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Genetics of Mother-Dependent Sex Ratio in Blue Mussels (Mytilus spp.) and Implications for Doubly Uniparental Inheritance of Mitochondrial DNA

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Manuscript received October 29, 2001
Accepted for publication May 29, 2002

ABSTRACT

Previous studies have shown that in most pair matings of *Mytilus edulis*, *M. trossulus*, and *M. galloprovincialis* there is a large sex-ratio bias in favor of either males or females. The degree of bias is a characteristic property of the female parent, as matings of the same female with different males produce the same sex ratio, but matings of the same male with different females produce different sex ratios. All three species possess the unusual feature of doubly uniparental inheritance of mitochondrial DNA (mtDNA); *i.e.*, they contain two distinct types of mtDNA, one that is transmitted matrilinearly and one that is transmitted patrilinearly. This coupling of sex and mtDNA transmission raises the possibility that the mechanism of sex-ratio determination in mussels might be under the control of the mtDNA of the female parent. Here we present data from pedigreed crosses that confirm the previous observations that in mussel matings there is a strong sex-ratio bias and that the bias is under the control of the female parent. In addition, these data strongly suggest that this control is exercised by the mother's nuclear rather than mitochondrial genotype. Making use of these findings we develop a model of mother-dependent sex determination and use data from crosses involving wild females to test the model's predictions at the population level.

ALL three members of the *Mytilus edulis* species complex, *M. edulis, M. galloprovincialis*, and *M. trossulus*, are known to share two rather unusual features. First, they have a system of biparental mitochondrial DNA (mtDNA) transmission (Skibinski *et al.* 1994a,b; Zouros *et al.* 1994a,b) in contrast to the maternal mtDNA inheritance that is the rule among animals. Second, the sex ratio among progeny from pair matings can be very different from 1:1 (Zouros *et al.* 1994a; Saavedra *et al.* 1997). It is highly probable that the two phenomena [to which we refer as doubly uniparental inheritance (DUI) and sex-ratio bias (SRB), respectively] are causally linked, but firm evidence for this connection has yet to be established.

At present, DUI has been detected in species from three families of bivalves: the sea mussels Mytilidae (Skibinski *et al.* 1994a; Zouros *et al.* 1994a), the freshwater mussels Unionidae (Hoeh *et al.* 1996; Liu *et al.* 1996), and the clams Veneridae (Passamonti and Scali 2001). It involves the presence of two independently evolving mtDNA genomes, one that is transmitted through the female lineage and the other that is transmitted through the male lineage. DUI represents a radical departure from the uniparental inheritance that is the rule in organelle transmission, whether mitochondrial or plastid (Birky 1995). In contrast, SRB is found in a great variety of organisms and has been the subject of many empirical and theoretical studies and exhaustive reviews (*e.g.*, Maynard Smith 1978; Bell 1982; Karlin and Lessard 1986).

In mussels SRB has been reported first by Zouros *et al.* (1994a,b) in the context of studying the phenomenon of DUI. In a later study, Saavedra *et al.* (1997) produced pair matings in which female and male parents were multiply crossed. They showed that the bias could take extreme values, with the percentage of male progeny varying from 0 to 97%, and that the bias was about the same among matings sharing the same female parent, but very different among matings sharing the same male parent.

These findings have a clear bearing on sex determination in mussels. In no bivalve species (many of which are simultaneous or sequential hermaphrodites) is the mechanism of sex determination known, nor have sex chromosomes been identified. In the Pacific oyster (*Crassostrea gigas*) Guo *et al.* (1998) suggested a one-locus system with the heterogametic condition corresponding to obligatory males and the homogametic condition corresponding to males that may or may not revert to females at a later age. In the soft shell clam *Mya arenaria*, Allen *et al.* (1986) observed that practically all triploids were females and suggested that femaleness

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depends on an X/autosome balance, as in Drosophila. In the dwarf surfclam *Mulinia lateralis*, Guo and Allen (1994) observed that gynogenetic diploids were females but triploids were of both sexes, which is consistent with a X/Y mechanism. In the mussel *M. galloprovincialis* Kiyomoto *et al.* (1996) have observed that all triploids were males, whereas the sex ratio in diploids was 1:1, and concluded that this species may have a Z/W sex determination mechanism (*i.e.*, females are heterogametic). But female heterogamy cannot explain the wide range of female-dependent SRB observed in this species (Saavedra *et al.* 1997). For this one would need auxiliary and rather unlikely postulates, such as an abundance of strong sex-ratio drivers of two types, one favoring the Z and the other the W chromosome.

