

DIFFERENTIAL PATTERNS OF MALE AND FEMALE MTDNA EXCHANGE ACROSS THE ATLANTIC OCEAN IN THE BLUE MUSSEL, *MYTILUS EDULIS*

CYNTHIA RIGINOS,¹ MICHAEL J. HICKERSON,² CHRISTINE M. HENZLER,³ AND CLIFFORD W. CUNNINGHAM⁴
Department of Biology, Box 90338, Duke University, Durham, North Carolina 27708

¹*E-mail: riginos@duke.edu*

²*E-mail: mhick@socrates.berkeley.edu*

³*E-mail: cmh20@duke.edu*

⁴*E-mail: cliff@duke.edu*

Abstract.—Comparisons among loci with differing modes of inheritance can reveal unexpected aspects of population history. We employ a multilocus approach to ask whether two types of independently assorting mitochondrial DNAs (maternally and paternally inherited: F- and M-mtDNA) and a nuclear locus (ITS) yield concordant estimates of gene flow and population divergence. The blue mussel, *Mytilus edulis*, is distributed on both North American and European coastlines and these populations are separated by the waters of the Atlantic Ocean. Gene flow across the Atlantic Ocean differs among loci, with F-mtDNA and ITS showing an imprint of some genetic interchange and M-mtDNA showing no evidence for gene flow. Gene flow of F-mtDNA and ITS causes trans-Atlantic population divergence times to be greatly underestimated for these loci, although a single trans-Atlantic population divergence time (1.2 MYA) can be accommodated by considering all three loci in combination in a coalescent framework. The apparent lack of gene flow for M-mtDNA is not readily explained by different dispersal capacities of male and female mussels. A genetic barrier to M-mtDNA exchange between North American and European mussel populations is likely to explain the observed pattern, perhaps associated with the double uniparental system of mitochondrial DNA inheritance.

Key words.—Coalescent, divergence population genetics, DUI, gene flow, interlocus contrasts, phylogeography.

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Gene flow is perhaps the most important factor affecting the evolutionary dynamics of populations within a species. The patterns of gene exchange across loci, however, often depend on the specific ecology of dispersal. For example, in plants, pollen may travel greater distances than seeds, and in animals male and female individuals may have different patterns of dispersal. Often a close examination of genes with different modes of inheritance can reveal such sex-related differences (e.g., Karl et al. 1992; Palumbi and Baker 1994; Larsen et al. 1996; Rassmann et al. 1997; Lyrhom et al. 1998; Seielstad et al. 1998; Escorza-Treviño and Dizon 2000; Pardini et al. 2001; Petit et al. 2001; Liepelt et al. 2002).

Different parts of a genome may also be more or less permeable to gene flow, leading to different rates of gene exchange among loci. This idea has been discussed extensively in the context of hybrid zones, where loci conferring reproductive isolation (or linked to such loci) should introgress less onto another species' genetic background than neutral loci (reviews in Barton and Hewitt 1985; Harrison 1990). The same concept can be applied between geographically separated populations of one species connected by some gene flow. Inferences regarding the permeability of a given locus can only be made in relation to other loci or genomic regions, so that multilocus data are required.

Here, we use multiple loci to examine patterns of gene flow and population divergence in a marine invertebrate. Adult blue mussels, *Mytilus* spp., are essentially sessile, and, as with many marine invertebrates, dispersal occurs in the planktonic larval stage. Mussels reproduce by spawning gametes into the water column. Fertilization and larval development occur in the water column and larvae are dispersed as they drift on ocean currents. Larvae can increase their dispersal capacity by delaying metamorphosis (Bayne 1965) and postlarvae can remain planktonic using long monofila-

ment threads to increase viscous drag (Sigurdsson et al. 1976), such that two to three months (and up to six months) of total drifting are possible (Lane et al. 1985). The prolonged planktonic duration of mussels may permit occasional long-range dispersal, such as across the Atlantic Ocean. However, empirical estimates of mussel dispersal distances suggest that larvae typically disperse only 20–50 km (Hilbish 1985; McQuaid and Philips 2000; Gilg and Hilbish 2003). No sex-related differences in dispersal capacity are known that would differentially affect maternally and paternally inherited genetic markers.

Mussels have an unusual system of mtDNA transmission, involving both maternally and paternally inherited mitotypes. These mitotypes are independently assorting and, thus, should provide independent records of female and male population histories. In addition to the normal matrilineally inherited (female) mtDNA, mussels have a second independently assorting mtDNA; the male (or M-type) mtDNA is found nearly exclusively in males and transmitted patrilineally via the sperm (Skibinski et al. 1994; Zouros et al. 1994a). In general, female mussels only have female mtDNA (F-mtDNA), whereas male mussels carry both male (M-) and female (F-) types of mtDNA. This system of inheritance has been described as double uniparental inheritance (DUI; Zouros et al. 1994a).

The most common type of M-mtDNA is paralogous to F-mtDNA, with the mtDNA duplication having occurred before the divergence of the congeners *Mytilus edulis*, *M. galloprovincialis*, and *M. trossulus* (Rawson and Hilbish 1995; Stewart et al. 1995; Hoeh et al. 1996, 1997). However, in some populations there are high frequencies of M-mtDNA that share a more recent common origin with F-mtDNA (Quesada et al. 1999; Ladoukakis and Zouros 2001). The mode of inheritance of these newly “masculinized” M-mtDNA

appears to match that of the older or “standard” M-mtDNA, but the dynamics and frequency of masculinization are not well understood (Hoeh et al. 1997; Saavedra et al. 1997; Ladoukakis and Zouros 2001). In this study, we focus on the “standard” M-mtDNA, which is easily distinguished from F-mtDNA at the DNA sequence level. For the remainder of this paper, M-mtDNA will refer to “standard” M-mtDNA.

An important consideration when using F- and M-mtDNA as markers of demographic history is that these loci may not be evolving in a strictly neutral manner. Specifically, the rate of substitutions at M-mtDNA exceeds that of F-mtDNA. In general, there are more fixed differences (both synonymous and nonsynonymous) between species and more polymorphisms (both synonymous and nonsynonymous) within species for M-mtDNA than F-mtDNA, so that direct comparisons of the two types of mtDNA show deviations from neutral expectations (Stewart et al. 1995, 1996; Quesada et al. 1998a, 1999). F-mtDNA is likely to be under purifying selection, as evidenced by a paucity of nonsynonymous differences between species (Skibinski et al. 1999), and is thus similar to most animal mtDNA (Rand and Kann 1998). M-mtDNA probably has fewer selective constraints because, aside from gonadic tissue, M-mtDNA is heteroplasmic with F-mtDNA (Stewart et al. 1995, 1996), but it may also experience some purifying selection (Skibinski et al. 1999; Skibinski 2000). Although the two types of mtDNA may be subject to different selective pressures, as long as evolution *within* the F- and M-lineages is consistent and unaffected by selective sweeps, they can be used as independently assorting markers of population history with different neutral rates of mutation. Furthermore, because mtDNA (both F- and M-mtDNA, in this case) are not linked to nuclear genes, they should be more permeable to genetic exchange between divergent taxa than typical nuclear genes (Barton and Jones 1983).

In the present study, we investigate the dynamics of trans-Atlantic population divergence between North American and European *M. edulis* and subsequent gene flow across the Atlantic Ocean. Although many rocky intertidal taxa colonized the North American coastline from Europe following the last glacial maximum (Fig. 1 and Cunningham and Collins 1994; Wares and Cunningham 2001), an earlier study of F-mtDNA in *M. edulis* suggested that *M. edulis* have continuously resided in North America for longer than 20,000 years (Wares and Cunningham 2001). We compare estimates of population divergence time and gene flow for two distinct mitotypes (F- and M-mtDNA) with a biparentally inherited nuclear locus, ITS. We find that overall gene flow of *M. edulis* across the Atlantic is low, but that there are striking differences in gene flow between markers. These differences in recent trans-Atlantic genetic exchange lead to discordant estimates of trans-Atlantic population divergence times among loci. We suggest that the complete absence of M-mtDNA gene flow across the Atlantic Ocean may result from some genetic barrier to gene flow for this locus, rather than an inherent difference in the dispersive capacities of male and female mussels.

