doi:10.1111/j.1558-5646.2007.00073.x

ADAPTIVE GAMETE-RECOGNITION DIVERGENCE IN A HYBRIDIZING *MYTILUS* POPULATION

Stevan A. Springer[1,2] and Bernard J. Crespi[1]

[1] *Department of Biosciences, Behavioural Ecology Research Group, Simon Fraser University, Burnaby, British Columbia, V5A 1S6, Canada*

Received July 5, 2006
Accepted December 10, 2006

Gamete-recognition proteins often evolve rapidly, but it is not known if their divergence occurs within species and corresponds with the evolution of reproductive isolation, or if divergence typically accumulates between already isolated lineages. We examined the evolution of a candidate gamete-recognition protein in several sympatric and allopatric populations of *Mytilus* blue mussels, species that hybridize in nature. Within a single species, *Mytilus galloprovincialis*, we found adaptive divergence of Lysin-M7, a sperm acrosomal protein that dissolves the egg vitelline envelope during fertilization. *Mytilus galloprovincialis* Lysin-M7 alleles group into two distinct clades (termed G and G_D), and individual alleles in these clades are separated from each other by at least three and up to eleven amino-acid substitutions. Maximum-likelihood estimates of selective pressure ($dN/dS = \omega$) implicate selection in the divergence between *M. galloprovincialis* Lysin-M7 clades, and within the G_D clade. Exact tests of population differentiation indicate that the relative frequency of G and G_D Lysin-M7 alleles differs significantly among *M. galloprovincialis* populations. Compared with allopatric Mediterranean samples, Lysin-M7 alleles in the G_D clade are found at elevated frequency in samples from the East Atlantic and California, areas of secondary contact and hybridization between *Mytilus* species, and Australia, an area of unknown species composition. Adaptive divergence between the alleles most common in allopatry and those found at elevated frequency in samples from sympatry suggests that selection pressures acting in hybridizing populations, likely following Pleistocene secondary contact with *M. edulis* in the East Atlantic, drove the divergence of Lysin-M7 in *M. galloprovincialis*.

KEY WORDS: **Adaptive evolution, gamete-recognition, hybridization, *Mytilus,* prezygotic isolation, reinforcement, sexual conflict.**

Gamete-recognition proteins are gene products that mediate sperm–egg interactions during fertilization. Gamete incompatibility can result from a mismatch between sperm and egg recognition proteins, and these failures during fertilization are sufficient to reproductively isolate even closely related taxa (Metz et al. 1994; Palumbi 1998; Swanson and Vacquier 2002). Studies of the molecular evolution of gamete-recognition proteins yield a pattern which is therefore quite remarkable: both sperm and egg recognition proteins are often subject to positive selection that drives rapid divergence between species (Swanson and Vacquier 2002). Surveys of variation at loci encoding gamete-recognition proteins indicate that adaptive protein divergence is often found between species that are broadly sympatric: abalone (Vacquier et al. 1997; Yang et al. 2000), *Tegula* (Hellberg and Vacquier 1999), and sea urchins (Geyer and Palumbi 2003; Zigler and Lessios 2003; McCartney and Lessios 2004). This pattern is not always evident between species that diverged in allopatry (Metz et al. 1998). The divergence of gamete-recognition proteins is sufficient to produce strong reproductive isolation between species, as evidenced by fertilization or vitelline envelope dissolution assays in sea urchins (Metz et al. 1994; McCartney and Lessios 2002), abalone (Swanson and Vacquier 1997), and *Tegula* (Hellberg and Vacquier 1999). Gametic isolation due to the divergence of gamete-recognition proteins may therefore be a primary mechanism maintaining

[2] Present address: Department of Biology, University of Washington, Seattle, Washington 98195. E-mail: stevan@u.washington.edu.

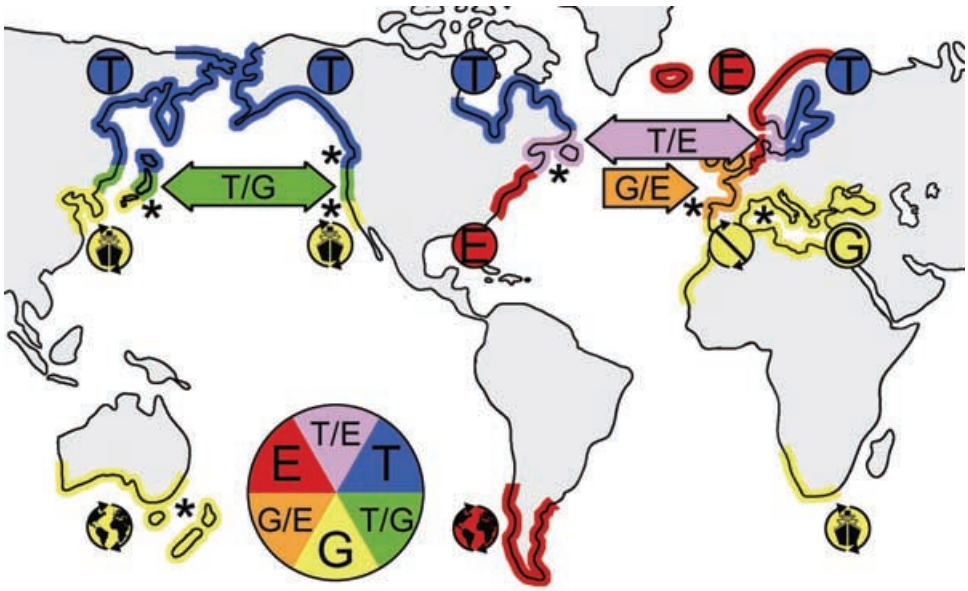


Figure 1. Global distribution of hybridizing *Mytilus* species. Primary colors represent allopatric populations: *Mytilus galloprovincialis* (yellow), *M. edulis* (red), *M. trossulus* (blue). Hybrid populations are shown with the appropriate secondary color. Circled letters are adjacent to populations in their natural range, globes represent populations transported during the Pleistocene transatlantic exchange (~1.5 million years ago; Hilbish et al. 2000), ships identify populations introduced by human activities (McDonald and Koehn 1988; Geller et al. 1994), the voided arrows denote an oceanographic barrier that divides genetically distinct *M. galloprovincialis* populations (Quesada et al. 1995; Sanjuan et al. 1997; Daguin and Borsa 1999). Two *M. trossulus*, *M. galloprovincialis* hybrid zones exist in the Pacific and two *M. trossulus*, *M. edulis* hybrids zones in the Atlantic. The origin of Baltic *M. trossulus* and the species composition of southern hemisphere populations are uncertain. Asterisks indicate sampled regions; sampling details for each region are in Table 1.

species boundaries in sympatric populations of broadcast-spawning taxa.

In addition to maintaining existing species boundaries, gamete-recognition divergence could also contribute to the origin of new species, but only if adaptive divergence contributes to initial reductions in reproductive compatibility between incipient species, and not just species differences following speciation (Templeton 1981; Butlin 1987). Gamete-recognition protein divergence accumulates more rapidly among taxa with sympatric ranges than those that likely diverged in allopatry (Yang et al. 2000; Zigler and Lessios 2003), but adaptive gamete-recognition protein divergence can, and does, continue to accumulate in sympatric populations after reproductive isolation is complete (Geyer and Palumbi 2003). It is therefore difficult to determine if gamete-recognition divergence coincides with the evolution of reproductive isolation, or if divergence typically accumulates between lineages that are already reproductively isolated; yet this is a crucial distinction for studies of speciation. Evaluating the divergence of gamete-recognition proteins in naturally hybridizing populations could provide direct evidence that gamete-recognition evolution contributes to the early stages of the evolution of reproductive isolation (Templeton 1981; Butlin 1987). However, natural hybrids are rare among most species whose gamete-recognition proteins have thus far been studied (Palumbi and Metz 1991; Brown 1995).

