is it actually decided that a zygote will develop into a male or a female?, remains. We emphasize that maleness or femaleness is determined by the presence of the corresponding gonad, so that a more appropriate way to ask the question is, how is it decided whether a gonad will produce sperm or eggs? The observation that the female gonad of hermaphrodites has only the F genome but the male gonad has both the M and the F genomes may suggest a causal relationship between presence/absence of the M mtDNA type and maleness/femaleness of the gonad. This simple hypothesis is, however, incompatible with the existence of male gonads in M-negative sons. The sperm of the fathers that produced these sons contained the M mtDNA genome. A hypothesis that would explain these observations is that presence of a sperm-derived mitochondrial factor in the early germ cells is necessary to commit these cells to producing a male gonad, but continuous presence of this factor is not necessary for the maleness of the gonad. The hypothesized factor can be the M mtDNA genome itself. It is, however, equally possible, given the findings of Kaneda *et al.* (1995) in mice, that the hypothesized mechanism involves mitochondrial signals coded by the nuclear genotype of the father. Given that the sperm carries perhaps <10 mitochondria (Longo and Dornfeld 1967), when the egg carries thousands, the probability that sperm-derived mitochondria will enter the first germ cells accidentally is negligible. Either there should be a mechanism that directs these mitochondria into the germ cells or these mitochondria have a strong and immediate replicative advantage over the maternal mitochondria. Results on the mtDNA content of early larvae (Sutherland 1996) argue in favor of the second possibility. The realization of this advantage, and therefore the probability of the sperm's mitochondria to enter the germ line, must depend on the mother's genotype: it is very small in eggs produced by female-bias mothers, high in male-bias mothers and intermediate in mothers with no sex-bias.

The model that we put forward as a working hypothesis for mitochondrial and sex inheritance in mussels (Figure 4) assumes that presence in the early germ cells of a factor associated with sperm mitochondria is necessary for the development of a male gonad and that replication of M mtDNA in the embryo is controlled by a paternally coded mitochondrial factor that provides a replicative advantage and a maternally coded egg factor that suppresses this advantage. We note that the pater-