MATERIALS AND METHODS

Populations Sampled

Mytilus edulis mussel populations were surveyed from North America, Iceland, and northern Europe (Fig. 1). In

Europe, we avoided areas where *M. edulis* hybridizes with other mussels, namely the Baltic Sea (hybridization with *M. trossulus*: Väinölä and Hvilsum 1991; Riginos et al. 2002) and southwestern Europe (hybridization with *M. galloprovincialis*: Skibinski and Beardmore 1979). Because *M. edulis* mtDNA is known to assymmetrically introgress in both hybrid zones (Quesada et al. 1995; Rawson and Hilbish 1998; Quesada et al. 1999), this sampling strategy should avoid including any mussels whose nuclear background is not *M. edulis*. Although *M. edulis* and *M. trossulus* are found in sympatry in the northern part of the North American coastline, hybridization is less frequent than in Europe and there is no evidence for mtDNA introgression (Saavedra et al. 1996; Comesaña et al. 1999). A small number of *M. trossulus* were also sampled for molecular clock calibrations.

DNA Amplification and Sequencing

Total genomic DNA was extracted from mantle tissue using a standard phenol-chloroform protocol. Because male mussels contain two distinct forms of mtDNA, separate primers targeting female and male mitochondrial cytochrome oxidase I (COI) and cytochrome oxidase III (COIII) genes were used to amplify these regions using the polymerase chain reaction (PCR), with Promega *Taq* polymerase. All male primers targeted the standard M-mtDNA. The complete list of primers used is given in Table 1, and specific cycling conditions can be obtained on request from the authors.

PCR products were purified using Qiagen's Qiaquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and sequenced in both forward and reverse directions with PCR primers using the ABI PRISM BigDye Terminators version 2.5 Cycle Sequencing Kit (Applied Biosystems, Foster-City, CA). Sequencing reactions were run out in an ABI PRISM 3700 DNA Analyzer. Sequence chromatograms were visualized and edited using SEQUENCHER version 4.1 software (Gene Codes Corp., Ann Arbor, MI). Sequences of the internal transcribed spacer region (ITS) of rDNA were taken from a previously published ITS dataset (Riginos et al. 2002); similarly our final datasets include some F-COI sequences from Wares and Cunningham (2001) and M-COIII *M. trossulus* sequences from Stewart et al. (1995). (A complete list of sequence sources can be found in the Appendix Tables available online only at: <http://dx.doi.org/10.1554/04-183.1.s1>). All mtDNA datasets were manually aligned. As many of the ITS sequences were identical within an individual, the number of taxa in the ITS dataset was reduced by only considering the two most divergent alleles within each individual.

Tests of Selection

Mussel F- and M-mtDNA are known to be under different selective regimes with respect to each other (see Introduction). Our concern here is whether there are substantial deviations from neutral expectations *within* each gene lineage that might bias estimates of divergence times and gene flow. To address this issue, we conducted McDonald-Kreitman (1991) tests examining relationships among all major groupings (*M. edulis* groups from the Canadian Maritimes, Atlantic USA, Iceland, and Europe, and *M. trossulus*) and conducted a Tajima's (1989) D test for each group. McDonald-Kreitman

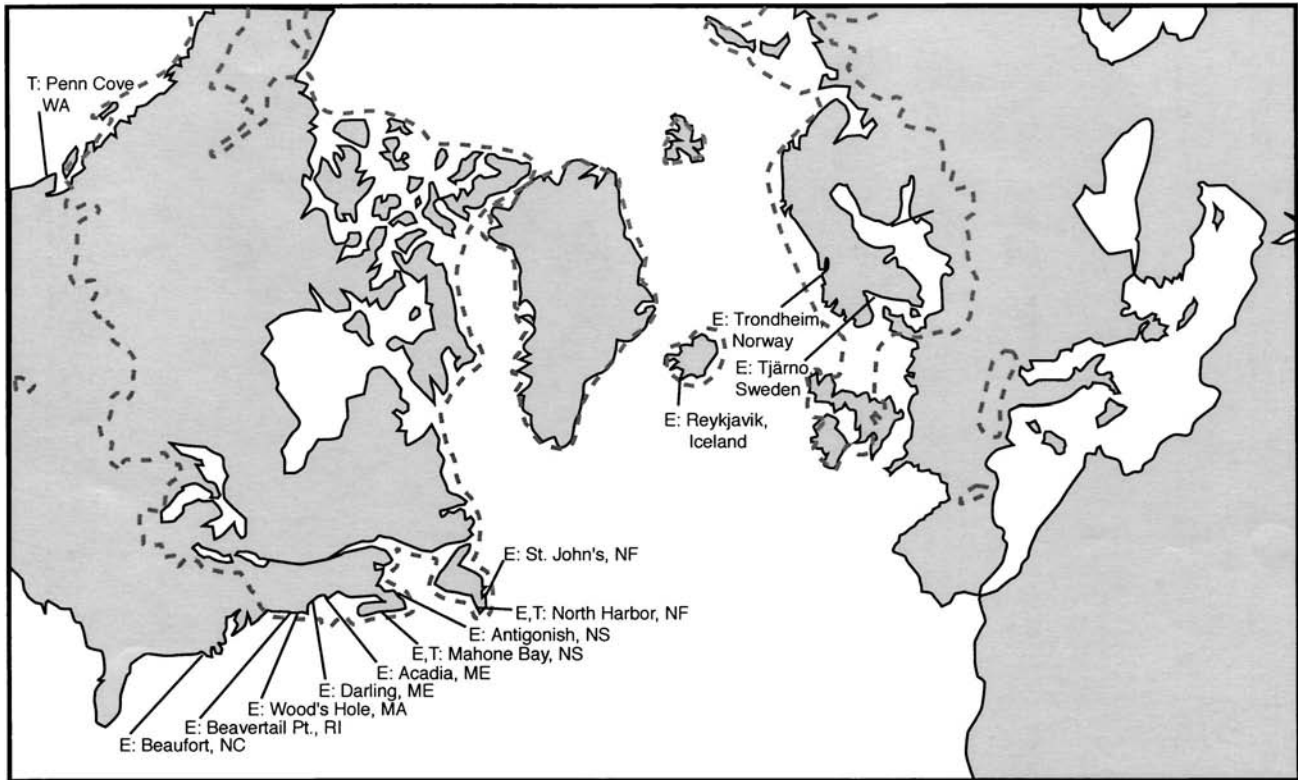


FIG. 1. Mussel collecting sites. The species identity of mussels from each site is indicated by an E or T, for *Mytilus edulis* and *M. trossulus*. The extent of glacialiation at the last glacial maximum (~ 20,000 years before present) is shown by a dashed line.

tests examine the ratios of polymorphic and fixed nonsynonymous and synonymous substitutions within and between species; under a neutral model of evolution, ratios of polymorphisms to fixed differences should be approximately equal for both types of mutations. Tajima's test uses the frequency spectrum of within species polymorphisms to detect deviations from neutral conditions. McDonald-Kreitman and Tajima's tests were conducted in DnaSP version 3.51 (Rozas and Rozas 1999).

Phylogenetic Analyses

Genealogical relationships for mtDNA and ITS were estimated using maximum likelihood. The most appropriate model of evolution was determined by ModelTest (Posada and Crandall 1998) and then implemented with Paup* ver. 4.0b10 (Swofford 1998), using a heuristic search with TBR branch-swapping.

Estimation of Trans-Atlantic Population Divergence Time and Gene Flow

Gene duplication of the male and female mtDNA lineages took place before the speciation of *M. trossulus* and *M. edulis*, so that each lineage represents an independent historical record of that speciation and more recent events within *M. edulis*. Nuclear ITS is also an independently assorting locus, giving a total of three unlinked markers used in this study. Speciation between Pacific *M. trossulus* and the ancestor of Atlantic/Mediterranean *M. edulis* and *M. galloprovincialis*

was probably allopatric and associated with the trans-Arctic interchange where many Pacific species, including Pacific mussels, colonized the northern Atlantic Ocean (Vermeij 1991; Cunningham and Collins 1994; Rawson and Hilbish 1995). We employed a molecular clock calibrated by the opening of the Bering Strait 3.5 million years ago (MYA), which created a passageway for Pacific taxa to disperse to the northern Atlantic Ocean. Although there is some controversy over the exact date of the Bering Strait opening (Marincovich and Gladenkov 1998), the best evidence for the arrival of Pacific migrants to the Atlantic Ocean remains at 3.5 million years (Wares and Cunningham 2001).

Here, we followed three approaches to dating divergence time between North Atlantic and European *M. edulis* populations: (1) a phylogenetic approach that allows for rate differences among lineages (local molecular clocks: Langley and Fitch 1974; penalized likelihood: Sanderson 1997, 2002); (2) a classical approach based on simple genetic distance where extant polymorphism was subtracted from the total genetic divergence (Nei and Li 1979); and (3) a method that jointly estimates divergence time and gene flow between two populations within a coalescent framework (Nielsen and Wakeley 2001). Population divergence refers to the splitting of two populations (North American and European *M. edulis*, in this case) and is not necessarily contemporaneous with the coalescence of a given locus. Polymorphism within the ancestral population (e.g., the ancestor to all Atlantic *M. edulis*) would make coalescence times for each locus older than population divergence time (see Edwards and Beerli 2000), but

TABLE 1. Primers used for mtDNA amplifications.