Mytilus blue mussels are globally distributed marine bivalves common to temperate and polar rocky intertidal shores, and share many of the life-history traits common to the broadcast-spawning marine invertebrates discussed above. However, *Mytilus* species are only partially reproductively isolated and produce natural hybrids in areas of sympatry (Hilbish et al. 2000; Fig. 1). Three *Mytilus* species hybridize in nature: *Mytilus trossulus*, native to the North Atlantic and Pacific, and the sister-species *M. edulis,* native to the Atlantic coasts of Europe and North America, and *M. galloprovincialis*, native to the Mediterranean (Hilbish et al. 2000; Fig. 1). Range overlap following movement of *M. trossulus* from the Pacific has produced a pair of hybrid zones between *M. edulis* and *M. trossulus* one in the western North Atlantic, and another in the Baltic Sea (Riginos and Cunningham 2005). Relaxation of a barrier to oceanographic exchange separating the Mediterranean and Atlantic Ocean basins resulted in secondary contact of *M. edulis* and *M. galloprovincialis* during the Pleistocene (Gardner 1994; Quesada et al. 1995). These two taxa now form an extensive hybrid zone on the Atlantic coast of Europe between Spain and Britain (Bierne et al. 2003b). The natural ranges of *M. galloprovincialis* and *M. trossulus* do not overlap, but transport in shipping vessel ballast water has introduced *M. galloprovincialis* to the Pacific, creating secondary contact with *M. trossulus* and resulting in a pair of hybrid zones, one in California

(McDonald and Koehn 1988; Geller et al. 1994; Rawson et al. 1999) and another in Japan (Suchanek et al. 1997; Skurikhina et al. 2001).

Mytilus species remain genetically distinct, despite hybridization and considerable larval dispersal potential, because of the combined action of a number of barriers to interspecific gene exchange. Partial postzygotic isolation due to endogenous hybrid incompatibility has been documented between *M. edulis* and *M. galloprovincialis* (Bierne et al. 2006) and similar barriers likely exist between these species and the more distantly related *M. trossulus*. Partial prezygotic isolation between *Mytilus* species results from spatial separation due to habitat isolation (Gosling and McGrath 1990; Bierne et al. 2003a), asynchronous spawning (Toro et al. 2002; Bierne et al. 2003a), and assortative gamete interaction (Bierne et al. 2002; Rawson et al. 2003).

No specific molecular interaction has yet been implicated in creating assortative gamete interaction or fusion in *Mytilus*, but biochemical assays examining the effect of egg compounds added to sperm–egg suspensions on fertilization rates suggest that variation in carbohydrate recognition may be an important component of the sperm–egg recognition process (Focarelli et al. 1991; Togo and Morisawa 1997). The most thoroughly characterized gamete-recognition protein candidates in *Mytilus* are the lysins, a family of sperm acrosomal proteins named for their lytic activity against the egg vitelline envelope (Takagi et al. 1994). Three members of the lysin gene family, Lysin-M3, M6, and M7, together comprise approximately 70% of *Mytilus* acrosomal protein content (Takagi et al. 1994). Conserved features of their primary amino-acid sequence indicate that *Mytilus* lysins are C-type lectins, members of a protein family whose carbohydrate-binding activity has been subject to detailed structural and functional investigations (Takagi et al. 1994; Drickamer and Fadden 2002). A previous study examined the molecular evolution of Lysin-M7 between allopatric populations of *Mytilus* species and found evidence of a selective sweep, resulting in a single amino-acid replacement fixed between *M. edulis* and *M. galloprovincialis* alleles (Riginos and McDonald 2003).

Here we examine the possibility that selective factors acting in naturally hybridizing populations drive the evolution of gamete-recognition proteins. In surveys of Lysin-M7 polymorphism in multiple sympatric and allopatric *Mytilus* populations, we found two distinct Lysin-M7 clades within a single species, *M. galloprovincialis*. Sequence differences between these two clades appear to result from the adaptive divergence of one of the two clades (G_D). Lysin-M7 G_D alleles are more abundant in samples from the East Atlantic than they are in allopatric *M. galloprovincialis* samples, and the divergence of G_D may have been a consequence of secondary contact and hybridization between *M. galloprovincialis* and *M. edulis*.

Methods

SAMPLING

Individual mussels >30 mm shell length were collected from seven regions worldwide: four regions where *Mytilus* species are known to co-occur and hybridize, and three regions known to contain only a single *Mytilus* species (Hilbish et al. 2000). Subsamples were collected from geographically separated locations in three of these regions; sampling locations and dates are detailed in Table 1. All sampled mussels were collected from subtidal low wave-action sites, and reproductive maturity was checked by visual inspection of gonad tissue. Immature and spent individuals were not retained for analysis. Archival mantle tissue from sampled individuals was stored in 95% ethanol at −20°C, and gonad tissue from reproductively mature individuals was preserved in

Table 1. Sampling locations and dates.

Population allo/sympatric	Location	*N*	Sampling date	Coordinates
Mediterranean	Venice Italy (VE)	10	February, 2003	45°25′45″N 12°19′40″E
G (Allopatric)	Rapallo Italy (RP)	10	February, 2003	44°20′55″N 9°13′51″E
	Iraklion Greece (IK)	9	February, 2003	35°20′33″N 25°08′06″E
Atlantic-East G/E (sympatric)	Vigo Spain (VG)	26	May, 2004	42°13′45″N 8°42′50″W
Pacific-California T/G (sympatric)	Eureka, CA (EK)	8	January, 2002	40°48′33″N 124°07′30″W
	San Raphael, CA (RF)	4	January, 2002	37°53′05″N 122°26′35″W
	Oyster Point, CA (OP)	5	January, 2002	37°35′18″N 122°19′02″W
	Morro Bay, CA (MB)	12	January, 2002	35°19′45″N 120°49′57″W
	San Diego, CA (SD)	12	January, 2002	32°38′02″N 117°06′32″W
Pacific-Japan T/G (sympatric)	Kobe Japan (KO)	14	April, 2002	34°40′44″N 135°10′32″E
Australia G (unknown)	Sydney Australia (AU)	8	March, 2003	33°52′29″S 151°13′51″E
Atlantic-West T/E (sympatric)	Clarenville, NF (NF)	14	July, 2001	48°09′00″N 53°53′56″W
Pacific-North T (allopatric)	Haynes, AK (HA)	4	April, 2002	59°14′23″N 135°26′32″W
	Oyster Bay, BC (OB)	17	August, 2000	49°57′53″N 125°12′23″W

RNALater (Ambion, Applied Biosystems, St. Austin, TX, USA at −20°C.

GENOTYPING AND SEQUENCING

All sampled individuals were genotyped at two marker loci: GLU (Rawson et al. 1996), which is diagnostic for all three *Mytilus* species, and EFbis (Bierne et al. 2002), which can resolve *M. galloprovincialis* and *M. edulis*, but produces bands of similar size for *M. edulis* and *M. trossulus* on agarose gels. The GLU and EFbis loci were amplified using published PCR primers and reaction conditions. Primers that amplify the 540 bp (base pairs) coding region of Lysin-M7 and flanking regions from cDNA (M7-143F, 5′ GTC TTT TGT GCA GCA CTT ATT GT 3′ and M7-874R 5′ TTC ACT CTT CAC TTG TCA TGT G 3′) were designed using published Lysin-M7 cDNA sequence (Takagi et al. 1994). PCR amplifications of Lysin-M7 (35 cycles of 94°C, 55°C, and 72°C for 30 sec each, followed by a 5 min 72°C extension) were performed on cDNA reverse transcribed (Amersham RT-PCR Beads) from the total RNA of mature gonad tissue (Qiagen RNeasy). PCR products were cloned (Qiagen PCR Cloning^{plus}) and at least five clones per individual were sequenced on both strands (Perkin Elmer Big Dye Terminator, ABI automated sequencer) using SP6 and T7 primers. A maximum of two alleles were recovered from any one individual suggesting that these PCR primers amplify a single expressed locus. Lysin-M7 sequences are reproducible across reverse transciption polymerase chain reaction (RT-PCR) reactions, clones, and individuals, and are therefore not the product of PCR template switching or RT-PCR artefacts. Sequences have NCBI GenBank Accession numbers: Lysin-M7, EF079677–EF079825, and D14731 (Takagi et al. 1994); Lysin-M6, JX0349 (Takagi et al. 1994); and AY131183 (Riginos and McDonald 2003).