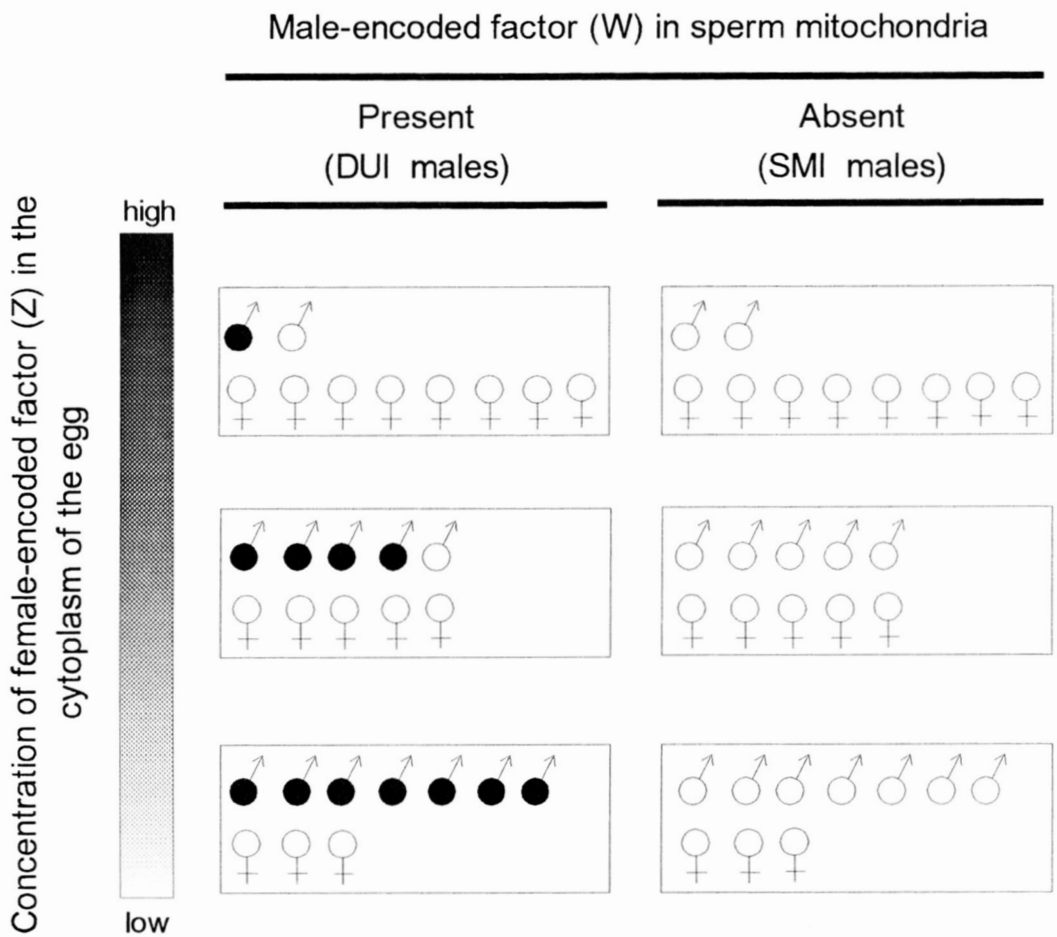

FIGURE 4.—Schematic presentation of a model for sex-determination and mtDNA inheritance in Mytilus. The presence in the primordial germ cells of a factor X, which is transmitted into the egg via the sperm's mitochondria, is necessary to masculinize the germ line. A male-expressed genetic polymorphism causes some males to supply and others not to supply the sperm mitochondria with a factor W, which provides these mitochondria with a replicative advantage. Males of the first type (which represent the majority of males) produce sons with F and M mtDNA (males with DUI). Males of the second type produce sons only with the F mtDNA (males with SMI) because the sperm's mtDNA is eventually lost in the developing embryo. A female-expressed genetic polymorphism is responsible for the amount of a factor Z with which females supply the egg. This factor inhibits the replicative advantage of sperm mitochondria. Females that supply their eggs with a high dose of Z are almost sonless. If some sperm mitochondria (or their descendants) escape the action of this factor and enter the primordial germ cells (thus causing them to produce a male gonad), they will most likely succumb to the abundance of factor Z at a later time. The result will be that these males will become M-negative, in agreement with the observation that the very few sons produced by highly-female biased mothers are mostly M-negative. Females that supply their eggs with a low dose of Z (or not at all) produce a very high percentage of sons and almost never produce M-negative sons when mated to a DUI male. Each box represents a pair mating. ●, presence of M type of mtDNA; ○, absence of M type of mtDNA. The varying number of the minority sex in the two sex-ratio biased females is to indicate that female bias is more extreme than male bias.

nal mitochondrial factor could be coded either by the paternal mtDNA or by paternally expressed nuclear genes. Under the model, hermaphrodites result from rare events when, during the critical early rounds of germ cell division, a few germ cells fail to incorporate or retain paternally derived mitochondria thus producing a female gonad. SMI males are assumed to have a genotype that fails to provide the sperm mitochondria with a sustainable replicative advantage. These males would be able to pass their paternal mtDNA into eggs, produce sons in the ratio expected from the genotype of the mother that provided the eggs, but upon development, these sons will contain no M mtDNA. The high frequency of M-negative sons among strongly daughter-biased mothers is also explainable. If there is a race between sperm-derived mtDNA to enter the germ line by realizing an early replicative advantage and egg-derived factors to prevent this from happening, in cases in which the race is in favor of the egg factors, the outcome would be that the sperm mtDNA would fail to enter the germ line in most of the eggs (thus the bias in favor of daughters), and in the few eggs in which they would succeed, they would be vulnerable to subsequent elimination (thus the presence of M-negative sons). An early replicative advantage of the M genome in male-to-be embryos would explain why in most males this genome is also present in somatic tissues. The small and varying amount of the M genome in females can

be understood as the result of stochastic survival of M molecules that failed to enter the germ line. The model predicts that M-positive females and males with high somatic M content would be more common among male-biased broods. There are no sufficient data to test this prediction. Another prediction is that the majority of hermaphrodites should contain both types of mtDNA, but ~20% (equal in frequency to the M-negative males) should contain only the F type. RAWSON and HILBISH (1995) observed two hermaphrodites of which one was homoplasmic for F and the other was heteroplasmic. In combination with our two heteroplasmic hermaphrodites, the overall frequency of M-negative ones is not different from expected. The strongest prediction of the model is that eggs from male-biased mothers should develop into females if fertilized with mitochondria-deprived sperm. This experiment has not been attempted yet.