Gene	Species	Forward primer	Reverse primer	Reference
Female COI	<i>Mytilus edulis</i>	MytF: 5'-GGT TTT ATA ATG TGG TTG TTA C-3'	MytR: 5'-TCA AAA AAT GTT GTG TTA AAA T-3'	Wares and Cunningham 2001
	<i>M. trossulus</i>	FCOI tr F: 5'-CTG AGG AGG GTT GTT TGG AG-3'	FCOI tr R: 5'-CTC CTG CGG GAT CGA AAA-3'	Present study
Female COIII	<i>M. edulis</i>	FOR1: 5'-CCA AAC CCG TCA TCT ACT AG-3'	REV1: 5'-ATG CTC TTC TTG AAT ATA AGC GTA CC-3'	FOR1: Quesada et al. 1998a REV1: Zouros et al. 1994b
	<i>M. trossulus</i>	FOR2: 5'-GTA ACT CAA GCC CAT AAG AG-3'	REV1: 5'-ATG CTC TTC TTG AAT ATA AGC GTA CC-3'	FOR2: Stewart et al. 1995 REV 1: Zouros et al. 1994b
Male COI	<i>M. edulis</i>	MCOI F: 5'-GGT TAC AAC MCA TGC WTT AAT GA-3'	MCOI R: 5'-RAA GTT TCG GTC AAA MAR RAT TAT- 3'	Present study
	<i>M. trossulus</i>	MCOI F: 5'-GGT TAC AAC MCA TGC WTT AAT GA-3'	MCOI R: 5'-RAA GTT TCG GTC AAA MAR RAT TAT- 3'	Present study
Male COIII ^a	<i>M. edulis</i>	FOR2: 5'-GTA ACT CAA GCC CAT AAG AG-3'	REV2: 5'-AGC CTT TTT GTC ATC ATT CTG T-3'	FOR2: Stewart et al. 1995 REV2: Skibinski et al. 1994a REV1: Zouros et al. 1994b
			REV1: 5'-ATG CTC TTC TTG AAT ATA AGC GTA CC-3'	

^a Male COIII genes were amplified using a nested PCR protocol following Quesada et al. 1998a, where primers FOR2 and REV2 were used for the initial amplification and then a second round of amplification was performed using primers FOR2 and REV1.

gene flow following population divergence can make coalescence more recent than population divergence (Rosenberg and Feldman 2002).

The methods employed here make assumptions that may contribute to different possible biases depending on the real history of Atlantic mussels. For example, simple genetic distance provides estimates and corrects for ancestral polymorphism but assumes no gene flow following divergence, whereas Nielsen and Wakeley's (2001) coalescent method estimates both ancestral polymorphism and gene flow. However, both of these methods assume rate constancy within lineages; penalized likelihood and local molecular clocks allow for rate variation but do not take ancestral polymorphism or gene flow into consideration.

We restricted all trans-Atlantic comparisons to Atlantic USA–Europe for two reasons. First, there is some population structure of *M. edulis* F-mtDNA along the North American coastline (between the Canadian Maritimes and Atlantic USA; C. Riginos unpubl.). Second, the sampling of M-mtDNA and ITS was not as good in the Canadian Maritimes (as compared to Atlantic USA), so that robust estimates of Canadian Maritimes–Europe divergence times were not possible for these loci. In all possible direct comparisons within loci (e.g., Atlantic USA–Europe vs. Canadian Maritimes–Europe), estimated divergence times were compatible.

Penalized likelihood and local molecular clocks

Because it is well established that M-mtDNA evolves faster than F-mtDNA, variable rates were taken into account in the r8s1.6 program (Sanderson 1997, 2002) on a Macintosh G5 computer. Two methods were used to estimate dates: (1) penalized likelihood, which minimizes changes in local rates along the branches of a genealogy, and (2) the Langley-Fitch (1974) local molecular clock method, which permits a dis-

crete number of rates of evolution along a genealogy. These methods can handle deviations from clock-like expectations, but, as previously mentioned, do not take ancestral polymorphism or gene flow into account. Therefore, these methods estimate the most recent common ancestor (MRCA), or the coalescence time, of each locus.

Although r8s can indicate areas of a genealogy where rates differ, we also verified that substitution rates remained roughly constant within each gene lineage (F-mtDNA, M-mtDNA, ITS) by applying a series of log-likelihood ratio tests comparing maximum-likelihood scores with and without an enforced molecular clock (Felsenstein 1988). For each locus, one parsimony tree was randomly chosen. The likelihood scores for each of these trees were estimated in PAUP* under the best-fit model (1993) of sequence evolution with and without an enforced molecular clock.

Genetic distance (no gene flow)

Net population distances were estimated using a Tamura-Nei (1993) distance model in MEGA 2.1 (Kumar et al. 2001), with pairwise deletion for missing data. Bootstrapping was used to estimate standard errors. Although multiple substitutions at any one site were a concern, particularly for the quickly evolving male lineages, the use of Tamura-Nei distances that correct for multiple hits, should alleviate this problem. To estimate divergence for the split between *M. trossulus* and *M. edulis*, the average net genetic distance between *M. trossulus* and each major *M. edulis* regional group (see above) was used. For each locus, the estimates of genetic distance were very close regardless of the *M. edulis* group used.

Coalescent estimates of divergence and gene flow

The program MDIV (Nielsen and Wakeley 2001) was used to jointly estimate population divergence and gene flow under

the finite sites HKY model (Hasegawa et al. 1985). This program uses Markov Chain Monte Carlo procedures to obtain joint posteriors of the standard coalescent parameters θ (heterozygosity), M (gene flow), and T (divergence) for a prior range of M and T values; for mtDNA $\theta = 2N\mu$, $M = 2Nm$ and $T = t/N$; for nuclear DNA $\theta = 4N\mu$, $M = 4Nm$ and $T = t/2N$, where N is the effective population size, μ is the gene-specific mutation rate per generation, m is the migration rate, and t is generations. By simple multiplication, $\theta T = 2\mu t$, where $2\mu t$ is the genetic distance between two populations. From this relationship standard molecular clock mechanics were used to infer the date of trans-Atlantic population divergence. All nonzero gene flow estimates were tested for significant differences from zero using log-likelihood ratio tests of the posterior probabilities for the most likely value of M and the probability associated with $M = 0$ (see Nielsen and Wakeley 2001). In order to compare gene flow estimates for nuclear ITS and haploid mtDNA, an equal sex ratio was assumed and M_{ITS} was converted to its haploid equivalent by dividing by 4 ($M_{mtDNA} = 2N_{haploid} m$; $M_{ITS} = 4N_{diploid} m$, assuming $N_{diploid} = 2N_{haploid}$). (All reported gene flow values for ITS are converted haploid equivalents.)

Discrepancies between estimated population divergence times and gene flow among loci prompted us to explore these inconsistencies. Specifically, estimates of trans-Atlantic *M. edulis* gene flow and divergence were substantially different for M-mtDNA relative to F-mtDNA and ITS (see Results). The maximum-likelihood framework of MDIV allowed us to test whether the data were better accommodated by a single gene flow estimate or by two separate gene flow rates (one for M-mtDNA, a second for F-mtDNA and ITS) by calculating the likelihood ratio,

$$LR = \frac{\max\{L(M_{F-mtDNA,ITS}, M_{M-mtDNA})\}}{\max\{L(M)\}},$$

where the likelihood function of a single gene flow rate, $L(M)$, was estimated by multiplying all three likelihood functions together. Similarly, the likelihood function of $L(M_{F-mtDNA,ITS})$ was estimated by multiplying the likelihood functions for $M_{F-mtDNA}$ and M_{ITS} . And, $\max\{L(M_{F-mtDNA,ITS}, M_{M-mtDNA})\} = \max\{L(M_{F-mtDNA,ITS})\} \times \max\{L(M_{M-mtDNA})\}$. The same mechanics were used to test for two independent trans-Atlantic population divergence times versus a single population divergence time. The significance of the LR was tested by assuming that twice the log-likelihood ratio (2 LLR) is distributed as a chi-square with one degree of freedom (see Nielsen and Wakeley 2001).