ANALYSIS

A network of genealogical relationships was estimated by statistical parsimony at 95% parsimony probability using TCS (Clement et al. 2000). Phylogenetic relationships and topology confidence estimates were generated by PAUP 4.0b10 (neighbor-joining uncorrected p distance, 100,000 bootstrap replicates, 80% majority-rule consensus) (Swofford 2001). Clade specific maximum-likelihood estimates of nonsynonymous (dN) and synonymous (dS) substitution rate ratios (dN/dS = ω) and associated likelihood ratio test statistics, were generated using PAML 3.13 (Yang 1997). Pairwise maximum-likelihood ω-ratio estimates were also made with PAML (codon frequency = F3X4, κ estimated).

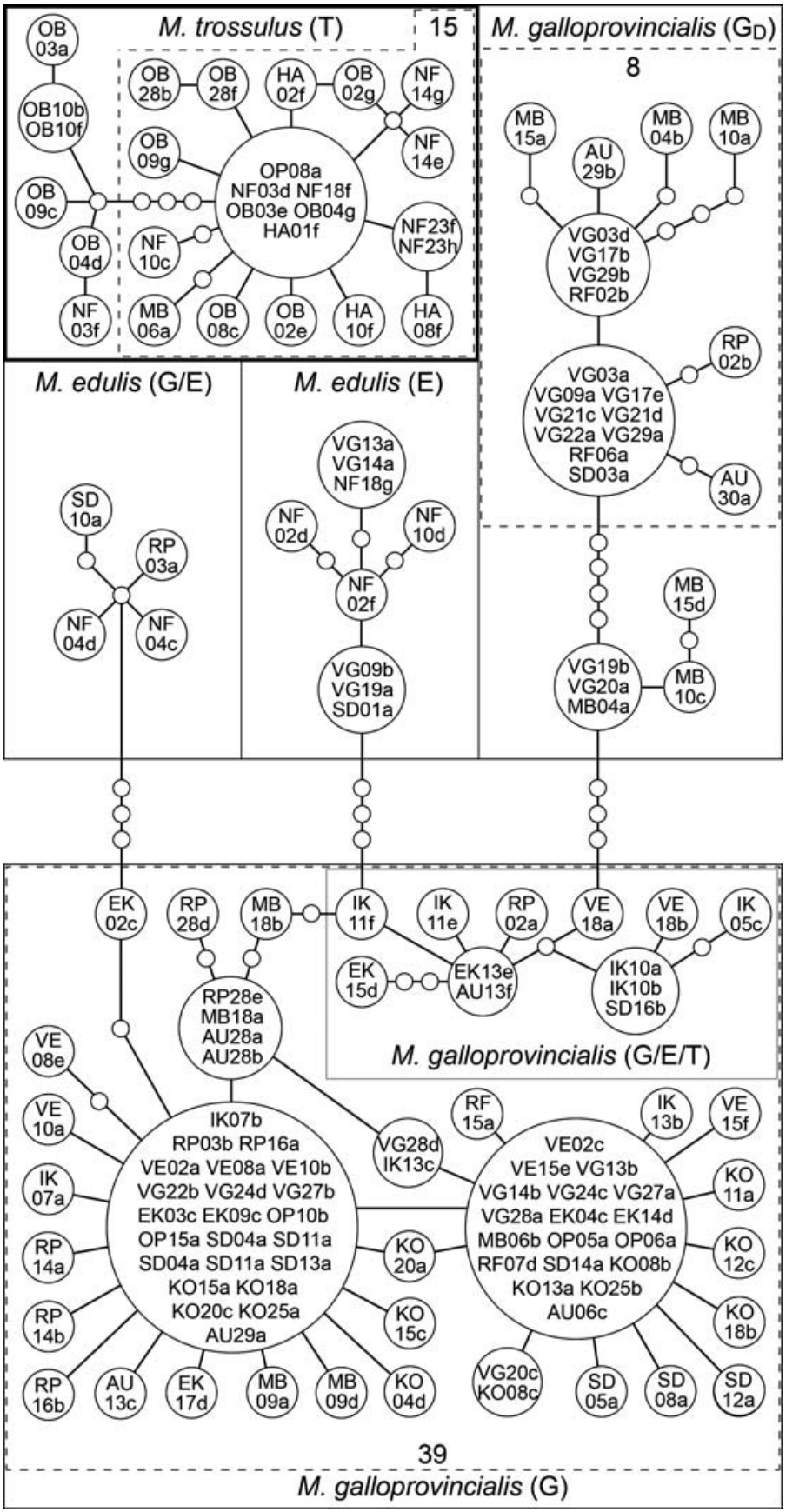
Protein sequence alignments of the C-type lectin carbohydrate-recognition domain of human tetranectin (PDB: 1TN3) and Lysin-M7 were generated by ESyPred3D (Lambert et al. 2002). Homology between these two carbohydrate-recognition domains was used to model the three-dimensional structure of Lysin-M7 based on the crystal structure of human tetranectin (percent amino-acid identity with human tetranectin carbohydrate-recognition domain: T 25.9%, G_D 27.3%). Structural models were generated by ESyPred3D and visualized in Pymol (Delano 2002).

Exact tests of sample differentiation (10,000 Markov chain, 1,000 dememorization steps) (Raymond and Rousset 1995) were conducted using Arlequin 2.001 (Schneider et al. 2000) based on the frequency of G, and G_D alleles in the California, Japan, Australia, East Atlantic, and Mediterranean population samples. The possible presence of recombination events was assessed with the four-gamete test using DNAsp 3.0 (Hudson and Kaplan 1985; Rozas and Rozas 1999), and also using a Genetic Algorithm for Recombination Detection (GARD) implemented in HyPhy on the Datamonkey server (Pond and Frost 2005; Pond et al. 2006).

Results

Lysin-M7 alleles group into five clades whose species composition were inferred using two diagnostic species markers, GLU and EFbis (Individual genotypes at all three loci are included as supplementary material). The data reveal two divergent *M. galloprovincialis* clades (G and G_D), one *M. trossulus* clade (T), and two *M. edulis* clades (E and G/E) (Figs. 2 and 3). Analyzing the two *M. edulis* clades separately or together does not significantly change parameter estimates for other clades, and we report estimates assuming a single ω-ratio for all *M. edulis* alleles. Branch-specific maximum-likelihood estimates of selection (Yang 1997) on the complete Lysin-M7 coding region detect adaptive evolution ($\omega > 1$) among divergent *M. galloprovincialis* (G_D) alleles and between these alleles and previously identified *M. galloprovincialis* (G) alleles (Takagi et al. 1994; Riginos and McDonald 2003; Tables 2 and 3, Fig. 3). Pairwise maximum-likelihood estimates are consistent with this result, finding positive selection only in the G_D radiation; within clades (G_D, max $\omega = 2.762$; G, max $\omega = 1.039$; E, max $\omega = 0.575$; T, max $\omega = 0.679$) and between clades (G_D|G, max $\omega = 3.893$, G_D|E, max $\omega = 1.006$; G_D|T, max $\omega = 0.546$; all other comparisons, max $\omega = 0.453$).

