No evidence for absence of paternal mtDNA in male progeny from pair-matings of
the mussel *Mytilus galloprovincialis*

Ioannis Theologidis*, Carlos Saavedra† and Eleftherios Zouros*

*Department of Biology, University of Crete, 71409 Heraklion, Crete, Greece
†Instituto de Acuicultura de Torre la Sal, Consejo Superior de Investigaciones
Científicas, Ribera de Cabanes, E 12595, Castellón, Spain
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Corresponding author: Eleftherios Zouros, Department of Biology, University of Crete, 71409 Heraklion, Crete, Greece. E-mail: zouros@biology.uoc.gr, phone +302810 394074, fax: +302810 394408
ABSTRACT

The claim that in controlled crosses a *Mytilus galloprovincialis* male failed to transmit mtDNA to its sons is shown to be false. At present there is no evidence for mussel males lacking a paternal mtDNA. This makes unlikely the hypothesis that maternal genomes may become paternally transmitted by invading the germ line of males that lack a paternal genome.
SAAVEDRA et al. (1997) studied sex inheritance and mtDNA transmission in pair-matings of the sea mussel *Mytilus galloprovincialis*, a species with doubly uniparental inheritance (DUI) of mtDNA (SKIBINSKI et al. 1994a; ZOUROS et al. 1994a). Progeny were scored for sex by microscopic observation of gonads and for presence of the maternally and paternally transmitted mitochondrial mtDNA. The latter was done by examining the restriction pattern of a 860 bp fragment of the COIII gene, after amplification from gonadal DNA and cleavage with *BamHI* and *EcoRI*. They found that there was a strong sex ratio bias in cohorts from pair matings, ranging from 0% to 97% males, and that the bias was mother-specific. They also reported that, with few exceptions, daughters contained only the mother’s mtDNA and sons contain the mtDNA of both parents, a result that was previously established (SKIBINSKI et al. 1994a; ZOUROS et al. 1994a). An unexpected observation was that 45 of the 46 sons sired by one male (M70) contained only the mother’s mtDNA (one son contained the mtDNA of both parents). This was independent of the female parent (M70 was crossed to four different females). The apparent absence of the paternal mitochondrial genome in these males was doubly checked. Sperm from M70 was also examined and showed presence of paternal mtDNA, even though the diagnostic zones were faint (Fig.3 in SAAVEDRA et al. 1997). SAAVEDRA et al. (1977) concluded that “the sons of M70 did not receive (or did not retain) the M mtDNA molecule of their father. In addition, because their gonads contained the F genome of their mother, the possibility exists that if they produce sons, these sons will inherit F molecules from both parents”.

In a recent study VENETIS et al. (2006) re-addressed the question of whether the maternal mtDNA can invade the male germline in mussels. In sperm purified by active swimming through a solution of Percoll these authors recovered only the
paternal mtDNA in all 36 males they examined. This result is not in line with the results from crosses involving M70 in SAAVEDRA et al. (1997). We, therefore, revisited the DNA extracted from sons of M70 and used primers that recognized the control region of the standard paternal genome (genome M), but not that of the maternal genome (genome F) (details for all primers used are given in the legend of Fig. 1). In all males we recovered the M genome (Fig. 1A). We then repeated the assay of SAAVEDRA et al. (1997) and obtained their result: we recovered the expected product from the targeted part of the COIII gene for the F genome, but not for the M genome (Fig. 1B). Finally, we applied a similar assay for the COI gene and recovered products from both genomes (Fig. 1C). These results show that the sons of M70 received the paternal mtDNA. The failure of the COIII assay to detect the M genome in these males is most likely due to a mutation at one of the binding sites of the primers used. The faint bands that were observed when the same assay was used for the sperm of M70 might be due to the fact that, unlike the gonads of sons, the sperm of M70 did not contain the F genome and thus the primers were not offered other binding sites expect those of the M genome (see GREEN et al. 2006 for an example of competition for binding sites). DNA from M70 was not available for further examination. In addition to the sons of M70, four of the 66 sons of male M54 and two of the 41 sons of male M46 were scored as lacking the paternal mtDNA in SAAVEDRA et al. (1997). We tested all these six males and found them positive for the control region of the M genome (data not shown).