The program MDIV was run on a UNIX workstation. Because MDIV eliminates all sites for which there is missing data (even if that site is missing from a single sequence), it was necessary to prune the F-mtDNA dataset so that only individuals for which we had both COI and COIII sequences were included. Similarly, the M-mtDNA dataset was split into separate M-COI and M-COIII datasets and analyses were run independently for these two genes. For each locus-by-taxa pairwise comparison, we used an arbitrary initial sampling range for the parameters M and T ($M = [0, 5]$; $T = [0, 10]$). After the initial run, the priors for M and T were adjusted to reasonable intervals and the runs were repeated three times for each comparison to verify that a consistent estimate could

be obtained. In every case, 2,000,000 steps of the Markov Chain were sampled with a burn-in of 500,000 steps.

RESULTS

DNA Amplification and Sequencing

In general, PCR-amplification of *Mytilus* F-mtDNA was straightforward and sequence data were easily obtained for all individuals attempted. Male mtDNA genes were much harder to PCR-amplify. In some instances, an individual mussel that yielded a PCR product with M-COI primers was not amplifiable for M-COIII or vice versa. By combining our new sequences with previously published sequences, in all we present here the results from 132 F-haplotypes, 56 M-haplotypes, and ITS sequences from 21 individuals. A complete list of sources and Genbank accession numbers is given in the Appendix Tables available online.

Tests of Selection

The general patterns of molecular evolution within each lineage matched previous descriptions. Both M- and F-mtDNA genes showed patterns typical of animal mtDNA, where there were fewer nonsynonymous polymorphisms and fixed differences than synonymous, although this pattern was more extreme (but not statistically significant) for F-mtDNA. For example, there were only two nonsynonymous fixed differences between all *M. edulis* and *M. trossulus* F-mtDNA and two nonsynonymous polymorphisms (combined COI and COIII), whereas in the same contrast for M-mtDNA there were 18 nonsynonymous fixed differences and 13 nonsynonymous polymorphisms (synonymous polymorphisms and fixed differences were roughly the same between F- and M-mtDNA for the *M. edulis*-*M. trossulus* comparison: 109 and 89 for F-mtDNA and 126 and 88 for M-mtDNA, respectively). Our failure to find statistically significant McDonald-Kreitman tests for comparisons restricted to either F- or M-mtDNA contrasts with some of the results of Quesada et al. (1998a; 1999), who did find a signature of purifying selection for F-COIII (e.g., fewer than expected nonsynonymous fixed differences between *M. edulis* and *M. trossulus*).

An excess of rare polymorphisms was typical of North American and European *M. edulis* populations, resulting in negative Tajima's D values (Table 2). F-mtDNA haplotypes from Europe yielded a significant Tajima's test, but this result was not significant following a Bonferroni correction for multiple tests. Although these results may indicate that both F- and M-mtDNA conform to a mildly deleterious model of evolution it may also point to recent population expansions.

Phylogenetic Analyses

The combined phylogenetic analysis of M- and F-mtDNA lineages is shown as a chronogram from the r8s analysis (Fig. 2, see description below; F-mtDNA is shown in greater detail in Fig. 3). This combined analysis found a clear pattern of reciprocal monophyly between F- and M-mtDNA lineages. This pattern confirms that all M-mtDNA included in this study are standard male mitotypes and not recently masculinized. (Although recently masculinized M-mtDNA may be present in any of the populations sampled, we would not

TABLE 2. Gene diversity within *Mytilus edulis*.

Locus	Region	π (%)	θ (%)	Tajima's D
M-mtDNA ^{a,b}	Canadian Maritimes	0.57	0.51	1.09
	Atlantic USA	1.18 (2.71)	2.05 (2.83)	-1.73 (-0.22)
	Northern Europe	1.91 (2.08)	3.72 (2.45)	-2.05* (-0.80)
F-mtDNA ^a	Canadian Maritimes	0.98 (1.65)	1.08 (1.56)	-0.29 (0.22)
	Atlantic USA	0.71 (1.17)	1.46 (1.36)	-1.71 (-0.48)
	Iceland	0.37 (0.87)	0.46 (0.99)	-0.78 (-0.54)
	Northern Europe	1.35 (1.48)	1.50 (1.67)	-0.37 (-0.43)
ITS ^b	Atlantic USA	0.97	0.95	0.18
	Northern Europe	0.87	1.63	-1.79

* $P < 0.05$; not significant following a Bonferroni correction for multiple tests.

^a Values for COI given outside parentheses, COIII given inside parentheses.

^b For M-mtDNA, there were an insufficient number of COI haplotypes from the Canadian Maritimes and COI and COIII haplotypes from Iceland to estimate gene diversity. ITS was not surveyed from either the Canadian Maritimes or Iceland.

expect to detect them with the targeted PCR methods employed here.) *Mytilus trossulus* and *M. edulis* were also reciprocally monophyletic in F-mtDNA, M-mtDNA, and ITS lineages (Figs. 2 and 4). Reciprocal monophyly was additionally evident within the M-mtDNA lineage between European and all North American haplotypes, a pattern found with much smaller sampling by Quesada et al. (1998a; 1999). In F-mtDNA, some geographic structure between North America and Europe was apparent (Fig. 3), but some individual haplotypes clustered with haplotypes from the other side of the Atlantic (particularly clade 6), a pattern consistent either with incomplete lineage sorting or recent trans-Atlantic gene flow. As would be expected from a nuclear locus, the ITS genealogy was less resolved than either F- or M-mtDNA (Fig. 4).

Iceland is located in the middle of the northern Atlantic Ocean and mussels residing there had genetic affinities with both Europe and North America. The three M-mtDNA haplotypes from Iceland fell within the European clade. F-mtDNA haplotypes from Iceland clustered with both European and North American haplotypes (Fig. 3).

Estimation of Trans-Atlantic Population Divergence Time and Gene Flow

Penalized likelihood and local molecular clocks

Our r8s analyses were consistent with the assumption that the male and female lineages experienced simultaneous subdivision during the trans-Arctic interchange causing speciation between *M. edulis* and *M. trossulus*. When we fixed the F-mtDNA divergence between *M. trossulus* and *M. edulis* at 3.5 MYA, the penalized likelihood method found a date for M-mtDNA trans-Arctic divergence of 3.6 MYA (2.9–4.7 confidence interval). Similarly, when the M-mtDNA *M. trossulus* and *M. edulis* divergence was fixed at 3.5 MYA, the penalized likelihood method estimated F-mtDNA trans-Arctic divergence at 3.5 MYA (2.7–4.4 CI).

Within the M- and F-mtDNA lineages, the rates assigned by penalized likelihood were fairly constant. Using the Langley-Fitch local molecular clock method, we allowed r8s to assign four rates of evolution, but it chose only two, one for the male clade, and one for the female clade (4.1% and 2.6% per million years (MY), respectively), consistent with clock-like rates of substitution within each lineage. Also, log-like-

likelihood tests did not reveal significant deviations from clock-like behavior within either of the mtDNA lineages or ITS. The rate constancy within the F-mtDNA, M-mtDNA and ITS lineages allowed us to use methods that assume a constant substitution rate and to calibrate molecular clocks separately for F-mtDNA, M-mtDNA and ITS based on a trans-Arctic divergence time of 3.5 MYA.

Although the trans-Arctic divergence appears to have been contemporaneous in the male and female mtDNA lineages, this was not the case for the trans-Atlantic divergence within *M. edulis* (Fig. 2). Using penalized likelihood, the MRCA of North American and European M-mtDNA was estimated at 0.95 MYA (0.66–1.40 CI; Table 3), whereas the MRCA of all F-mtDNA haplotypes was 0.21 MYA (confidence search failed) and 0.47 MYA (0.24–0.87 CI) for ITS. (All results were similar with the local molecular clock method.) For F-mtDNA and ITS, however, there is no meaningful way to distinguish between coalescence of North American and European haplotypes/alleles versus coalescence of all *M. edulis* haplotypes/alleles. We can only estimate the latter as reported above.

Genetic distance (no gene flow)

Consistent with a higher substitution rate for the male mtDNA lineage, the estimate of net genetic distance between *M. trossulus* and *M. edulis* M-mtDNA was 21.4%, yielding a divergence rate of 6.12% per MY (6.01% for M-COI and 7.82% for M-COIII), whereas F-mtDNA genetic distance was 17.1% (4.89% per MY). *Mytilus trossulus* and *M. edulis* ITS distance was 3.87% (1.11% per MY). These molecular clock calibrations resulted in different estimates of *M. edulis* trans-Atlantic (Atlantic USA–Europe) divergence, with a substantially older date estimated from M-mtDNA (1.5 MYA) as compared to F-mtDNA (0.068 MYA) and ITS (0 MYA) (Table 3). (The per locus calibrated rates of molecular evolution described above were also used to calibrate the MDIV results that follow.)