It is premature to speculate on the genetic and molecular aspects of the model. Its main merit is that it is consistent with all current observations about DUI and, at the same time, it is one of the least complex models that could account for these observations. Another positive element of the model is that it has strong parallels with the findings of KANEDA *et al.* (1995) on mtDNA inheritance in mice. These authors have demonstrated the existence of a mechanism apparently involving two nuclearly encoded factors, each expressed in one of the two sexes. The male-expressed factor resides in the outer surface of the mitochondria of the sperm and the female-expressed factor in the egg's cytoplasm. In homospecific crosses, elimination of sperm mitochondria results from recognition of the sperm factor by the egg factor. In heterospecific crosses, the recognition is not perfect and elimination of paternal mtDNA is less efficient. In experiments involving males whose nuclear and mitochondrial DNA came from different species, the recognition mechanism depended on the male's nuclear rather than mtDNA genome.

Reversal of transmission routes of mtDNA genomes: An important question about the "leaky" transmission of paternal mtDNA in females is whether it could result in the production of eggs with M mtDNA and whether these maternally transmitted M mtDNA molecules would behave henceforth as F molecules. If this happened, egg-derived M molecules would become functionally indistinguishable from F molecules (even though quite different at the DNA sequence level), a process that we may call "feminization" of the M molecule. The same question can be asked for F molecules in M-negative males. As noted, SMI males produce only M-negative sons and DUI males produce such sons with a frequency of 0.025. In our two studies, we encountered two male parents of the first kind among 12 tested. Thus the best current estimate of the population frequency of M-negative males is (1/6) + 0.025 (5/6), or 0.187. As noted, gonads from M-negative sons from both SMI and DUI fathers contain the F genome of their mother. Thus, it is very likely that M-negative males will pass their maternal mtDNA to their offspring. If sperm-derived F molecules behave the same way as sperm-derived M molecules, it would lead to the "masculinization" of the F molecule.

M-positive females are rare, their M content is much lower than that of F, and there is no report, as yet, of M-positive eggs. Thus, feminization remains only a remote possibility. On the opposite, M-negative males may appear in the population with a frequency as high as 20% and their mtDNA content, including that of the gonad, is 100% of type F. Hence, masculinization is a distinct possibility. Two additional types of observation strongly argue in favor of the occurrence of masculinization. HOEH *et al.* (1996) have studied the distribution of DUI in a collection of species from the families Mytilidae and Unionidae. The molecular phylogeny they obtained could be explained by assuming three independent origins of DUI, one for the Unionidae and two for the Mytilidae (one for the genus Mytilus and one for the distantly related genus Geukensia). If accepted, this interpretation would suggest that DUI is a phenomenon with a fairly high probability of spontaneous occurrence, which might be difficult to reconcile with the underlying complexity of the nuclear-mtDNA interactions and with its restricted and spotty distribution among bivalves. An alternative explanation suggested by HOEH *et al.* (1996) is reversal of transmission routes. If masculinization occurs with appreciable frequency, then it is possible that, stochastically or otherwise, a masculinized F molecule will be fixed in the population replacing all the "old" M lineages. STEWART *et al.* (1996) have argued that the M lineage evolves much faster than the F lineage because it is under relaxed selective constraints. This implies that in the time lapsed between the masculinization event and the time of fixation, the masculinized lineage will evolve faster than the nonmasculinized lineage from which it sprung, but it would still be much closer to it than to the M lineage that it has replaced. This would result in resetting the molecular distance between the two gender-associated mtDNA lineages that coexist in the population. In a collection of taxa, the phylogenetic footprint of this resetting would be identical to that of independent origins. The second independent evidence for masculinization is the existence in natural populations of sequences isolated from male gonads that in terms of DNA sequence affiliate with the F rather than the M lineage (HOEH *et al.* 1997). We suggest that the results of WENNE and SKIBINSKI (1995) in *M. trossulus* from the Baltic Sea can also be explained in terms of masculinization events.