Figure 2. Genealogical relationships among *Mytilus* Lysin-M7 alleles. Black boxes delimit the clades identified by neighbor-joining bootstrap at 80% consensus (see Methods). The alleles in dashed boxes are represented by triangles in Figure 3. *Mytilus galloprovincialis* G/E/T alleles are shown in a gray box to facilitate comparison with Table 5. *Mytilus trossulus* alleles form an independent network, the number of differences between *M. trossulus* and other *Mytilus* clades exceeds the 95% probable parsimony limit of 10 mutational steps. Individual genotypes at all three loci are available as supplementary material.


M. trossulus (T)
15
OB 03a
OB10b OB10f
OB 09c
OB 04d
NF 03f
OB 28b
OB 28f
HA 02f
OB 02g
NF 14g
NF 14e
OB 09g
OP08a NF03d NF18f OB03e OB04g HA01f
NF 10c
NF23f NF23h
MB 06a
OB 08c
OB 02e
HA 10f
HA 08f
M. galloprovincialis (GD)
8
MB 15a
AU 29b
MB 04b
MB 10a
VG03d VG17b VG29b RF02b
RP 02b
VG03a VG09a VG17e VG21c VG21d VG22a VG29a RF06a SD03a
AU 30a
MB 15d
VG19b VG20a MB04a
MB 10c
M. edulis (G/E)
SD 10a
RP 03a
NF 04d
NF 04c
M. edulis (E)
VG13a VG14a NF18g
NF 02d
NF 10d
NF 02f
VG09b VG19a SD01a
EK 02c
RP 28d
MB 18b
IK 11f
IK 11e
RP 02a
VE 18a
VE 18b
IK 05c
EK 15d
EK13e AU13f
IK10a IK10b SD16b
M. galloprovincialis (G/E/T)
RP28e MB18a AU28a AU28b
VE 08e
VE 10a
IK 07a
RP 14a
RP 14b
RP 16b
AU 13c
EK 17d
MB 09a
MB 09d
KO 04d
KO 15c
KO 20a
VG28d IK13c
IK07b RP03b RP16a VE02a VE08a VE10b VG22b VG24d VG27b EK03c EK09c OP10b OP15a SD04a SD11a SD04a SD11a SD13a KO15a KO18a KO20c KO25a AU29a
RF 15a
IK 13b
VE 15f
KO 11a
KO 12c
KO 18b
VE02c VE15e VG13b VG14b VG24c VG27a VG28a EK04c EK14d MB06b OP05a OP06a RF07d SD14a KO08b KO13a KO25b AU06c
VG20c KO08c
SD 05a
SD 08a
SD 12a
39
M. galloprovincialis (G)

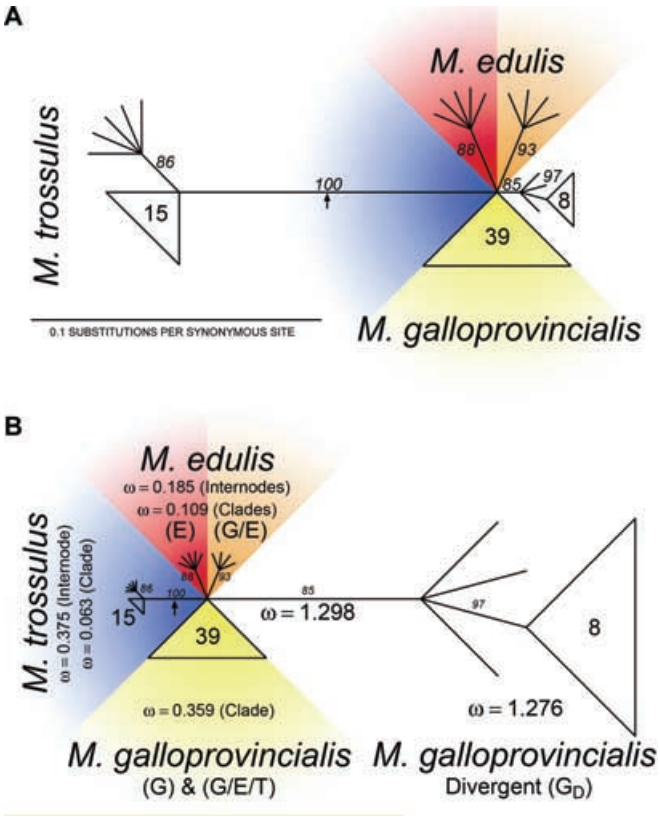


Figure 3. **Phylogenetic relationships among unique *Mytilus* Lysin-M7 alleles. Tips represent individual alleles; clades with more than five alleles are represented by triangles each enclosing the indicated number of unique alleles (Fig. 2). (A) Branch lengths represent the number of substitutions per synonymous site, a measure of neutral evolution. Bootstrap support values are italicized; arrows indicate the position of the root, determined with Lysin-M6 (Riginos and McDonald 2003). (B) The same topology with branch-lengths scaled to represent clade-specific estimates of selection. Estimates of selective pressure (Yang 1997) exceed the neutral expectation ($\omega = 1$) in the divergent *Mytilus galloprovincialis* clade indicating that G_D is a product of adaptive divergence.**

Exact tests of population differentiation indicate that G_D alleles are significantly more abundant in samples from the East Atlantic, California, and Australia than they are in Mediterranean samples (Raymond and Rousset 1995; Table 4).

The three acrosomal lysin proteins, M3, M6, and M7 found in *Mytilus* sperm result from two gene duplication events (Takagi et al. 1994), and the possibility that the divergence of the G_D clade also follows a gene duplication event within *M. galloprovincialis* therefore deserves consideration. Our sampling of multiple clones of Lysin-M7 PCR product has thus far yielded a maximum of two alleles per individual, and a cDNA library of multiple *M. galloprovincialis* individuals from an allopatric Mediterranean population uncovered five G alleles and zero G_D alleles. Eleven ESTs matched recognized sperm lysins: five M7, one M6, and five M3; as well as two ESTs that indicate a possible duplication of M3 (Venier et al. 2003). These data suggest that a single Lysin-M7 locus is expressed, and confirm the rarity of G_D alleles in another sample from the Mediterranean.

Methods of detecting recombination among Lysin-M7 alleles give conflicting results. The four-gamete test, which evaluates pairs of sites, infers a minimum of eight recombination events among Lysin-M7 alleles. However, breakpoint analysis methods, which look for discordance between topologies of trees constructed using subsets of the sequence, find no evidence of recombination. The apparent signal of recombination in Lysin-M7 may therefore be due to concerted evolution, or parallel amino-acid substitution (Zhang 2006). Independent evolution of the same amino acid at a given site could positively mislead methods of detecting recombination that only evaluate pairs of sites, but may not impact methods that search for breakpoints using larger blocks of sequence (Pond et al. 2006).

Repeated use of the same amino acids at specific positions is evident among *Mytilus* lysins. Three amino-acid replacements in G_D, relative to the most common G allele (98Y, 141R, 144P), are identical to those of Lysin-M6 alleles and not likely to have resulted from recombination, although these substitutions could be a product of concerted evolution. Position 56(S) is found in both *M. edulis* and *M. galloprovincialis* alleles, and position 180(A) is typical of *M. trossulus*, but is also present in derived products of the G_D radiation (Table 5). It is important to note that even if some of the variation in Lysin-M7 alleles is a product of recombination, the divergence of the G_D clade cannot be explained by recombination alone. If we consider only Lysin-M7 alleles, nine of the 12 amino-acid replacements observed in G_D are found exclusively in *M. galloprovincialis,* and seven of these are unique to the G_D clade (Table 5). Four amino-acid replacements (53A, 125G, 132I, 158S) are completely unique to the G_D clade and not observed in any other lysin allele sampled to date.