To explain the coupling between DUI and SRB in mussels Saavedra *et al.* (1997) proposed a model of sex determination according to which femaleness is the default state and maleness results from the presence of sperm mitochondria in the primordial germ cells. A revised version of the model is given by Zouros (2000). The model departs from the assumption that in mussels, as in all other animals, a mechanism prevents sperm mtDNA from establishing itself in the fertilized embryo. It is assumed that this mechanism entails the recognition of a male-specific factor W that resides in the outer surface of sperm mitochondria by a female-specific factor X that resides in the egg cytoplasm. Recent studies have produced evidence for the existence of such a recognition mechanism in a number of mammalian species (Kaneda *et al.* 1995; Shitara *et al.* 1998; Sutovsky *et al.* 1999, 2000). Saavedra *et al.* (1997) have further assumed that in mussels this system has been undermined by the appearance of a third female-specific factor, Z, which is present in the egg cytoplasm and acts as a suppressor of factor X. Factor Z is controlled by a locus with two alleles, the active allele *Z* that produces the factor and the inactive allele *z* that does not. The last and more demanding part of the model is that if sperm mitochondria find their way into the primordial germ cells of the embryo, they will cause the masculinization of the resulting gonad. This part of the hypothesis would predict the presence of three types of females in the population: those of genotype *zz* that produce eggs in which the sperm mitochondria cannot persist and will develop into females, those of genotype *ZZ* that produce eggs in which the sperm mitochondria persist and will develop into males, and those of genotype *Zz* that will produce daughters and sons in an intermediate ratio. The model requires that all eggs receive sperm mitochondria, regardless of the sex to which they will develop. Sutherland *et al.* (1998) have shown that this is indeed the case. Another requirement of the model is that SRB must be controlled by nuclear rather than mitochondrial genes of the female parent. Our study provides firm evidence for this requirement of the model. In addition, it provides a simple formulation of the hypothesis and uses this formulation to examine the model's conformity with empirical data.

MATERIALS AND METHODS

We used two types of animals of the species *M. edulis*: wild and pedigreed. Wild animals were randomly selected from a natural population in Nova Scotia, Canada, and maintained in the laboratory until spawned. They were coded by the year of collection (the last two digits, *e.g.*, 99 for 1999), the letter W for wild, the letter M or F for male or female, and an identification number. After spawning a wild animal was either discarded or maintained for further use in other crosses. Pedigreed animals were produced in the laboratory and subsequently used as parents. Female animals were coded alphabetically, while male animals were coded numerically, both being referenced back to their original wild parents in their coding.

The general methods of spawning, gamete collection, fertilization, and rearing of larvae and juveniles have been previously described (Zouros *et al.* 1992; Saavedra *et al.* 1997) and do not differ from common procedures employed with other bivalves. Animals were encouraged to spawn naturally at room temperature without temperature shock treatment. This was done to avoid immature gametes and possible influence of temperature on sex ratio. Matings were done by mixing eggs from one female with sperm from one male. Sperm from the same male could be used to fertilize eggs from several females and vice versa. Sperm densities of 10–15 sperm per egg were targeted and verified microscopically.

A procedure of maintaining crosses in individual buckets combined with prolonged soaking of screens and cleaning apparatus in a bleach solution was adopted to kill any larvae that may have been attached to containers or screens before reuse. Postlarvae were maintained in the buckets until 2.5-mm shell length at which time they were placed in individual silos in an upwelling unit. The upwelling unit optimized growth and facilitated feeding in this species. The animals in each silo were repeatedly counted as a precautionary measure; however, no evidence of movement was detected. All seawater used with the animals was sand filtered, followed by 2-μm and 10-μm bag filtration and UV sterilization. These measures were adopted to eliminate the possibility of cross-contamination of families, which arose in earlier studies (*cf.*, Zouros *et al.* 1992, 1994b).

Sexing of mature progeny was done directly by examining the gonads of adults for the presence of sperm or eggs. Animals could be sexed ~1 year from birth using our enhanced feeding protocols. Only crosses in which at least 10 progeny were scored were included in the data set. Four *M. edulis* × *M. edulis* crosses previously reported in the context of studying DUI (Zouros *et al.* 1994b) were also used, as were the data from the crosses of *M. galloprovincialis* that were reported in Saavedra *et al.* (1997).

The homogeneity of sex ratio among pair matings was tested by a simple chi-square test. The population genetics model for the distribution of sex ratio in natural populations was explicitly solved and tested numerically with the EXCEL software program. The estimation of the model's parameter from empirical data was done by the maximum-likelihood method.

RESULTS

Pedigreed crosses: Table 1 presents the results from 49 pair matings involving 10 female parents and 31 male parents. Six female parents were daughters from a pair

TABLE 1

Results from pair matings of daughters of a sonless and of a son-bearing female mussel

	Females										
	90WF4 × 90WM4 (0/20)						90WF5 × 90WM5 (16/25)				
Males	A	B	D	E	X	Z	F	H	I	J	Total
90WF5 × 90WM5 (16/25)											
1		0/23									0/23
2		0/37					7/10				7/47
3							20/24				20/24
4		0/41							24/72		24/113
10			0/52					24/50			24/102
11								35/50		22/29	57/79
12			0/11								0/11
13			0/10					44/61		44/51	88/122
104						33/48					33/48
105					0/50						0/50
106					0/51						0/51
107					0/57						0/57
108									11/17		11/17
90WF7 × 90WM7 (32/40)											
5		0/37					16/30		26/60		42/127
6		0/19					29/38		18/27		47/84
7		1/46					18/33				19/79
9		0/46					13/20		6/18		19/74
14								24/61			24/61
15								6/12		39/52	45/64
16								29/34		38/47	67/81
17										42/51	42/51
100						45/52					42/52
101						30/51					30/51
102					0/20	39/50					39/70
103						33/50					33/50
Z101A	10/11			51/55							61/66
Z103A				19/20							19/20
98WM8	29/37			53/69							82/106
98WM11				26/30							26/30
00WM22				23/30							23/30
00WM24				19/28							19/28
Total	39/48	1/249	0/73	191/232	0/178	180/251	103/155	162/268	103/155	185/230	996/1878