Coalescent estimates of divergence and gene flow

The variation among trans-Atlantic genetic distances (above) can be explained by differences in trans-Atlantic gene flow among loci, where any gene flow would reduce estimates of standard genetic distances. Using MDIV, M-mtDNA (both

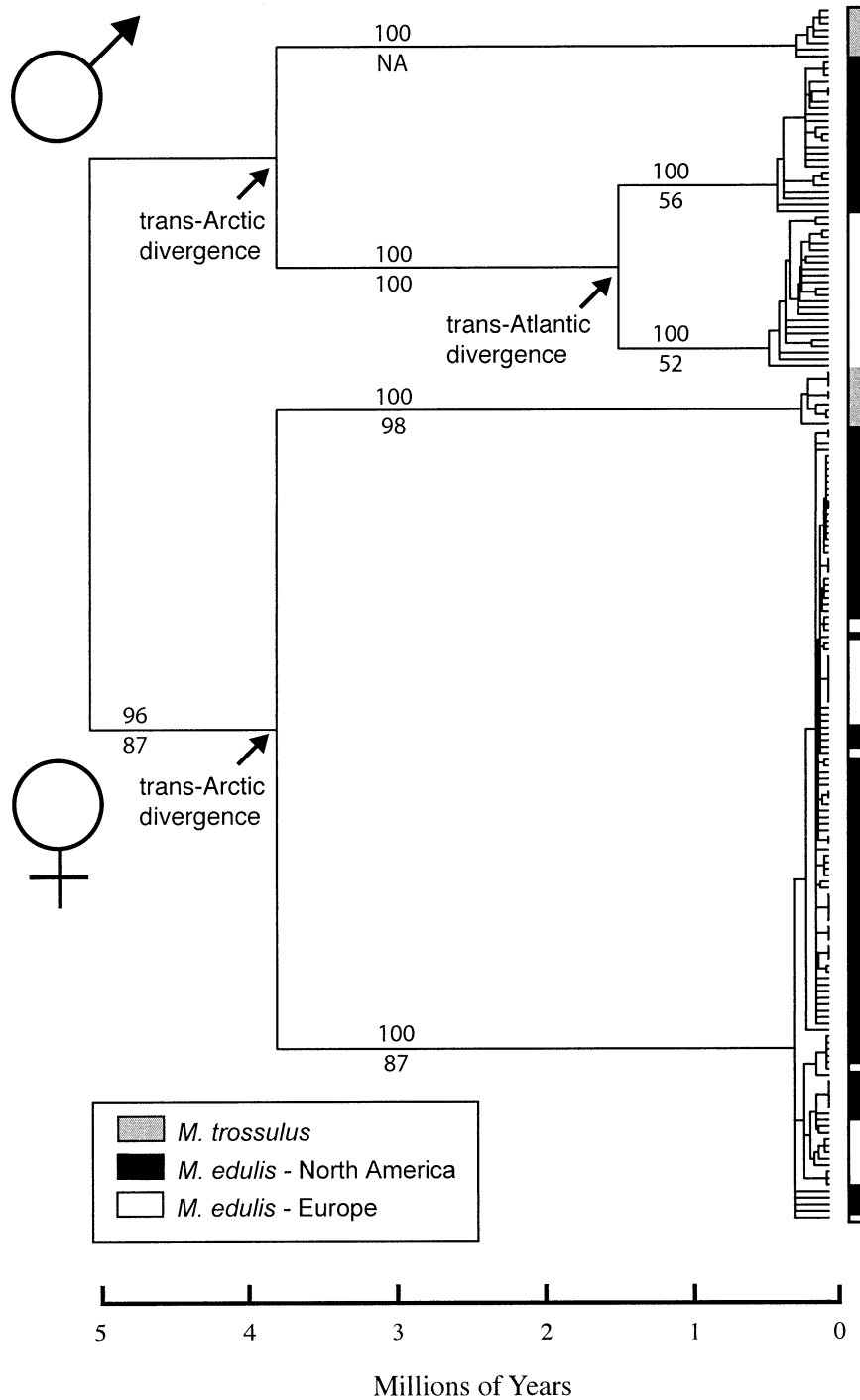


FIG. 2. Chronogram of all mtDNA haplotypes based on a Tamura-Nei plus gamma plus invariant sites model of evolution, with midpoint rooting. The nodes for trans-Arctic divergence are fixed at 3.5 MYA. Bars to the right of the tree show taxon identity. Bootstrap percent support based on neighbor-joining using Tamura-Nei distance are given for branches defining major clades; values above branches are based on COI and values below branches are based on COIII. (There was only a single COIII *M. trossulus* M-mtDNA sequence, thus it is not possible to determine bootstrap support for that node with COIII.)

M-COI and M-COIII) gave a strong signal of zero gene flow, whereas both F-mtDNA and ITS showed clear evidence of gene flow between Atlantic USA and Europe (Fig. 5a: F-mtDNA: $M = 0.3$, $P < 0.05$; ITS: $M = 1.5$, $P < 0.01$). Our maximum-likelihood analyses found that the data across the three loci were significantly better fitted to two rates of gene

flow than to a single rate for all three loci (2 LLR = 4.5; $P < 0.04$, $M_{ITS,F-mtDNA} = 0.5$; 2 LLR = 10.9; $P < 0.001$).

Although the posterior probability surfaces for *M. edulis* trans-Atlantic divergences were qualitatively different among the three loci, the 95% credibility intervals were large and depended on the Bayesian priors. This property of MDIV

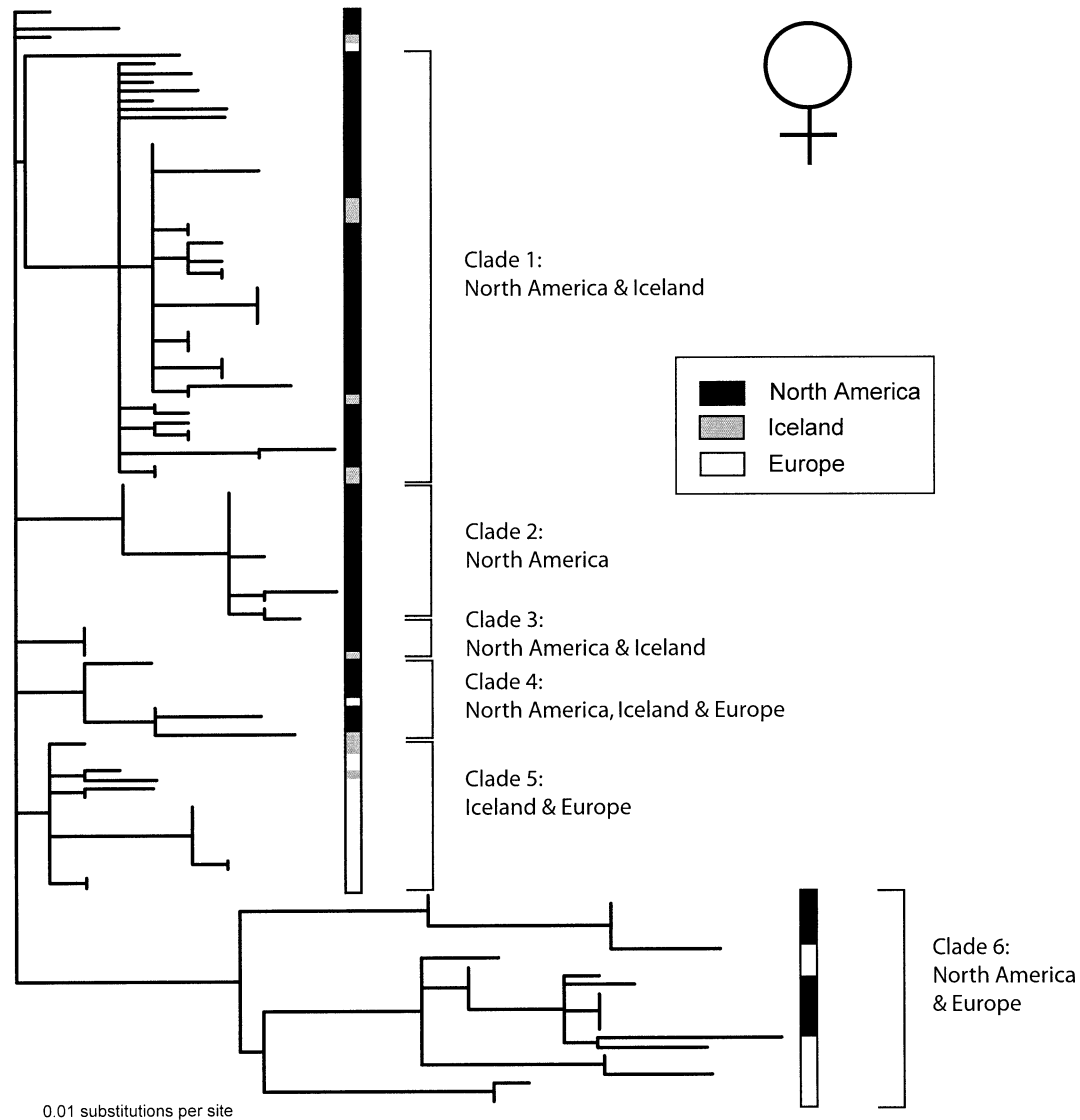


FIG. 3. Genealogy of *M. edulis* F-mtDNA haplotypes based on a Tamura-Nei plus gamma plus invariant sites model of evolution with midpoint rooting. Bars to the right of the tree show species identity and major clades within the female tree are numbered (see Results).