Homology models (Lambert et al. 2002) predicting the three-dimensional structure of the carbohydrate-recognition domain of Lysin-M7 indicate that amino-acid substitutions accumulated between species and during the intraspecific G_D radiation occur in or near regions whose functional importance has been confirmed in other C-type lectins by mutagenesis (Streicher and Sharon 2003; Fig. 4). Several amino-acid substitutions between Lysin-M7 alleles occur close to the inferred carbohydrate-binding region, including three positions (125, 144, and 158) that have been substituted once between *M. trossulus* and *M. galloprovincialis* and again within *M. galloprovincialis* in the G_D radiation (Fig. 4). Amino acid 144 is a particularly likely determinant of carbohydrate specificity, occurring two residues N-terminal of the functionally conserved group of amino acids that bind one of the two calcium molecules that coordinate lectin carbohydrate interaction (Drickamer and Fadden 2002). A substitution at residue 144 (S–A)

Table 2. Tests of selection on Lysin-M7, log-likelihood and parameter estimates under specific models of evolution. The best fit model is shown in boldface. Asterisks indicate comparisons that consider internode branches separately from their respective clades. For each model: lnL indicates the log-likelihood value obtained by PAML; np indicates the number of parameters; and κ indicates the estimated transition/transversion rate ratio. ωN_C reports the estimated dn/ds rate ratio for the respective clade under each model, and ωN_I the dn/ds rate ratio for the respective internode where appropriate.

Model	lnL	np	κ	ωT_C	ωT_I	ωE_C	ωE_I	ωG_C	ωG_{DC}	ωG_{DI}
A. 1 ratio: $\omega T = \omega E = \omega G = \omega G_D$	−2355.372393	84	3.821	0.282	—	0.282	—	0.282	0.282	—
B. 2 ratio: $\omega T = \omega E = \omega G$, ωG_D	−2342.183942	85	3.824	0.208	—	0.208	—	0.208	1.282	—
C. 4 ratio: ωT, ωE, ωG, ωG_D	−2336.164184	87	3.827	0.145	—	0.116	—	0.359	1.281	—
D. 7 ratio: ωT, ωE, ωG, ωG_D*	**−2330.144177**	**90**	**3.820**	**0.063**	**0.375**	**0.109**	**0.185**	**0.359**	**1.276**	**1.298**
E. 17 ratio: ωT, ωE, ωG, Free ωG_D*	−2325.954071	100	3.820	0.063	0.375	0.109	0.185	0.359	free	1.298
F. Free ratio model	−2290.024833	165	3.822	free	free	free	free	free	free	free

Table 3. Tests of selection on Lysin-M7, likelihood ratio test statistics (χ^2) comparing specific models of evolution. Asterisks indicate that internode branches are considered separately from their respective clades. ωN is the dn/ds rate ratio for the respective clade or internode. Boldface rows are comparisons with significantly different log-likelihood estimates as indicated by χ^2 tests.

Models	Null	Alternate	2ΔlnL	df	*P*
A vs. B	**$\omega T = \omega E = \omega G = \omega G_D$**	**$\omega T = \omega E = \omega G$, ωG_D**	**26.376902**	**1**	**0.001**
B vs. C	**$\omega T = \omega E = \omega G$, ωG_D**	**ωT, ωE, ωG, ωG_D**	**12.039516**	**2**	**0.002**
C vs. D	**ωT, ωE, ωG, ωG_D**	**ωT, ωE, ωG, ωG_D***	**12.040014**	**3**	**0.007**
D vs. E	ωT, ωE, ωG, ωG_D*	ωT, ωE, ωG, Free ωG_D*	8.380212	10	0.591
D vs. F	ωT, ωE, ωG, ωG_D*	free ratio model	80.238688	75	0.318

was previously identified as a potential target of selection during the divergence of Lysin-M7 alleles between *M. galloprovincialis* and *M. edulis* (Riginos and McDonald 2003) and position 144 is substituted a second time (A–P) within *M. galloprovincialis* in derived alleles of the G_D radiation (Table 5).

Overall the spatial arrangement of substitutions in Lysin-M7 within *M. galloprovincialis* is similar to that between *M. trossulus* and *M. galloprovincialis*, and also to adaptive substitutions observed in the C-Type lectin domains of the sea urchin receptor for egg jelly (Fig. 4; Mah et al. 2005). Carbohydrate interaction has been identified as an important component of fertilization in *Mytilus* (Focarelli et al. 1991), and the presence of substitutions in putative functional regions of Lysin-M7 and repeated substitutions at the same amino-acid sites suggests a role for at least some of these substitutions in modified carbohydrate recognition by Lysin-M7.

Discussion

INTRASPECIFIC DIVERGENCE OF LYSIN-M7

Adaptive gamete-recognition protein divergence has been documented between the species of a number of broadcast-spawning invertebrate taxa (Palumbi 1998; Swanson and Vacquier 2002). Implicating this divergence in the evolution of reproductive isolation ultimately requires examples of incipient gamete-recognition divergence within species. Adaptive divergence of a sperm

Table 4. Exact test of sample nondifferentiation. Boldface comparisons have significantly different frequencies of G and G_D alleles ($P = 0.05$). Counts of G and G_D alleles in each population are on the diagonal. Crosses represent populations with significantly elevated G_D frequency relative to allopatric Mediterranean samples. Global probability of nondifferentiation $P = 0.000$.

Population allo/sympatric	**Mediterranean** G (allopatric)	**Atlantic-East** G/E (sympatric)	**Pacific-California** T/G (sympatric)	**Pacific-Japan** T/G (sympatric)	**Pacific-Australia** G (unknown)
Mediterranean	$n = 28$ (27G, $1G_D$)	**0.00000 ± 0.0000**	**0.01475 ± 0.0027**	1.00000 ± 0.0000	**0.00700 ± 0.0007**
Atlantic-East	+	$n = 22$ (10G, $12G_D$)	**0.01230 ± 0.0022**	**0.00000 ± 0.0000**	0.09905 ± 0.0050
Pacific-California	+		$n = 37$ (28G, $9G_D$)	**0.05080 ± 0.0030**	0.66335 ± 0.0047
Pacific-Japan				$n = 14$ (14G, $0G_D$)	**0.00000 ± 0.0000**
Pacific-Australia	+				$n = 8$ (6G, $2G_D$)

Table 5. Lysin-M7 variation in *Mytilus* and alleles sampled in each population and location.