The grandparental cross is given above the parents, with the sex ratio in parentheses. The first number of the progeny from each pair mating gives the number of males and the second the total number of progeny scored. The last four male parents were collected in the wild.

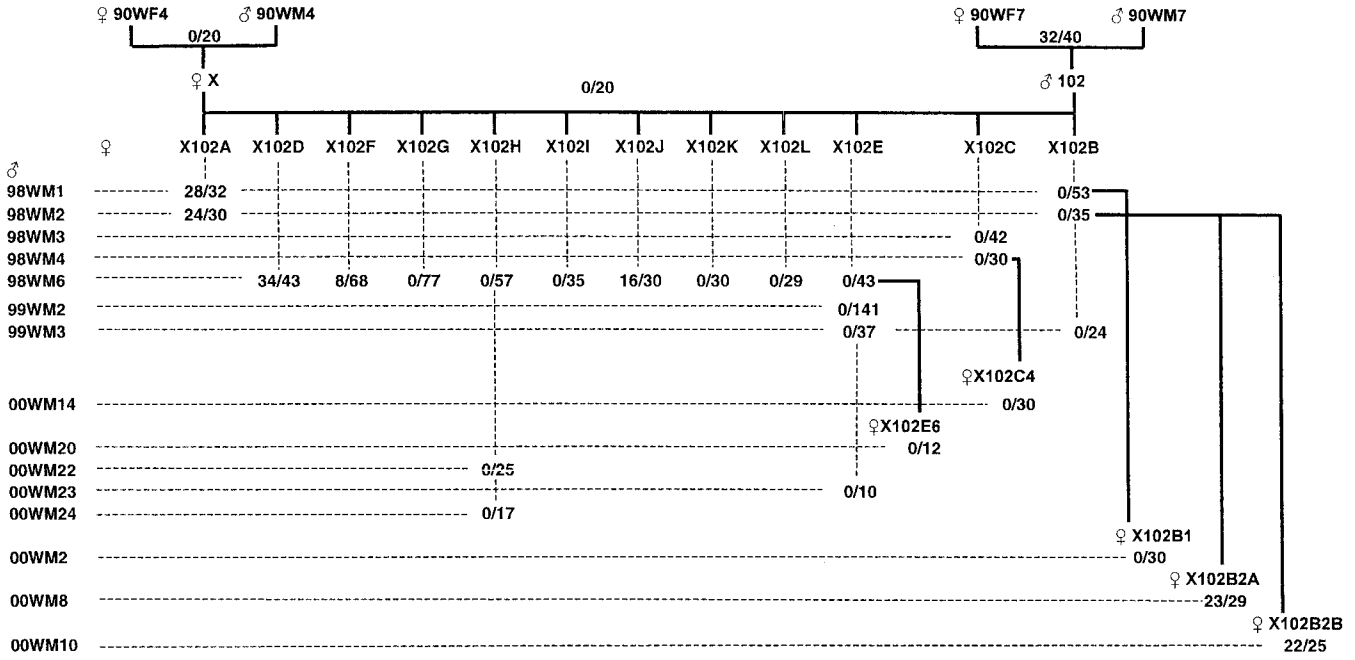

FIGURE 1.—Crosses of pedigreed females (and five of their daughters) to wild males. In each sibship, the first number is the number of males and the second is the total number of offspring sexed. Boldface lines indicate pair matings; dashed lines are to assist in identifying parents.

mating of a sonless female (90WF4) and 4 were daughters from a pair mating of a son-bearing female (90WF5). The male parents were sons from 2 pair matings or were drawn from a wild population. Note that six crosses (involving females F, H, I, J and males 2, 3, 4, 10, 11, 13) were brother-sister matings. The crosses of Table 1 confirm the findings of SAAVEDRA *et al.* (1997) for *M. galloprovincialis* that the sex ratio may vary from zero sons to >90% sons and that this ratio is a characteristic property of the female parent. We make the distinction between females that produce no sons or produce sons at a very low rate (<5%; we refer to these as "sonless" females) and females that produce males at a high frequency, normally >5% (we refer to these as "son-bearing" females). None of the 3 sonless females of Table 1 (we include female B in this class, even though it produced one son among 249 progeny scored) reverted to son bearing as a result of being crossed to a different male. The same is true for the 7 son-bearing females.