The high frequency of M-negative males seen in our crosses does not match with the low frequency of such males in natural populations. Indeed, we have seen no such males in our previous studies of natural populations

of *M. edulis* and *M. trossulus* (STEWART *et al.* 1995; SAAVEDRA *et al.* 1996). However, RAWSON, SECOR and HILBISH (1996) have reported a high frequency of such males among hybrids between *M. trossulus* and *M. galloprovincialis*, as well as *M. edulis* and *M. galloprovincialis*. In *M. trossulus*, we have reported a high frequency of males that were apparently homoplasmic for a restriction pattern we have called type O (SAAVEDRA *et al.* 1996), but upon closer examination, these males were found to be heteroplasmic for two closely related types resembling the common F type of *M. trossulus* (SAAVEDRA *et al.* 1996; HOEH *et al.* 1997). We have no explanation for the discrepancy between results from crosses and population surveys regarding the frequency of M-negative males in samples from pure species. High early mortality in the wild is an obvious possibility. We also emphasize that our empirical demonstration of masculinization is not yet complete. A second round of crosses is required to show that M-negative males, which carry only the maternal F molecule in their gonad, do actually produce sons carrying this molecule.

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LITERATURE CITED

ALLEN, S. K., and P. M. GAFFNEY, 1993 Genetic confirmation of hybridization between *Crassostrea gigas* (Thunberg) and *Crassostrea rivularis* (Gould). Aquaculture **113:** 291–300.

BIRKY, C. W., JR., 1995 Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. Proc. Natl. Acad. Sci. USA **92:** 11331–11338.

BISHOP, Y. M. M., S. E. FIENBERG and P. W. HOLLAND, 1975 *Discrete Maltivariate Analysis: Theory and Practice.* The MIT Press, Cambridge, MA.

EDWARDS, C. A., and D. O. F. SKIBINSKI 1987 Genetic variation of mitochondrial DNA in mussel (Mytilus) populations in South West England and South Wales. Mar. Biol. **94:** 547–556.

EVERITT, B. S., and G. DUNN, 1992 *Applied Multivariate Data Analyis.* Oxford Univ. Press, New York.

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

FOLTZ, D. W., 1986 Segregation and linkage studies of allozyme loci in pair crosses of the oyster *Crassostrea virginica.* Biochem. Genet. **24:** 941–956.

GOSLING, E. M., 1992 Genetics of Mytilus, pp 309–382 in *The Mussel Mytilus: Ecology, Physiology, Genetics and Aquaculture,* edited by E. M. GOSLING. Elsevier, Amsterdam.

GYLLENSTEN, U. B., D. WHARTON, A. JOSEFSSON and A. C. WILSON, 1991 Paternal inheritance of mitochondrial DNA in mice. Nature **325:** 255–257.

HOEH, W. R., K. H. BLAKLEY and W. M. BROWN, 1991 Heteroplasmy suggests limited biparental inheritance of *Mytilus* mitochondrial DNA. Science **251:** 1488–1490.

HOEH, W. R., D. T. STEWART, B. W. SUTHERLAND and E. ZOUROS, 1996 Multiple origins of gender-associated mitochondrial DNA lineages in bivalves (Mollusca: Bivalvia). Evolution **50:** 2276–2286.

HOEH, W. R., D. T. STEWART, C. SAAVEDRA, B. W. SUTHERLAND and E. ZOUROS, 1997 Phylogenetic evidence for role-reversals of gender-associated mitochondrial DNA in Mytilus (Bivalvia:Mytilidae). Mol. Biol. Evol. (in press).

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.

KANEDA, H., J.-I. HAYASHI, S. TAKAHAMA, C. TAYA, K. F. LINDAHL *et al.,* 1995 Elimination of paternal mitochondrial DNA in intraspesific crosses during early mouse embryogenesis. Proc. Natl. Acad. Sci. USA **92:** 4542–4546.