Population Allo/Sympatric	Clade	n	Amino Acid 012 014 018 029 046 053 056 064 086 094 098 108 109 112 125 132 138 141 143 144 158 163 175 180	Sublocation(n)*Singletons*
			G M I T I S P F T P F N K I S T S K S A A L V P	
Mediterranean G (Allopatric)	G	14	· ·	**IK**(2)*4H* **RP**(7)*5D,82T,151R,179R* **VE**(5)*59G-165N*
		5	· · V ·	**IK**(2)*85R-172H* **VE**(3)*97A*
	G/E/T	6	· I · · · · S · · · · · · · · · · · · · · · · ·	**IK**(4)*12D,72T* **RP**(1) **VE**(1)
		1	· I V · · · S · · · · · · · · · · · · · · · · ·	**VE**(1)
		1	· · · · · · S · · · · · · · · · · · · · · · · ·	**IK**(1)
	GD	1	· · V A · A S · · · Y · · · G · · R · P S · · A	**RP**(1)*77R*
	G/E	1	· · · · · · S · · · · · · · · · · · · · · · · ·	**RP**(1)*32L*
Atlantic-East G/E (Sympatric)	G	3	· ·	
		7	· · V ·	**VG**(7)*175A*
	GD	2	· · V · · A S · · · Y · · · · · · · · · · · · ·	
		7	· · V · · A S · · · Y · · · G · · R · P S · · A	
		3	· · V · · A S · · · Y · · · G I · R · P S · · A	
	E	2	· · · · · · S Y · · · · · · · · · · · S · · · ·	
		2	· · · · · · S · · · · · · · · · · · · S · · · ·	
Pacific-California T/G (Sympatric)	G	12	· ·	**EK**(4) **MB**(3)*75R* **OP**(2) **SD**(3)
		1	· F ·	**MB**(1)
		12	· · V ·	**EK**(2) **OP**(2) **MB**(1) **RF**(2) **SD**(5)*7K,73N,137S*
	G/E/T	3	· I · · · · S · · · · · · · · · · · · · · · · ·	**EK**(2)*159R* **SD**(1)
	GD	1	· · V · · A S · · · Y · · · · · · · · · · · · ·	**MB**(1)
		2	· · V · · A S · · · Y · · · · · · · · · · F ·	**MB**(2)
		1	· · V · · A S · · · Y · · · G I · R · P S · F A	**MB**(1)
		3	· · V · · A S · · · Y · · · G I · R · P S · · A	**MB**(2)*21D-73E,35R-152A-159R* **RF**(1)
		2	· · V · · A S · · · Y · · · G · · R · P S · · A	**RF**(1) **SD**(1)
	G/E	1	· · · · · · S · · · · · · · · · · · · · · · · ·	**SD**(1)
	E	1	· · · · · · S · · · · · · · · · · · · S · · · ·	**SD**(1)
	T	2	A I · S V · · · Q S · Q T T N · T · T S T F · A	**OP**(1) **MB**(1)
Pacific-Japan T/G (Sympatric)	G	7	· ·	**KO**(7)*96S*
		7	· · V ·	**KO**(7)*5C,18L,48S,175A*
Pacific-Australia G (Unknown)	G	4	· ·	
		1	· · V ·	
	G/E/T	1	· I · · · · S · · · · · · · · · · · · · · · · ·	
	GD	2	· · V · · A S · · · Y · · · G I · R · P S · · A	**AU**(2)*1S,29A*
Atlantic-West T/E (Sympatric)	E	4	· · · · · · S Y · · · · · · · · · · · S · · · ·	**NF**(4)*97A*
	G/E	2	· · · · · · S · · · · · · · · · · · · · · · · ·	**NF**(2)*173K*
	T	8	A I · S V · · · Q S · Q T T N · T · T S T F · A	**NF**(8)*50K,113P*
Pacific-North T (Allopatric)	T	17	A I · S V · · · Q S · Q T T N · T · T S T F · A	**HA**(4)*165H* **OB**(13)*26K,130R,166K*
Lysin-M6	M6 G		N N · · · · E · Q · Y Q R T N · T R · P T · I H	

Note: Dots represent identity with consensus. Colouring conventions follow Figure 1. Gray-shaded amino acids are identical to *Mytilus galloprovincialis* Lysin-M6. Location abbreviations are in bold. The number of times a given allele was sampled in each location is bracketed. Singleton amino-acid replacements are in italics, dashes separate singletons from the same allele.

acrosomal protein, Lysin-M7, is evident among populations of the blue mussel *M. galloprovincialis* (Fig. 3). Divergent (G_D) Lysin-M7 alleles exist at elevated frequency, relative to allopatric samples, in two sympatric hybridizing *M. galloprovincialis* populations: with *M. edulis* in the East Atlantic, and with *M. trossulus* in California, and in one population of unknown species composition, Australia (Table 4).

The initial divergence of *M. galloprovincialis* Lysin-M7 likely occurred in the East Atlantic following a Pleistocene introduction across the Almeria-Oran front (Gardner 1994; Quesada

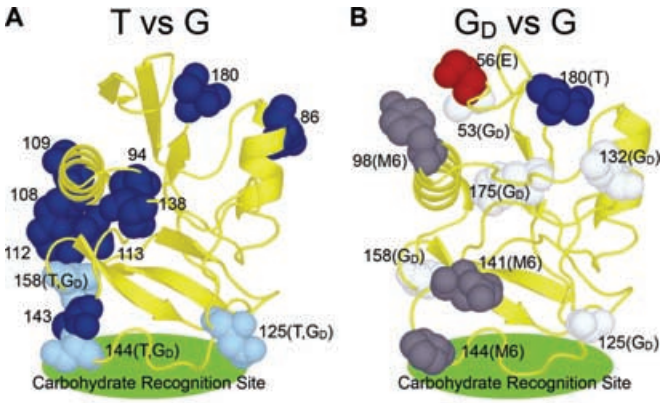


Figure 4. Spatial locations of amino-acid substitutions in the carbohydrate-recognition domain of *Mytilus* Lysin-M7. (A) Pairwise differences between T and G. Blue residues are typical of *Mytilus trossulus*, light blue (125, 144, 158) are substituted a second time in G_D. (B) Pairwise differences between G_D and G. White residues (53, 125, 132, 158, 175) are unique to G_D alleles, red (56) are identical to *M. edulis*, blue (180) to *M. trossulus,* and gray (98, 141, 144) to Lysin-M6 alleles from *M. galloprovincialis*. Yellow ribbons are sites identical to *M. galloprovincialis*. Numbers refer to the mature peptide; singletons are not shown (Table 5). In both comparisons amino-acid substitutions occur in or near regions known to affect carbohydrate specificity (Drickamer and Fadden 2002; Streicher and Sharon 2003).

et al. 1995) an oceanographic dispersal barrier that divides Atlantic and Mediterranean species assemblages and environments. Moderate divergence has accumulated between *M. galloprovincialis* populations on either side of this dispersal barrier even at apparently neutral loci, and the distribution of G and G_D Lysin-M7 alleles observed in this study is concordant with intraspecific allozyme, mitochondrial, and nuclear DNA discontinuities (Quesada et al. 1995; Sanjuan et al. 1997; Daguin and Borsa 1999). Divergence at multiple neutral loci suggests that genetic exchange was limited between the *M. galloprovincialis* populations that were introduced into sympatry and those that remained in allopatry following this Pleistocene introduction. Adaptive divergence of Lysin-M7 is evident in the G_D clade (Fig. 3), and G_D alleles are more abundant in the sympatric hybridizing populations of the East Atlantic than they are in samples from allopatric Mediterranean populations (Table 4). The initial divergence of Lysin-M7 G_D alleles could therefore be a response to selection pressures acting in this isolated *M. galloprovincialis* population following secondary contact and hybridization with *M. edulis* in the East Atlantic.

Divergent *M. galloprovincialis* Lysin-M7 alleles also occur outside the East Atlantic probably as a result of contemporary transport in the ballast water of shipping vessels, the same mechanism responsible for the initial (McDonald and Koehn 1988) and continued (Geller et al. 1994) introduction of *M. galloprovincialis* to the Pacific. Ballast introduction of *M. galloprovincialis* is pervasive. Ten percent of individuals in Australia derive from contemporary introductions (Hilbish et al. 2000) and transport of Lysin-M7 alleles is evident in all but two of the populations sampled here (Table 5). The sources of worldwide *M. galloprovincialis* introductions are difficult to identify with certainty. For example, the most probable source of the Californian *M. galloprovincialis* population depends on which loci are examined. This study finds an association between California and the East Atlantic, but existing allozyme studies indicate that Californian populations more closely resemble Mediterranean *M. galloprovincialis* (McDonald and Koehn 1988; Sanjuan et al. 1997). One reason for this difference might be that introduced populations are mosaics resulting from continual reintroduction of individuals from a number of other populations.