Conversely, there were nine cases of males producing both sonless broods and broods of both sexes. In all cases whether a male would produce a sonless or a mixed brood could be predicted from the brood its mate produced when crossed to another male. In four son-bearing females (E, Z, H, and I) the sex ratio from different crosses was statistically different, which suggests that other factors beyond the mother's genotype (*e.g.*, environmentally induced sex-specific mortality) may affect the male-to-female ratio in broods of mixed sex.

The novel observation from Table 1 is that 3 daughters (B, D, X) of the sonless female 90WF4 were themselves sonless and 3 (A, E, Z) were son bearing, while all daughters of the son-bearing female 90WF5 were also son bearing. To further study the phenomenon of reversion from sonless to son bearing, we produced two more generations of crosses using descendants of the original crosses shown in Table 1. Together with the two-generation crosses of Table 1, these data extend the observations from pedigreed crosses to four generations (Figure 1). To produce the third generation, 12 daughters from the sonless cross "female X to male 102" (Table 1) were crossed to males collected from the wild. Eight of these daughters were themselves sonless and 4 were son bearing. Four of the 8 sonless daughters were crossed to more than one wild male and were sonless in all cases.

The fourth generation involved five crosses of daughters from sonless mothers of the third generation (Figure 1). One daughter of female X102E and male 98WM6 was crossed to another wild male (00WM20) and produced a sonless brood. The same was observed with a daughter of female X102C and male 98WM4. The other three crosses involved daughters of the sonless female X102B. One of her daughters (female X102B1) produced a sonless brood, but the other two daughters produced broods of mixed sexes. Interestingly, the last two daughters shared the same father (98WM2), which was different from the one (98WM1) that sired her sonless sister. Over all four generations we observed three transitions from a sonless mother to son-bearing daughters: from 90WF4 to A, E, and Z; from X to X102A,

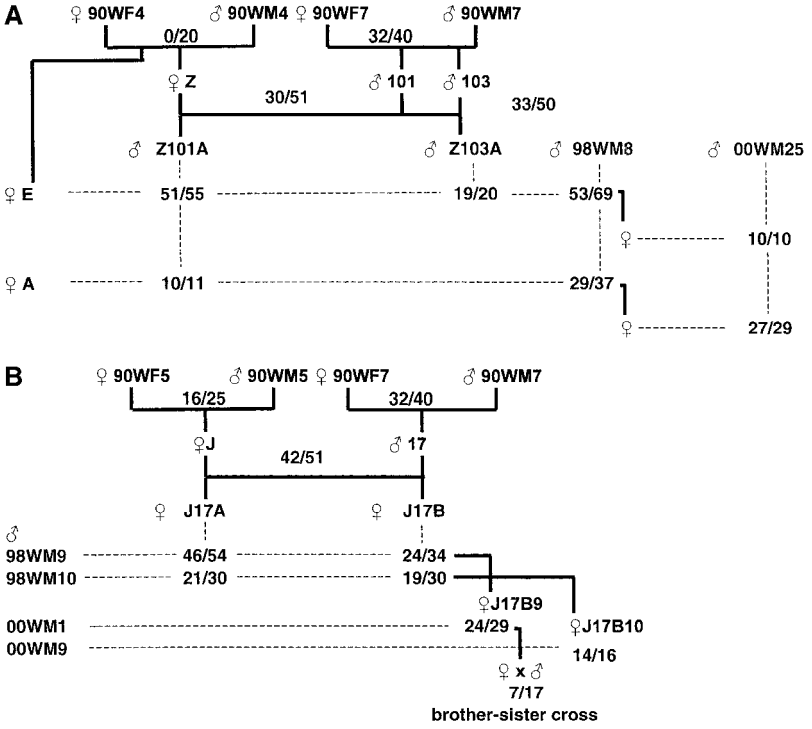

FIGURE 2.—Crosses of pedigreed females to pedigreed and wild males. (A) Females and males originating from son-bearing mothers. (B) Females originating from a sonless mother and males from a son-bearing mother. Boldface lines indicate pair matings; dashed lines are to assist in identifying parents.

X102D, X102F, and X102J; and from X102B to X102B2A and X102B2B.

Crosses using descendants of the original son-bearing females 90WF5 and 90WF7 are shown in Figure 2A. Two daughters of the son-bearing female J (Table 1), itself a daughter of a son-bearing female (90WF5), were son bearing when crossed to wild males. Two daughters from one of these two females (female J17B) were also son bearing. Finally a granddaughter of J17B was also son bearing when crossed to one of her brothers. Thus, the son-bearing trait was transmitted for five successive generations of females. When the son-bearing daughters E and A of the original sonless female 90WF4 (Table 1) were crossed to sons from their sister Z they were also son bearing (Figure 2B). Interestingly, a daughter of female E with the wild male 98WM8 was daughterless, and a daughter of female A with the same male was almost daughterless (it produced two daughters in a brood of 29). This is a strong indication that male 98WM8 tends to produce daughters that produced broods with a strong male bias.