KONDO, R., Y. SATTA, E. T. MATSURA, H. ISHIWA, N. TAKAHATA *et al.,* 1992 Incomplete maternal transmission of mitochondrial DNA in Drosophila. Genetics **126:** 658–663.

LIU, H.-P., J. B. MITTON and S.-K. WU, 1996 Paternal mitochondrial DNA differentiation far exceeds maternal mitochondrial DNA and allozyme differentiation in the freshwater mussel, *Anodonta grandis grandis.* Evolution **50:** 952–957.

LONGO, F. J., and E. J. DORNFELD, 1967 The fine structure of spermatid differentiation in the mussel, *Mytilus edulis.* J. Ultrastruct. Res. **20:** 462–480.

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.

MALLET, A. L., E. ZOUROS, K. E. GARTNER-KEPKAY and K. R. FREEMAN, 1986 Genetics of growth in blue mussels: family and enzyme-heterozygosity effects. Mar. Biol. **92:** 475–482.

QUESADA, H., C. ZAPATA and G. ALVAREZ, 1995 A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. Mar. Ecol. Prog. Ser. **116:** 99–115.

QUESADA, H., D. A. G. SKIBINSKI and D. O. F. SKIBINSKI, 1996 Sex-biased heteroplasmy and mitochondrial DNA in the mussel *Mytilus galloprovincialis* Lmk. Curr. Genet. **29:** 423–426.

RAWSON, P. D., and T. J. HILBISH, 1995 Evolutionary relationships among the male and female mitochondrial DNA lineages in the *Mytilus edulis* species complex. Mol. Biol. Evol. **12:** 893–901.

RAWSON, P. D., C. L. SECOR and T. J. HILBISH, 1996 The effects of natural hybridization on the regulation of doubly uniparental mtDNA inheritance in blue mussels (*Mytilus* spp.). Genetics **144:** 241–248.

RAYMOND, M., and F. ROUSSET, 1995 An exact test for population differentiation. Evolution **49:** 1280–1282.

SAAVEDRA, C., D. T. STEWART, R. R. STANWOOD and E. ZOUROS, 1996 Species-specific segregation of gender-associated mitochondrial DNA types in an area where two mussel species (*Mytilus edulis* and *Mytilus trossulus*) hybridize. Genetics **143:** 1359–1367.

SASTRY, A. N., 1979 Pelecypoda (excluding Ostreidae), pp 113–292 in *Reproduction of Marine Invertebrates,* edited by A. C. GIESE and J. S. PEARSE. Academic Press, New York.

SKIBINSKI, D. O. F., C. GALLAGHER and C. M. BEYNON, 1994 Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis.* Genetics **138:** 801–809.

SOKAL, R. R and F. J. ROHLF, 1995 *Biometry.* Ed. 3. Freeman, New York.

STEWART, D. T., C. SAAVEDRA, R. R. STANWOOD, A. O. BALL and E. ZOUROS, 1995 Male and female mitochondrial lineages in the blue mussel (*Mytilus edulis*) species group. Mol. Biol. Evol. **12:** 735–747.

STEWART, D. T., E. R. KENCHINGTON, R. K. SINGH and E. ZOUROS, 1996 Degree of selective constraint as an explanation of the different rates of evolution of gender-specific mitochondrial DNA lineages in the mussel Mytilus. Genetics **143:** 1349–1357.

SUTHERLAND, B., 1996 The fate of paternal mitochondrial DNA during the development of *Mytilus edulis.* Masters Thesis, Dalhousie University, Halifax, NS, Canada.

WENNE, R, and D. O. F. SKIBINSKI, 1995 Mitochondrial DNA heteroplasmy in European populations of the mussel *Mytilus trossulus.* Mar. Biol. **122:** 619–625.

WILKINSON, L, 1987 *SYSTAT: The System for Statistics.* SYSTAT Inc., Evanston, IL.

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.

ZOUROS, E., A. O. BALL, C. SAAVEDRA and K. R. FREEMAN, 1994 An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus.* Proc. Natl. Acad. Sci. USA **91:** 7463–7467.

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