Transport of *M. galloprovincialis* to California within the last several hundred years (Geller 1999) has resulted in the introduction and persistence of nearly the entire suite of G_D alleles (Table 5). This diversity could have been achieved by a single large introduction, but multiple introductions over time are more consistent with the increasing scale of ballast transport over the last century, and with the continued transport of marine invertebrate larvae in shipping vessels today (Geller et al. 1994; Drake and Lodge 2004). Interestingly, G_D alleles have not substantially reinvaded allopatric *M. galloprovincialis* populations despite close proximity and intense contemporary ballast exchange between the East Atlantic and the Mediterranean (Drake and Lodge 2004), nor are they present in our samples from sympatric populations in Japan, an area where hybrid individuals are rare (Suchanek et al. 1997; Table 5). The persistence of G_D alleles in California and their relative absence in Japan and the Mediterranean (Table 5), suggests either that hybridizing populations in California have received migrants from the East Atlantic to a greater extent than Japan and the Mediterranean, a pattern not consistent with allozyme data (McDonald and Koehn 1988; Sanjuan et al. 1997), or that individuals carrying G_D alleles may suffer some selective disadvantage in allopatric *M. galloprovincialis* populations and areas where hybrid individuals are rare.

An alternative to the hypothesis that G_D alleles diverged following secondary contact with *M. edulis,* is that G and G_D alleles diverged from each other due to intraspecific selection pressures while still in the Mediterranean. The differentiation observed in this study would then reflect local variation in allele frequencies, which itself could be maintained by intra- or interspecific selection pressures. These two scenarios may be difficult to disentangle, because the scale and intensity of ballast transport of *Mytilus* larvae between the East Atlantic and the Mediterranean are unknown, and difficult to estimate without a large number

of variable markers. Our sampling finds instances of G_D alleles in allopatry and G alleles in sympatry (Table 5); other surveys of these populations will certainly uncover more (Riginos et al. 2006). In the presence of ballast exchange, the relevant difference between these two scenarios is the pattern of historical divergence rather than the contemporary distribution. Our assertion, that Lysin-M7 divergence in *M. galloprovincialis* first occurred following introduction to the East Atlantic, predicts the existence of a discordance or cline in Lysin-M7 G and G_D allele frequencies at the mouth of the Mediterranean, matching those observed at other allozyme, mitochondrial, and nuclear loci (Quesada et al. 1995; Sanjuan et al. 1997; Daguin and Borsa 1999). There should be no such cline if the introduction of *M. galloprovincialis* into the East Atlantic had no effect on patterns of intraspecific variation in Lysin-M7. We also predict that G_D alleles are recent relative to most alleles in the G clade and should therefore exhibit reduced polymorphism in their adjacent non-coding regions.

LYSIN-M7 DIVERGENCE AND THE EVOLUTION OF REPRODUCTIVE ISOLATION

The effects of Lysin-M7 divergence on gametic compatibility between *Mytilus* species are currently unknown. If gamete-recognition protein divergence results in reduced sperm–egg compatibility in *Mytilus*, as it often does in other invertebrate species (Metz et al. 1994; Vacquier et al. 1995; Hellberg and Vacquier 1999), the divergence of Lysin-M7 could contribute to the reductions in gametic compatibility between *Mytilus* species, such as those observed between *M. galloprovincialis* and *M. edulis* (Bierne et al. 2002). Divergence of Lysin-M7 could also create reproductive isolation between allopatric *M. galloprovincialis* populations and those in sympatry as an indirect consequence of selection acting in sympatry (Howard 1993). A similar scenario has been proposed as a possible case of reinforcement in sea urchins (Geyer and Palumbi 2003).

Reproductive character displacement among sympatric non-hybridizing taxa is evident in abalone and urchins (Yang et al. 2000; Geyer and Palumbi 2003; Zigler and Lessios 2003), and our data indicate that sympatry may also exaggerate gamete-recognition divergence in populations that produce natural hybrids. The pattern reported here is explicitly consistent with the predictions of reinforcement given that *M. edulis*/*M. galloprovincialis* hybrids show endogenous reductions in hybrid fitness (Bierne et al. 2006). However, we emphasize that our data are not sufficient to implicate any particular selection mechanism in Lysin-M7 divergence, because we do not yet know the reasons for or the phenotypic effects of Lysin-M7 divergence. The adaptive divergence of Lysin-M7 in the East Atlantic is therefore equally consistent with other proposed selective mechanisms that could plausibly create selection on gamete recognition in sympatry, or even as a consequence of simple historical isolation between *M. galloprovincialis* populations. Mechanisms such as sexual conflict over fertilization rate and polyspermy avoidance (Palumbi 1998; Swanson and Vacquier 2002), or sexual selection via female choice or competition for fertilization opportunities (Palumbi 1999), are all consistent with exaggerated gamete-recognition divergence in hybridizing populations.

Conclusion

We report an example of adaptive divergence of a gamete-recognition protein, Lysin-M7, within a single species, *M. galloprovincialis*. The existing evidence suggests that geographic isolation between subpopulations of *M. galloprovincialis* following a historical introduction may have allowed Lysin-M7 to diverge, and that selection pressures associated with secondary contact and hybridization between *M. galloprovincialis* and *M. edulis* may have provided the impetus for divergence. Adaptive evolution of Lysin-M7 occurs despite the fact that reproductive isolation is not yet complete between *Mytilus* species. Examining the contribution of this divergence to the early stages of speciation should therefore be possible.

ACKNOWLEDGMENTS

We thank the Simon Fraser University FAB-Lab, R. Harrison, D. Heath, and W. Swanson for discussion. T. Chapman, Y. Itani, S. and T. Hoover, P. Presa, A. Springer, D. Springer, J. Sutton, J. Tyerman, D. Wheeldon, P-M. Wilson, and E. Yoshioka assisted with field collection. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada to BJC and SAS, and a Garfield Weston/BC Packers Fellowship to SAS.

LITERATURE CITED

Bierne, N., P. David, P. Boudry, and F. Bonhomme. 2002. Assortative fertilization and selection at larval stage in the mussels *Mytilus edulis* and *M. galloprovincialis*. Evolution 56:292–298.

Bierne, N., F. Bonhomme, and P. David. 2003a. Habitat preference and the marine-speciation paradox. Proc. R. Soc. Lond. B 270:1399–1406.

Bierne, N., P. Borsa, C. Daguin, D. Jollivet, F. Viard, F. Bonhomme, and P. David. 2003b. Introgression patterns in the mosaic hybrid zone between *Mytilus edulis* and *M. galloprovincialis*. Mol. Ecol. 12:447–461.

Bierne, N., F. Bonhomme, P. Boudry, M. Szulkin, and P. David. 2006. Fitness landscapes support the dominance theory of post-zygotic isolation in the mussels *Mytilus edulis* and *M. galloprovincialis*. Proc. R. Soc. Lond. B 273:1253–1260.

Brown, L. D. 1995. Genetic evidence for hybridisation between *Haliotis rubra* and *H. laevigata*. Mar. Biol. 123:89–93.

Butlin, R. 1987. Speciation by reinforcement. Trends Ecol. Evol. 2:8–13.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9:1657–1659.

Daguin, C., and P. Borsa. 1999. Genetic characterisation of *Mytilus galloprovincialis* Lmk. in North West Africa using nuclear DNA markers. J. Exp. Mar. Biol. Ecol. 235:55–65.

Delano, W. L. 2002. The pymol molecular graphics system. Available at www.pymol.org. Accessed Sept. 28, 2005..

Drake, J. M., and D. M. Lodge. 2004. Global hot spots of biological invasions: evaluating options for ballast-water management. Proc. R. Soc. Lond. B 271:575–580.

Drickamer, K., and A. J. Fadden. 2002. Genomic analysis of C-type lectins. Pp. 59–72 *in* K. Drickamer and A. Dell, eds. Glycogenomics: the impact of genomics and informatics on glycobiology. Portland Press, London.

Focarelli, R., D. Rosa, and F. Rosati. 1991. The vitelline coat spikes—a new peculiar structure of *Mytilus galloprovincialis* eggs with a role in sperm-egg interaction. Mol. Rep. Dev. 28:143–149.

Gardner, J. P. A. 1994. The structure and dynamics of naturally-occurring hybrid *Mytilus edulis* Linnaeus, 1758 and *Mytilus galloprovincialis* Lamarck, 1819 (Bivalvia, Mollusca) populations—review and interpretation. Archiv. Fur. Hydrobiol. 99:37–71.