The observation that the sonless/son-bearing trait can be transmitted maternally for several generations strongly implies a hereditary basis to female-controlled sex-ratio bias in mussels. At the same time the observation that sonless mothers may produce son-bearing daughters makes it very unlikely that the sex ratio is controlled by the female's mtDNA. In mussels, females receive mtDNA only from their mother, as in other animals. If the maternal determination of sex ratio was under the influence of the maternally transmitted mtDNA, then all sisters must have their mother's sex ratio. At the same time the fact that a cross of a sonless mother to a single male can produce both sonless and son-bearing daughters also excludes the possibility that the father's dual mtDNA genotype affects this character in some unknown way. Finally, the observation that two full sisters (females X102B2A and X102B2B; Figure 1) of a sonless mother were both son bearing, yet their maternal half-sister was sonless, suggests that the father may contribute to the sex-ratio bias of his daughters. The same conclusion follows from the observation that two daughters of the same male (98WM8), each from a different mate, were almost daughterless (Figure 2B). Taken together these observations make a strong case for a control of the sonless/son-bearing trait through the mother's nuclear genotype.

The model: We assume a nuclear locus Z with two alleles segregating in the population, the active allele *Z* and the inactive allele *z*. We further assume that *zz* females produce no sons at all (sonless females) and *ZZ* females produce only sons. In reality we have observed only two completely daughterless crosses (Figure 2B and Table 3), but we have observed several with only one or two daughters (frequency of $<5\%$). Complete lack of males is, on the other hand, common. *Zz* females are assumed to produce daughters with probability k and sons with probability $1 - k$. Thus, both *ZZ* and *Zz* females are son bearing. Starting with arbitrary genotype frequencies for the female and male part of the population at generation t one may write the recursion equations for these frequencies at generation $t + 1$ (Table 2). The system converges rapidly to a stable equilibrium, which is given by the solution in k shown in Table 2.

TABLE 2

The model

	Females		
Males	*ZZ, d*	*Zz, h*	*zz, r*
ZZ, D	Males	Females with probability *k*;	Females
Zz, H	Males	males with probability	Females
zz, R	Males	$1 - k$	Females

Recursion equations

$$D' = [Dd + Hd/2 + Dh(1 - k)/2 + Hh(1 - k)/4]/[d + h(1 - k)]$$
$$R' = [Rh(1 - k)/2 + Hh(1 - k)/4]/[d + h(1 - k)]$$
$$H' = 1 - D' - R'$$
$$d' = [Dhk/2 + Hh(1 - k)/4]/(r + hk)$$
$$r' = [Rr + Hr/2 + Rhk/2 + Hhk/4]/(r + hk)$$
$$h' = 1 - d' - r'$$

Equilibrium frequencies	$k = 0.3$
$\hat{h} = [1 - (1 - 2k(1 - k))]^{1/2}/[2k(1 - k)]$	0.568
$\hat{d} = [1 - 2\hat{h}(1 - k)]/2$	0.102
$\hat{r} = (1 - 2\hat{h}k)/2$	0.330
$\hat{H} = (1 - 4\hat{d}^2)/2$	0.479
$\hat{D} = (1 + 2\hat{d})^2/4$	0.362
$\hat{R} = (1 - 2\hat{d})^2/4$	0.158
$\hat{z}_{♀} = (1 + \hat{h} - 2\hat{h}k)/2$	0.614
$\hat{z}_{♂} = (1 - 2\hat{d})/2$	0.398

Females of genotype *ZZ* produce only sons, *Zz* females produce sons with probability $1 - k$, and *zz* females produce no sons. The recursion equations for male and female genotype frequencies are obtained from the 3×3 matrix of crosses. Numerical equilibrium values are given for $k = 0.3$.

From this solution it can be seen that at equilibrium the frequencies of females ($\hat{f} = \hat{r} + \hat{h}k$) and males ($\hat{m} = \hat{d} + \hat{h}(1 - k)$) in the population are equal at 0.5. In the female population, the heterozygote frequency takes its maximum value ($\hat{h} = 0.586$) at $k = 0.5$ and its minimum ($\hat{h} = 0.5$) when k approaches 0 or 1. Heterozygous females are always in excess from Hardy-Weinberg, with the maximum excess of 0.125 at $k = 0$ or $k = 1$ and the minimum of 0.086 at $k = 0.5$. The male frequencies are at Hardy-Weinberg proportions. The frequency of *z* in the female population ranges from 0.75 (at $k = 0$) to 0.25 (at $k = 1$) and always exceeds that in the male population by an amount of $(1 - \hat{h})/2$. In the population as a whole the frequency of *z* is larger than that of *Z* for $0 < k < 0.311$ and smaller than that for $0.311 < k < 1$.

Fitting the model to sex ratios produced by wild-caught animals: Table 3 summarizes all currently available data of sex ratio in pair matings of female mussels taken from the wild. Families are arranged in descending percentage of male progeny. Females 7, 12, 18, and 28 were taken from Zouros *et al.* (1994b) and females 8, 15, 19, 22, and 26 from Saavedra *et al.* (1997). The remaining 27 females were tested for the needs of this study. All crosses were *M. edulis* × *M. edulis*, except the crosses from Saavedra *et al.* (1997), which were *M. galloprovincialis* × *M. galloprovincialis*. The hybrid crosses (*M. edulis* × *M. trossulus*) of Zouros *et al.* (1994b) were excluded because of the possibility that sex ratio might be affected by the hybrid nature of the cross.