The correction about the mitochondrial content of sons from pair matings that we present here has serious implications on our current understanding of the phenomenon of DUI. Several surveys of mtDNA restriction fragment length polymorphism (RFLP) in wild populations of mytilids have reported the presence of
males that lacked an M-type genome (M-less males) (ZOUROS et al. 1994b; SKIBINSKI et al. 1994b; RAWSON and HILBISH 1995; QUESADA et al. 1999; LADOUKAKIS et al. 2002). Some of these males carried two F-like genomes, one more common in the somatic tissues (the presumed maternal genome) and the other more common in the gonad. The latter genome was assumed to be of paternal origin and provided the basis for the “masculinization” (HOEH et al. 1997) or “role reversal” (QUESADA et al. 1999) hypothesis, according to which an F genome may become sperm-transmitted. But other M-less males appeared to be homoplasmic for the maternal genome and thought to have resulted from failure of the male to inherit a genome from its male parent. In turn, this suggested a way through which masculinization may occur: In the absence of a paternal genome, the maternal genome would invade the male germ line and be transmitted as a paternal genome thereafter. In due time these newly “masculinized” F genomes would diverge to the point that male heteroplasmy for two F-like genomes would be easily detected. The original interpretation of the mtDNA content of the sons of M70 (and of few sons of two other males) by SAAVEDRA et al. (1997) fitted this hypothesis. This is no longer the case. In addition, examination of apparently homoplasmic M-less males from a natural population (LADOUKAKIS et al. 2002) showed that they were mis-scored as homoplasmic, owing to the limited power of the RFLP assay to reveal the presence of two F-like genomes (I. THEOLOGIDIS, C. VENETIS, G. C. RODAKIS, and E. ZOUROS, personal communication). Combined with the finding that the maternal genome may not be able to invade the male germ line (VENETIS et al. 2006), this suggests that in species with DUI there exist no males without a paternal mtDNA or that such males are exceedingly rare.

The result we report here raises certain questions about how F-type genomes with a paternal transmission mode arise in the population. At present there are no
reports of male gonad or sperm that contained no genome with M-type sequences in its control region (CR). BURZYNSKI (2006) reported the presence in gonads of *M. trossulus* males of an F-type genome whose CR was longer than normal but did not apparently contain M sequences. The genome presented only a fraction of the mtDNA content of the gonad and there is no information as to whether it could be transmitted to male progeny of the individuals in which it was found. Thus, there is no evidence that this was a “masculinized” genome, a comment made also by the authors. At present, there is no exception to the observation that the CR of sperm-transmitted genomes is either of the standard M type (the typical M-type genomes) or it contains M-type motifs in combination with F-type motifs (masculinized genomes) (BURZYNSKI *et al.* 2003; BRETON *et al.* 2006; BURZYNSKI *et al.* 2006; C. VENETIS, I. THEOLOGIDIS, E. ZOUROS, and G. C. RODAKIS, personal communication). This observation leads up to the hypothesis – for which no direct evidence yet exists - that the transfer of M-type CR motifs into the CR of an F-type genome is the critical step in the process of masculinization. The transfer is apparently mediated by the F/M heteroplasmy that is the normal state in males of species with DUI. It remains a matter of speculation how often these transfers may occur. The fact that masculinized genomes have been observed in several distant populations of the *M. edulis* species complex (*M. edilus, M. galloprovincialis, M. trossulus*) suggests that masculinization may occur relatively often. Yet, this might be a premature conclusion. *M. edilus, M. galloprovincialis* and *M. trossulus* from the Baltic Sea share the same type of F and M genomes. It remains possible that masculinized genomes in these populations originated from one or a few recombination events between F and M genomes and diverged subsequently to their present variable forms. Related to this is the issue of whether the fate of a masculinized genome is simply stochastic or is driven by
selection. A newly arisen masculinized genome may have an advantage over the standard M genome, which, as paternally transmitted, is assumed to have been under relaxed selection and thus to have accumulated slightly deleterious mutations (STEWART et al. 1996). This selection may result from differential survival or replication when the masculinized genome is in the same mitochondrion with the typical M genome or it may emerge from competition between two types of sperm, one carrying the masculinized genome and the other the standard M genome. No evidence yet exists for either of these types of selection. Other types of selection are obviously possible. Clearly, we know at present very little about how a maternal genome may become paternally transmitted and how it may eventually replace the old paternal type in the population.
LITERATURE CITED


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Legend to Figure 1

PCR products form male progeny of male parents M70 and M54 (see SAAVEDRA et al. 1997 for details). All panels: lanes 1 & 2, sons of M70 X F19; lanes 3 & 4 sons of M70 X F66; lanes 5 & 6 sons of M54 X F20; lane 7 son of M54 X F19; lane 8 a wild female; M fragment size marker. Part A. A 370 bp fragment of the control region of the M genome amplified by the primers Lola1 and ssMdl2 (MIZI et al. 2005), of which the first binds to M and F genomes, and the second only to the M genome. Note the absence of the product in the female individual (lane 8). Part B. A 860 bp fragment of COIII amplified and restricted with BamHI as in SAAVEDRA et al. (1997). The F product contains no restriction site, but the M is cleaved in two fragments. Note that only the F product was obtained from the sons of M70 and that both F and M products were obtained from the sons of M54. Part C. A 1108 bp fragment of the COI gene amplified by the primer LCOI1491 (FOLMER et al. 1994) and COI-R (VENETIS et al. 2006). This primer pair recognizes both genomes. The product was restricted with BamHI. The F product contains no restriction site, but the M is cleaved in two fragments (659 bp and 449 bp). Note that both products were obtained from the sons of M70 and M54.
Figure 1.