divergence estimates can be visualized in Figure 5b, where the posterior probabilities for F-mtDNA and ITS population divergences flatten for divergences older than their most-likely peak. This property is particularly acute when there has been substantial gene flow (Nielsen and Wakeley 2001), because recent gene flow reduces signals of old divergence times. Therefore, for both trans-Atlantic *M. edulis* F-mtDNA and ITS, there was considerable cumulative probability for a divergence time close to that estimated by M-mtDNA, and in fact the probability of two separate dates (M-mtDNA vs. F-mtDNA and ITS) was not strongly supported (2 LLR = 2.27; $P = 0.1$). The most likely date for all three loci was 1.2 MYA (0.44–1.5 MYA CI). The cumulative probability for a postglacial (<20,000 years) trans-Atlantic divergence was extremely low (combined likelihood model; $P = 0$, M-mtDNA; $P = 0$, F-mtDNA; $P = 0$, ITS; $P < 0.02$).

The locus with the best regional sampling was F-mtDNA,

thus it is possible to describe gene flow and historical separation among geographic regions in greater detail for this locus. As might be suspected by geography (Fig. 1) and genealogy (Fig. 3), the smallest estimates of gene flow were between Atlantic USA and Europe ($M = 0.3$, ns), whereas the Canadian Maritime and Iceland populations gave higher estimates of gene exchange both with the USA and Europe. MDIV estimated gene flow between the Canadian Maritimes and USA as $M > 2$ ($P < 0.02$), and between the Canadian Maritimes and Europe as $M = 0.9$ ($P < 0.02$). Similarly, the mid-Atlantic Iceland populations were estimated to exchange migrants with proximate Canadian Maritime and European populations at rates of $M > 2$ ($P < 0.04$) and $M = 1.2$ ($P < 0.03$), respectively.

DISCUSSION

Within *Mytilus edulis*, the genealogies of M-mtDNA, F-mtDNA, and ITS are quite different and individually give

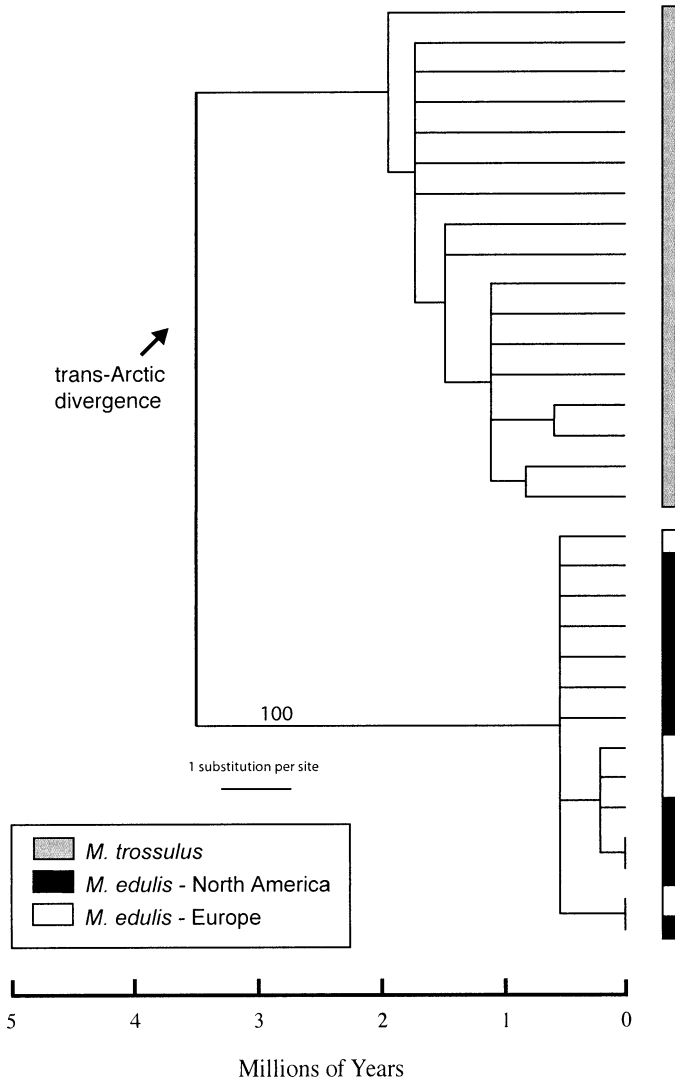


FIG. 4. ITS chronogram based on a Tamura-Nei plus gamma model of evolution with midpoint rooting. Bars to the right of the tree show taxon identity. Bootstrap percent support based on neighbor-joining using Tamura-Nei distance is given above the branch defining the major clades.

conflicting estimates of gene flow across the Atlantic Ocean and separation time between North American and European populations. In particular, recent coalescence of all *M. edulis* F-mtDNA haplotypes (Fig. 2) and ITS alleles (Fig. 4) would probably be interpreted as a recent historical division, especially if dating methods that assume no gene flow were employed (Table 3). Only by jointly estimating population divergence with gene flow (MDIV) and by considering the three loci together in a likelihood framework can we demonstrate that a single age of trans-Atlantic population divergence is plausible. The single date of 1.2 MYA suggests that the small trans-Atlantic genetic distances found for F-mtDNA and ITS loci (Table 3) result from the homogenizing effect of gene flow within *M. edulis*, which can yield a coalescence time more recent than the population divergence time (Rosenberg and Feldman 2002). Thus, it seems likely that trans-Atlantic genetic exchange of F-mtDNA and ITS has obscured our ability to detect a relatively old population divergence time, as reflected in the M-mtDNA lineage.

If discordances in genetic distances and coalescence times between loci are caused by gene flow, then the contrasting patterns of gene flow among loci are more difficult to reconcile. Simple inspection of the mtDNA genealogies point to qualitatively different patterns of trans-Atlantic gene flow for M- and F-mtDNA (Figs. 2 and 3), where recent gene flow is inconsistent with the topology of the M-mtDNA tree, but probable for F-mtDNA. These heuristic observations of apparent female gene flow are confirmed by the coalescent analyses; MDIV found statistically different rates of trans-Atlantic gene flow between M-mtDNA ($M = 0$) and F-mtDNA/ITS ($M = 0.5$; $P < 0.001$) (Fig. 5a).

Although our limited sampling of M-mtDNA and ITS precludes comparisons of gene flow and population divergence to F-mtDNA over a fine scale, such as along a single coastline, the pattern of greater geographic differentiation for M-mtDNA relative to F-mtDNA has also been reported within *M. edulis* from Great Britain (Skibinski et al. 1999) and within *M. galloprovincialis* (Ladoukakis et al. 2002). This pattern of greater M-mtDNA population differentiation has also been observed for freshwater unionid mussels, where M-mtDNA differentiation exceeded that found for either F-mtDNA or allozymes (Liu et al. 1996; Krebs 2004). Thus, it seems like

TABLE 3. *Mytilus edulis* trans-Atlantic divergence.