The ratio of males varied from 100% to zero and was not different from the 1:1 ratio in only 4 of the 36 families. Yet the sex ratio over all families was very close to 1:1 (number of males = 856 and number of females = 831), as predicted by the model and as is observed in natural populations (Sastry 1979). One may use the data of Table 3 in several ways to estimate the single parameter of the model *k*, the frequency of daughters among the progeny of a heterozygous female. For this one has to assign the 36 families into three classes: "sonless," "mixed sex," and "daughterless." One way of doing this is to consider a family as sonless or daughterless only when all progeny are of the same sex (the zero cutoff point). With this criterion, 1 family is daughterless, 10 are sonless, and 25 are of mixed sex. The overall frequency of daughters among the 1422 progeny from the latter 25 families is 0.415. If the female/male ratio among the 25 presumed heterozygous mothers was statistically similar, this value could be considered a reliable estimate of *k* ($k_1 = 0.415$). Given that this is not true (Table 3), a more reliable approach to estimating *k* from the sex ratio of families is to assume that there is an external cause of variation around the true *k*. In this case an estimate of *k* would be given by the mean of

TABLE 3

Progeny numbers of females from wild populations

No.	Female code	*M*	*N*	% *M*	*S* (1 − *k* = 0.7)
1	00WF11	24	24	100.0	—
4	97WF1	29	30	96.7	***
2	00WLF3	23	24	95.8	**
3	00WF16	19	20	95.0	*
5	00WF3	23	26	88.5	*
6	97WF2	24	30	80.0	NS
7	90WF7	32	40	80.0	NS
8	WGF19	124	156	79.5	NS
9	00WF3	19	24	79.2	NS
10	00WF7	19	24	79.2	NS
11	00WLF5	18	23	78.3	NS
12	90WF16	25	33	75.8	NS
13	00WF1	18	24	75.0	NS
14	00WF10	17	23	73.9	NS
15	WGF20	110	149	73.8	NS
16	00WF21	15	21	71.4	NS
17	99WF1	82	125	65.6	NS
18	90WF5	16	25	64.0	NS
19	WGF66	88	156	56.4	***
20	00WF14	13	24	54.2	NS
21	00WF9	11	24	45.8	**
22	WGF53	40	170	23.5	***
23	00WF2	3	21	14.3	***
24	00WF15	3	24	12.5	***
25	00WF18	3	30	10.0	***
26	WGF31	5	115	4.3	***
27	00WF19	0	10	0	—
28	90WF4	0	20	0	—
29	99WF7	0	23	0	—
30	00WF12	0	23	0	—
31	00WF5	0	24	0	—
32	00WF8	0	24	0	—
33	98WF2	0	28	0	—
34	00WF17	0	29	0	—
35	99WF6	0	30	0	—
36	00WF29	0	30	0	—

M is the number of male progeny in pure crosses; *N* is the total number of progeny scored; % *M* is the percentage of male offspring; *S* is the significance of the departure from fit of the observed sex ratio from the expected when $k = 0.3$, assuming the female parent was a heterozygote; NS is not significant; *significant at 0.05; **significant at 0.01; ***significant at 0.001. The following families are joined with statistically nondifferent sex ratios: 1–15, 5–17, 9–18, 11–20, 15–21, 22–25, 23–32, 24–36. Females 8, 15, 19, 22, and 26 are *M. galloprovincialis*; all others are *M. edulis*.

the ratios across families. This second estimate is $k_2 = 0.335$. Finally, a third estimate of k can be obtained by ignoring the observed sex ratios among broods and using instead the observed distribution of families into sonless, daughterless, and mixed-sex classes. This can be done through the maximum-likelihood method, using the explicit expressions for the expected frequencies of each type of family in the population (Table 2). This produces $k_3 = 0.211$. On face value these estimates of k are different, but this cannot be supported statistically given that we do not have expressions for the variance of these estimates.

The above exercise can be repeated by modifying the criterion of assigning a family into one of the three classes. We may consider that a small degree of "leakage" of the opposite sex is inevitable, as evidenced by the case of female B of Table 1 (one male among 249 offspring). When we use 5% as the cutoff point for the rare sex, the distribution of the 36 families of Table 3 becomes 3 daughterless, 11 sonless, and 22 of mixed sex and the three estimates of k are $k_1 = 0.478$, $k_2 = 0.333$, and $k_3 = 0.291$. Finally, when we use a 15% cutoff point, the family distribution is 5 daughterless, 14 sonless, and 17 of mixed sex, with $k_1 = 0.373$, $k_2 = 0.320$, and $k_3 = 0.305$. There is not much difference between the k_2 or k_3 estimates, whether one uses the 5 or 15% criterion, but there is a large difference for the values of k_1, which is another reason why this estimate is less reliable. It appears that for the purpose of this study 0.3 is as good a bold estimate for k as can be obtained from the available data. With $k = 0.3$, the expected number of *ZZ* (daughterless) females, in a random sample of 36, is 3.7; of *Zz* (mixed sex), 20.4; and of *zz* (sonless), 11.9. This distribution compares favorably with the observed 3, 22, and 11 when using the 5% cutoff point or the 5, 17, and 14 when using the 15% cutoff point.

We have used $k = 0.3$ to examine how many of the presumed *Zz* females have produced a sex ratio that is compatible with this estimate (Table 3). With zero as the cutoff point 12 (9 after the Bonferroni correction) of the 25 mixed-sex families produced a significantly different ratio. Using the 5% cutoff criterion, this number of families was 9 (7 after the Bonferroni correction) out of 22, and with 15% it was 4 (3 after the Bonferroni correction) out of 17. This testing assumes that the observed distribution of male to female progeny of *Zz* mothers is affected only by random sampling from the brood. It is, however, most likely that there would be several other sources of "noise" around the true value of k, such as leakage in the sex-determining mechanism or family-specific mortality differences between sexes, which may explain why the observed variance of k is larger than that predicted by the model alone.

Pedigreed families revisited: Having outlined a model for the determination of sex ratio in broods of individual females and having obtained an estimate of the parameter that determines the distribution of genotypes in the population, we may return to pedigree data with the aim to deduce the genotypes of the individuals involved. Using the 5% cutoff point we may conclude that of the 3 original wild females (Table 1), female 90WF4 was *zz* and the other 2, 90WF5 and 90WF7, were *Zz*. No other wild female was used in the pedigree data. From Table 2 the distribution of female genotypes with $k = 0.3$ is 1:5.6:3.2 for *ZZ*, *Zz*, and *zz*, respectively. Thus,

the observed distribution of 2 *Zz* and 1 *zz* females in a sample of 3 is fully consistent with the model. Of the pedigreed females, 11 were of type *zz* (all daughters of *zz* mothers) and 14 were of type *Zz*, a Mendelian distribution that is consistent with the parental genotypes.

By the nature of the model, genotype information about male parents can be obtained only from the broods of their daughters, which is not possible for most males in the pedigree data. Of the original wild males, 90WM4 is deduced to be of genotype *Zz*, since one-half of his daughters with a *zz* female were sonless and one-half were son bearing. No genotypic inference can be made for the other two males, 90WM5 and 90WM7, owing to the heterozygous state of their mates. Genotypic information can be deduced for only 5 of the 20 other wild males used in subsequent generations (Table 1, Figures 1 and 2). Males 98WM1, 98WM4, and 98WM6 are deduced to be of type *zz* or *Zz* and males 98WM2 and 98WM8 of type *Zz* or *ZZ*. The expected distribution of male genotypes in a wild population is 2.29:3.03:1 for *ZZ*, *Zz*, and *zz*, respectively (Table 2). Clearly, this information about genotypes of wild males cannot be used either to refute or to strengthen the model.

DISCUSSION

The information we provide here is fully consistent with the findings of Saavedra *et al.* (1997) that the sex ratio in pair matings of Mytilus may vary from one extreme to the other and that this is a characteristic property of the female parent. Saavedra *et al.* (1997) have made this observation in crosses of *M. galloprovincialis*. Our work extends it to *M. edulis* and suggests that it may apply to all species of the genus. The important new information from this study is that this property of females is heritable. The sonless/son-bearing trait did not appear to have a random distribution in pedigreed families. Rather, pedigrees that started from a sonless female continued to produce sonless females for the four generations for which we have observations and the analogous observation was made with pedigrees started from a son-bearing female.

There was no perfect fidelity in the transmission of the trait from mothers to daughters. We have observed three cases in which a sonless female produced in the same pair mating both sonless and son-bearing daughters. This is not consistent with the sex ratio being affected by a cytoplasmic factor. We first consider the possibility that this factor might be the mtDNA. This hypothesis is worth entertaining given the unusual mtDNA system of mussels. As it was argued in the previous section, where we provide justification for a model of nuclear control, a role for the paternal mtDNA is excluded from the fact that the same father may produce all-female or nearly all-male progeny depending on the female to which it is crossed. One can envisage two ways through which the maternal mtDNA might be involved. One is through a direct influence on the sex of the individual in which it resides. A polymorphism for the maternal mtDNA with one type producing daughters and the other producing sons is incompatible with the fact that the same maternal mtDNA is transmitted to both sons and daughters and also with the fact that the same female may produce offspring of both sexes. The second way through which the maternal mtDNA may be involved in sex determination would be that the mtDNA of a female determines whether she would be sonless or son bearing. This hypothesis would predict that all full or half daughters of the same mother would be of the same type as their mother. The three cases of sonless mothers that produced both sonless and son-bearing daughters contradict this hypothesis. Thus, it is unlikely that the female's mtDNA is responsible for the female's control of sex ratio. The same arguments can be used against the hypothesis that sex determination is under the control of a cytoplasmic symbiont. A sperm-transmitted symbiont is excluded on the basis that sperm from the same male may produce either sons or daughters depending on the female whose eggs it fertilizes. The more likely hypothesis that the symbiont is transmitted through the egg cannot be easily reconciled with the fact that most females we have scored produced either a high percentage of females or a high percentage of males, with only a minority of females having produced a 1:1 sex ratio. This pattern of strong bimodal bias of sex ratio is not known in any system of sex-ratio bias mediated by a cytoplasmic factor. These factors either would cause sterility of the affected individuals or would cause these individuals to produce a biased sex ratio but always in favor of the same sex (Hurst 1993).