Gene	Genetic distance (%) ^a		Divergence date, years before present (MYA) ^b		
	No gene flow (Tamura-Nei)	With gene flow (MDIV)	No gene flow (Tamura-Nei)	With gene flow (MDIV)	Penalized likelihood (r8s)
Male mtDNA	7.16 (4.46–9.85)	7.0 ^c (3.0–9.3)	1.46 (0.91–2.00)	1.2 (0.49–1.5)	0.95 (0.66–1.40)
Female mtDNA	0.33 (0.16–0.50)	0.7 ^d (0.3–3.2)	0.07 (0.03–0.10)	0.2 (0.1–1.5)	0.21 ^e
ITS	0 (0–0.09)	0 ^d (0–6.0)	0 (0–0.09)	0 (0–1.4)	0.47 (0.24–0.87)

^a Estimates the branch lengths separating two populations, $2\mu t$. In parentheses are the 95% confidence intervals for Tamura-Nei and r8s, and 95% credibility intervals for MDIV within the time frame of 0–1.5 MYA.

^b Dates calibrated by a 3.5 MYA *M. edulis*–*M. trossulus* divergence.

^c Based on M-COI. M-COIII yields similar estimates.

^d Probability of gene flow is substantially greater than zero; gene flow estimates for trans-Atlantic *M. edulis* are $M = 0.3$ for F-mtDNA ($P < 0.05$) and $M = 1.5$ for ITS ($P < 0.01$) for $M = [0, 2]$.

^e Failed in search for confidence interval.

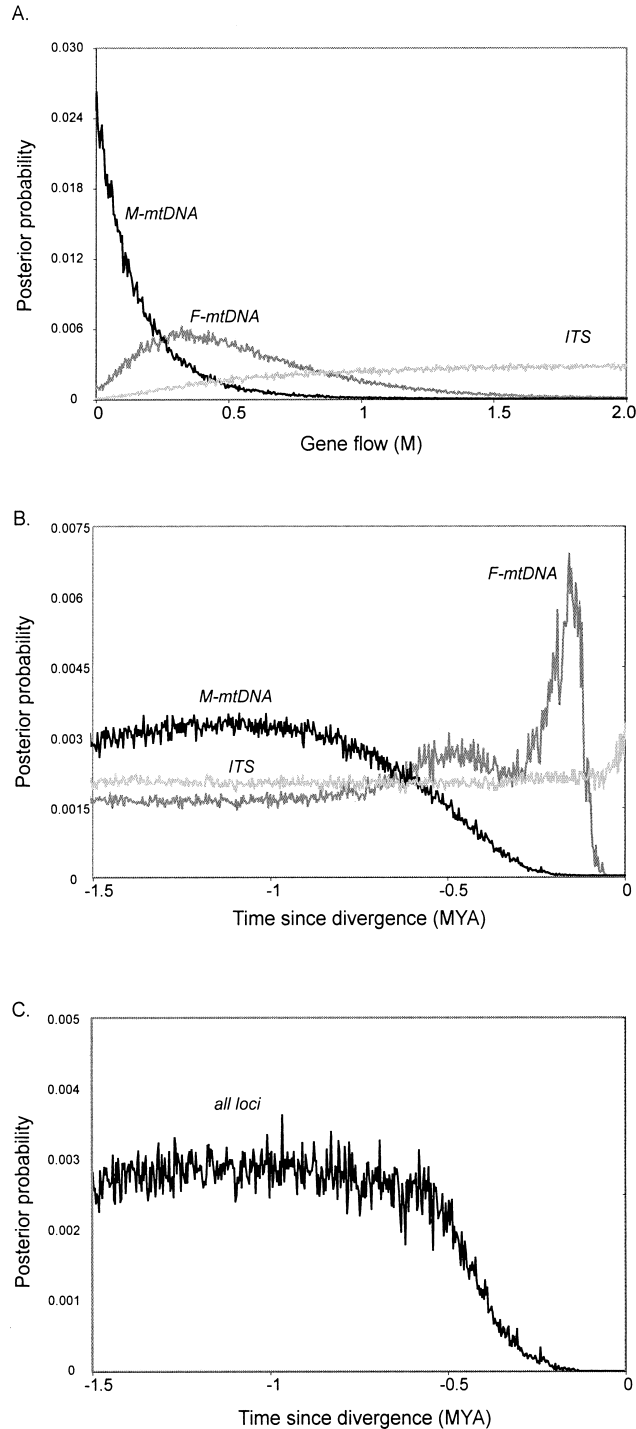


FIG. 5. Joint estimates of gene flow and time since divergence between North American and European *M. edulis* populations. (A) The posterior probability surfaces of gene flow ($M = 2Nm$) considering a range of priors from 0 to 2. (B) The posterior probability surfaces of population divergence dates for each gene, considering the time interval of 1.5 to 0 MYA. (C) The posterior probability surface of divergence dates for the multilocus model, combining M-mtDNA, F-mtDNA and ITS (see Methods and Results). In all cases, F-mtDNA probabilities are estimated from COI and COIII, and M-mtDNA probabilities from COIII (the M-COI probability distributions are nearly identical to the M-COIII probability distributions shown here).

a general feature of DUI for M-mtDNA to exhibit greater differentiation, and by inference lower gene flow, than F-mtDNA.

In contrast to vertebrates where sex-specific behavior can cause dispersal to differ between males and females, for mussels there are no obvious biological reasons why females (or maternally transmitted F-mtDNA) would have a higher gene flow than males (or paternally transmitted M-mtDNA) (see Introduction). It is difficult to imagine that female larvae consistently disperse further than male larvae, although this possibility cannot be completely excluded. In the following discussion, we consider specific demographic scenarios and selective pressures that could also cause the observed pattern of reduced M-mtDNA gene flow.

Demographic Explanations for Reduced Trans-Atlantic Gene Flow

Differences in effective population sizes between male and female mussels or between M- and F-mtDNA might account for differences in gene flow. A smaller male effective population size would also experience a greater rate of lineage sorting, leading to shorter coalescence times of haplotypes within each coastline. For mtDNA, gene flow is $M = 2Nm$, where N is effective population size and m is the migration rate. If the total effective population size of male mussels (N_M) were substantially less than the female effective population size (N_F), then gene flow ($2Nm$) estimated from paternally inherited M-mtDNA would be lower than estimates from maternally inherited F-mtDNA. Uneven sex ratios are unlikely to explain the reduction in *M. edulis* M-mtDNA gene flow, as ratios of females to males are not systematically biased (Rawson et al. 1996; Saavedra et al. 1996; Comesaña et al. 1999). In contrast, in pearl oysters, the effective population size of males is much greater than females and, thus, gene flow for F-mtDNA is reduced relative to biparentally inherited loci (Arnaud-Haond et al. 2003). Pearl oysters are protandrous hermaphrodites so that small males outnumber large females, and gene flow estimated from mtDNA is not representative of gene flow across the total genome. *Mytilus* spp. are primarily gonochoristic, but males may be more subject to “sweepstakes” reproduction (Hedgecock 1994), leading to a higher variance in reproductive success and a reduced male effective population size even if the census populations of males and females are equal. Either uneven sex ratios or greater variance in male reproductive success should affect all paternally inherited loci, not just M-mtDNA.

Alternatively, the effective population sizes of M-mtDNA (but not necessarily male mussels) might be reduced relative to F-mtDNA. While double uniparental transmission of mtDNA should result in equal population sizes of F- and M-mtDNA (F-mtDNA in male mussels are not transmitted to the next generation), sperm contain fewer copies of M-mtDNA than copies of F-mtDNA in eggs, increasing the potential effect of genetic drift on M-mtDNA (Stewart et al. 1996). In addition, some proportion of male mussels have nonstandard “masculinized” M-mtDNA (Quesada et al. 1999; Ladoukakis et al. 2002), possibly reducing the effective population size of the standard M-mtDNA lineage (which we have employed here) in comparison to F-mtDNA.

Regardless of specific scenarios, our data do not show a lower effective population size for M-mtDNA (or male mussels) compared to F-mtDNA (or female mussels). In general, estimates of *M. edulis* M-mtDNA gene diversity ($2N\mu$) are approximately twice as great as F-mtDNA (Quesada et al. 1998a; Skibinski et al. 1999; but for a different pattern see Ladoukakis et al. 2002). Even when gene diversity ($2N\mu$) is corrected for mutation rate (μ), the effective population sizes for American *M. edulis* M-mtDNA and F-mtDNA are about the same (assuming our molecular clock estimates are proportional to the actual neutral mutation rates), and the effective population size for European M-mtDNA is actually larger than for F-mtDNA (see Skibinski et al. 1999 for an extended discussion). Overall, there is no compelling evidence for a reduction in standard M-mtDNA effective population size relative to F-mtDNA, which would reduce estimates of gene flow.