Control of sex ratio through the mother's nuclear genotype appears to be the most likely alternative of sex-ratio bias in mussels. As demonstrated by the particular explicit model that we have presented, this hypothesis could provide a reasonably good quantitative fit to the empirical data. The basic tenet of the hypothesis is that the sex of the offspring is determined by the genotype of one of its parents and not by the gametes it receives from the parents. In its simplest form the hypothesis assumes an autosomal locus with two alleles. A parent (which in the particular case of mussels is the mother) produces progeny of only one sex if homozygous for one allele and of the other sex if homozygous for the alternative allele. A heterozygous parent produces both sexes in a ratio that depends on the degree of dominance of the two alleles. The model leads to a stable equilibrium with the two alleles at different frequencies in the two sexes and in the population as a whole, with genotype frequencies deviating from Hardy-Weinberg

within the female population, but with equal numbers of females and males in the whole population.

The model was inspired from the unusual linkage between sex and paternal mtDNA, which is the hallmark of DUI. The strong sex-ratio bias and its control by the female parent is an observation that is so far confined to mussels and, therefore, cannot be assumed to be an integral part of DUI. Even in mussels we do not know whether the two phenomena, maleness and inheritance of paternal mtDNA, are linked by cause or association. Saavedra *et al.* (1997) assumed the first. More explicitly they proposed that female is the default sex and that presence of paternal mitochondria in the gonad, during the developmental stage at which the gonad becomes committed to an egg- or sperm-producing organ, is necessary for the masculinization of the gonad. It must be noted that a sperm- or egg-producing gonad is the only known sex character in mussels. Further, Saavedra *et al.* (1997) proposed that whether sperm mitochondria will be present in the primordial germ cells is under the control of the female parent. The biological justification of the hypothesis is that DUI can be assumed to have derived from the standard maternal mtDNA inheritance that is prevalent in the animal kingdom. Even though details may vary among groups of species (Birky 1995), the basic feature of maternal inheritance is the presence of a mechanism that prevents sperm mitochondria from taking residence in the fertilized egg. The work of Kaneda *et al.* (1995) and Shitara *et al.* (1998) in mice has shown that sperm mitochondria are eliminated from the fertilized ovum before the formation of the diploid pronucleus. Interestingly, sperm mtDNA may persist in interspecific hybrids. By introgressing the mtDNA of one species into the other these authors were able to show that the control for the elimination of sperm mitochondria resides in the nuclear rather than the mtDNA genome. Sutovsky *et al.* (1999, 2000) have shown that in bovines the mechanism of sperm elimination involves ubiquitinization of sperm mitochondrial membranes and that the mechanism is also species specific. Taken together these studies clearly suggest that the elimination mechanism must involve at least two factors, an identification factor that resides in the surface of the sperm mitochondrion and a recognition factor that resides in the egg cytoplasm. In the mussel system we assume the presence of a third factor, which may be either present or absent in the egg cytoplasm. If we called W the factor that identifies sperm mitochondria and differentiates them from egg mitochondria, X the factor that recognizes W, and Z the third factor, then Z can be thought of as a suppressor of X. The amount of factor Z with which a female supplies her eggs would depend on her genotype. Females of genotype *ZZ* provide sufficient amounts for complete inactivation of X, so that the great majority of their eggs are destined to become males before fertilization. The opposite is true in eggs of females of genotype *zz* where the factor X is not inactivated. In heterozygous females the amount of factor Z may not be sufficient to inactivate all the amount of X, with the result that a percentage of eggs may develop feminine gonads after fertilization.

The simple one-locus two-allele system that we have modeled estimates the degree of inactivation of X by Z in heterozygous females at ~0.7 (percentage of female progeny at ~0.3). Even if the model is correct in its basic features, the real situation is likely more complex. It is possible that Z is controlled by more than one locus or that external factors may affect the final sex ratio in a sibship (*e.g.*, sex-dependent mortality from fertilization to the time of scoring). It is clear that beyond the obvious need to investigate at depth the nature of the link between sex inheritance and inheritance of paternal mtDNA, the most profitable way to study this link is not so much to concentrate on the model's quantitative predictions, but rather to see if it applies in general terms to other species that are known to follow the DUI system of mtDNA transmission.

We thank B. Bradford for spawning and rearing some of the mussels used in this study; A. Thompson, J. Johnson, D. Stewart, and B. Sutherland for help in sex determination; and C. Saavedra and M. Ladoukakis for helpful discussions. Grants from the Natural Sciences and Engineering Research Council of Canada to E.K. and E.Z. supported this research at various times.

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Communicating editor: M. A. Asmussen