Selective Explanations and Genetic Barriers to Trans-Atlantic Gene Flow

In the absence of any conclusive natural history or demographic explanations, selective causes for the contrasting patterns of F- and M-mtDNA must be considered. One obvious explanation for M-mtDNA trans-Atlantic reciprocal monophyly is directional selection on M-mtDNA. If M-mtDNA has been periodically subject to selective sweeps, within-population variation would be reduced, reciprocal monophyly between North American and European groups would be achieved faster than under neutral conditions, and historical signals of gene flow would be obscured. A significantly negative Tajima's D was found for European M-mtDNA (Table 2), but not for North American M-mtDNA, which also forms a unique clade. Our McDonald-Kreitman tests did not show any evidence for positive selection within male lineages (as would be expected from selective sweeps) nor have other studies (Quesada et al. 1998a; 1999; Skibinski et al. 1999). In fact, the overall pattern of genetic variation within the *Mytilus* M-mtDNA lineage points to a relaxation of selective constraint, where nonsynonymous substitutions are common (Stewart et al. 1995; 1996; Quesada et al. 1998a; 1999; but see Skibinski et al. 1999; Skibinski 2000 who suggest that purifying selection may also be affecting M-mtDNA, albeit at a lower degree than for F-mtDNA). Although it is not possible to exclude the possibility that a past selective sweep drove a clade to fixation and was followed by a period of relaxed selective constraint, the observed patterns of M-mtDNA genetic variation are inconsistent with repeated selective sweeps. Even if a selective sweep was responsible for early reciprocal monophyly in M-mtDNA, it does not explain why there has been no recent trans-Atlantic M-mtDNA exchange, as observed for F-mtDNA.

Whereas positive directional selection on M-mtDNA seems unlikely, selection in the form of genetic barriers could prevent trans-Atlantic gene exchange for M-mtDNA and have less effect on F-mtDNA and ITS. The boundaries between many species are probably semipermeable to gene flow (sensu Key 1968; Harrison 1990), with the strength of barriers differing among loci (Harrison 1986). For example, multilocus sequence studies of both fruit flies (Machado et al. 2002) and

mosquitoes (Besansky et al. 2003) have revealed gene flow at some, but not all, loci following speciation.

Although North American and European *M. edulis* are considered the same species, these allopatric populations may have evolved some degree of reproductive isolation that is sex-biased in its expression or directly involves mtDNA. In the former case, any number of pre- or postzygotic isolating mechanisms could differentially reduce the fitness of trans-Atlantic male migrants. For example, spawning might be asynchronous between migrant males and local females, hybrid male progeny might be inviable or infertile, etc. Any one of these scenarios should affect all paternally inherited loci and would yield a pattern of greatly reduced trans-Atlantic gene flow relative to biparentally and maternally inherited loci.

It is worth noting that males are the heterogametic sex in *Mytilus* (at least for mtDNA; sex chromosomes are unknown in mussels), and under Haldane's Rule, the heterogametic sex is expected to be less fit in hybrid crosses. Fitness differences between hybrid males and females could, in turn, affect M-mtDNA gene flow relative to biparentally and maternally inherited loci. For example, in birds, females are the heterogametic sex and (F-)mtDNA introgression among gulls (Crochet et al. 2003) and Chiffchaff species (Helbig et al. 2001) is reduced relative to nuclear loci, perhaps as a consequence of Haldane's Rule.

It is also possible that M-mtDNA are directly the targets of selection and that migrant M-mtDNA are purged from local populations. Because all mtDNA are unlinked to other (nuclear) genes, the introgression of mtDNA onto genetically disparate backgrounds should, theoretically, be less impeded than introgression of nuclear genes (Barton and Jones 1983). However, empirical studies of fruit flies and copepods have demonstrated coadaptation of mtDNA (or maternal cytoplasmic elements) and nuclear genes, where hybrids with mtDNA and nuclear genes from different geographic populations show a reduction in fitness (Clark and Lyckegaard 1988; Rawson and Burton 2002), the manifestation of which can vary between the sexes (Rand et al. 2001; Willet and Burton 2001).

Direct selection on mtDNA seems especially likely in mussels where patterns of mtDNA inheritance and sex determination are associated. Experimental crosses of *M. edulis* and *M. galloprovincialis* have demonstrated a strong maternal effect on the ratio of male to female progeny (Saavedra et al. 1997; Kenchington et al. 2002). These authors propose that the inheritance of M-mtDNA and male sex determination are controlled by a maternal nuclear factor found in the egg cytoplasm. It is possible that M-mtDNA is inhibited from introgressing onto novel nuclear backgrounds by this same female nuclear factor. In fact, in mussel hybrid zones, the normal DUI of mtDNA and sex determination frequently breaks down, with possibly greater effects on M-mtDNA inheritance than F-mtDNA inheritance (Zouros et al. 1994b; Rawson et al. 1996; Saavedra et al. 1996; Comesaña et al. 1999; Quesada et al. 1999). Similarly, in some hybrid zones *M. edulis* F-mtDNA asymmetrically introgresses onto *M. galloprovincialis* and *M. trossulus* nuclear backgrounds (Saavedra et al. 1996; Quesada et al. 1998b; Rawson and Hilbish 1998; Comesaña et al. 1999; Quesada et al. 1999), but asymmetric M-mtDNA introgression has not been observed. Furthermore,

in crosses between European *M. edulis* and *M. galloprovincialis*, M-mtDNA from *M. edulis* are disproportionately eliminated (Wood et al. 2003).

Our observation of zero M-mtDNA gene flow between trans-Atlantic populations of *M. edulis* is perfectly consistent with the general pattern that M-mtDNA introgression between species is more constrained than F-mtDNA introgression. However, we have only surveyed three loci. Allozymes also show a range of genetic differentiation across the Atlantic Ocean. In an extensive survey of six allozyme loci, Varvio et al. (1988) found four loci with negligible trans-Atlantic population structure (like ITS) and two loci (*Gpi* and *Ap*) with somewhat different allele frequencies between North America and Europe. This could reflect real differences in trans-Atlantic gene flow among these nuclear loci or be due to incomplete lineage sorting of ancestral polymorphism following population divergence. Detailed studies of several nuclear loci could help determine whether barriers to gene flow are common among nuclear loci or just restricted to M-mtDNA (see Machado et al. 2002; Besansky et al. 2003). Experimental crosses between North American and European *M. edulis* and examination of hybrid fitness would also be useful for testing the hypothesis that genetic barriers prevent trans-Atlantic M-mtDNA gene flow.

Recent History of Mytilus edulis in the North Atlantic

The detection of trans-Atlantic gene flow for F-mtDNA and ITS implies that individual mussels occasionally cross large distances in the Atlantic Ocean. Icelandic populations most likely serve as stepping stones between North America and Europe. For example, Icelandic F-mtDNA haplotypes nest within both North American and European clades (Fig. 3), suggesting gene flow between these regions (and supported by coalescent analysis of F-mtDNA; see Results). The three M-mtDNA haplotypes detected from Iceland fall within the European clade, but with such a small sample size and the suspicion of selection against some M-mtDNA, we cannot draw any conclusions from M-mtDNA regarding the movement of migrant mussels between North America and Iceland.

Although individual mussels may sometimes traverse the Atlantic Ocean, the relatively old coalescence time of M-mtDNA implies that North American and European M-mtDNA have had separate histories at least since about 1.2 MYA (Fig. 5c). This date could either reflect real divergence between North American and European *M. edulis* populations (colonization of one of the coastlines) or could reflect the origin of a genetic barrier to M-mtDNA exchange. In either case, North American and European lineages have persisted in situ for a long period of time, and this observation is not consistent with recent colonization of either region. Indeed, the cumulative probability for a recent, postglacial divergence (<20,000 years) is exceedingly low ($P = 0$; see Results).

It is well known that molecular clocks, such as those employed here are subject to tremendous variance and also rely on the accuracy of the dating of the calibrated node. The dates reported here represent best guesses. Nevertheless, it seems clear that mussels have been continuously populating both North American and European coastlines for much of

the Pleistocene. Thus, our conclusions confirm Wares and Cunningham's (2001) suggestion that *M. edulis* has survived on both coasts of the North Atlantic in glacial refugia for at least one, and probably several, glacial cycles.

Conclusions

In *M. edulis*, we find extreme differences in trans-Atlantic gene flow inferred from M-mtDNA, F-mtDNA, and ITS, which in turn greatly affect our estimates of population divergence times across the Atlantic Ocean. No aspect of mussel larval biology provides a satisfying explanation for the absence of M-mtDNA gene flow. We have suggested that genetic barriers, which may be associated with the DUI of mtDNA in mussels, prevent migrant M-mtDNA from invading genetically diverged populations. Although this explanation does invoke selection, this type of selection is not detectable at the nucleotide level and can only be discerned by comparisons to other independently assorting loci.

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