Geller, J. B., J. T. Carlton, and D. A. Powers. 1994. PCR based detection of mtDNA haplotypes of native and invading mussels on the Northeastern Pacific coast—latitudinal pattern of invasion. Mar. Biol. 119:243–249.

———. 1999. Decline of a native mussel masked by sibling species invasion. Conserv. Biol. 13:661–664.

Geyer, L. B., and S. R. Palumbi. 2003. Reproductive character displacement and the genetics of gamete recognition in tropical sea urchins. Evolution 57:1049–1060.

Gosling, E. M., and D. McGrath. 1990. Genetic-variability In exposed-shore mussels, *Mytilus* spp, along an environmental gradient. Mar. Biol. 104:413–418.

Hellberg, M. E., and V. D. Vacquier. 1999. Rapid evolution of fertilization selectivity and lysin cDNA sequences in teguline gastropods. Mol. Biol. Evol. 16:839–848.

Hilbish, T. J., A. Mullinax, S. I. Dolven, A. Meyer, R. K. Koehn, and P. D. Rawson. 2000. Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): routes and timing of transequatorial migration. Mar. Biol. 136:69–77.

Howard, D. J. 1993. Reinforcement: origin, dynamics, and fate of an evolutionary hypothesis. Pp. 46–69 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

Hudson, R. R., and N. L. Kaplan. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. Genetics 111:147–164.

Lambert, C., N. Leonard, X. De Bolle, and E. Depiereux. 2002. ESyPred3D: prediction of proteins 3D structures. Bioinformatics 18:1250–1256.

Mah, S. A., W. J. Swanson, and V. D. Vacquier. 2005. Positive selection in the carbohydrate recognition domains of sea urchin sperm receptor for egg jelly (suREJ) proteins. Mol. Biol. Evol. 22:533–541.

McCartney, M. A., and H. A. Lessios. 2002. Quantitative analysis of gametic incompatibility between closely related species of neotropical sea urchins. Biol. Bull. 202:166–181.

McCartney, M. A., and H. A. Lessios. 2004. Adaptive evolution of sperm bindin tracks egg incompatibility in neotropical sea urchins of the genus *Echinometra*. Mol. Biol. Evol. 21:745.

McDonald, J. H., and R. K. Koehn. 1988. The Mussels *Mytilus galloprovincialis* and *Mytilus trossulus* on the Pacific Coast of North-America. Mar. Biol. 99:111–118.

Metz, E. C., R. E. Kane, H. Yanagimachi, and S. R. Palumbi. 1994. Fertilization between closely-related sea-urchins is blocked by incompatibilities during sperm-egg attachment and early stages of fusion. Biol. Bull. 187:23–34.

Metz, E. C., G. Gomez-Gutierrez, and V. D. Vacquier. 1998. Mitochondrial DNA and bindin gene sequence evolution among allopatric species of the sea urchin genus *Arbacia*. Mol. Biol. Evol. 15:185–195.

Palumbi, S. R., and E. C. Metz. 1991. Strong reproductive isolation between closely related tropical sea-urchins (genus *Echinometra*). Mol. Biol. Evol. 8:227–239.

———. 1998. Species formation and the evolution of gamete recognition loci. Pp. 271–278 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, Oxford, U.K.

———. 1999. All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. Proc. Natl. Acad. Sci. USA. 96:12632–12637.

Pond, S. L. K., and S. D. W. Frost. 2005. Datamonkey: rapid detection of selective pressure on individual sites of codon alignments. Bioinformatics 21:2531–2533.

Pond, S. L. K., D. Posada, M. B. Gravenor, C. H. Woelk, and S. D. W. Frost. 2006. Automated phylogenetic detection of recombination using a genetic algorithm. Mol. Biol. Evol. 23:1891–1901.

Quesada, H., C. M. Benyon, and D. O. F. Skibinski. 1995. A mitochondrial DNA discontinuity in the mussel *Mytilus galloprovincialis* Lmk: Pleistocene vicariance biogeography and secondary intergradation. Mol. Biol. Evol. 12:521–524.

Rawson, P. D., K. L. Joyner, K. Meetze, and T. J. Hilbish. 1996. Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. Heredity 77:599–607.

Rawson, P. D., V. Agrawal, and T. J. Hilbish. 1999. Hybridization between the blue mussels *Mytilus galloprovincialis* and *M trossulus* along the Pacific coast of North America: evidence for limited introgression. Mar. Biol. 134:201–211.

Rawson, P. D., C. Slaughter, and P. O. Yund. 2003. Patterns of gamete incompatibility between the blue mussels *Mytilus edulis* and *M. trossulus*. Mar. Biol. 143:317–325.

Raymond, M., and F. Rousset. 1995. An exact test for population differentiation. Evolution 49:1280–1283.

Riginos, C., and J. H. McDonald. 2003. Positive selection on an acrosomal sperm protein, M7 lysin, in three species of the mussel genus *Mytilus*. Mol. Biol. Evol. 20:200–207.

Riginos, C., and C. W. Cunningham. 2005. Local adaptation and species segregation in two mussel (*Mytilus edulis* x *Mytilus trossulus*) hybrid zones. Mol. Ecol. 14:381–400.

Riginos, C., D. Wang, and A. J. Abrams. 2006. Geographic variation and positive selection on M7 Lysin, an acrosomal sperm protein in mussels (*Mytilus* spp.). Mol. Biol. Evol. 23:1952–1965.

Rozas, J., and R. Rozas. 1999. DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.

Sanjuan, A., C. Zapata, and G. Alvarez. 1997. Genetic differentiation in *Mytilus galloprovincialis* Lmk. throughout the world. Ophelia 47:13–31.

Schneider, S., D. Roessli, and Excoffier, L. 2000. Arlequin: a software for population genetics data analysis.Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva, Switzerland.

Skurikhina, L. A., Y. F. Kartavtsev, A. Y. Chichvarkhin, and M. V. Pan'kova. 2001. Study of two species of mussels, *Mytilus trossulus* and *Mytilus galloprovincialis* (Bivalvia, Mytilidae), and their hybrids in Peter the Great Bay of the Sea of Japan with the use of PCR markers. Russ. J. Genet. 37:1448–1451.

Streicher, H., and N. Sharon. 2003. Recombinant plant lectins and their mutants. Methods Enzymol. 363:47–77.

Suchanek, T. H., J. B. Geller, B. R. Kreiser, and J. B. Mitton. 1997. Zoogeographic distributions of the sibling species *Mytilus galloprovincialis* and *M. trossulus* (Bivalvia: Mytilidae) and their hybrids in the North Pacific. Biol. Bull. 193:187–194.

Swanson, W. J., and V. D. Vacquier. 1997. The abalone egg vitelline envelope receptor for sperm lysin is a giant multivalent molecule. Proc. Natl. Acad. Sci. USA 94:6724–6729.

———. 2002.Reproductive protein evolution. Annu. Rev. Evol. Syst. 33:161–179.

Swofford, D. L. 2001. PAUP*: phylogenetic analysis using parsimony * and other methods. Ver. 4. Sinauer, Sunderland, MA.

Takagi, T., A. Nakamura, R. Deguchi, and K. Kyozuka. 1994. Isolation, characterization, and primary structure of three major proteins obtained from *Mytilus edulis* sperm. J. Biochem. 116:598–605.

Templeton, A. R. 1981. Mechanisms of speciation - a population genetic approach. Ann. Rev. Ecol. Syst. 12:23–48.

Togo, T., and M. Morisawa. 1997. Aminopeptidase-like protease released from oocytes affects oocyte surfaces and suppresses the acrosome reaction in establishment of polyspermy block in oocytes of the mussel *Mytilus edulis*. Dev. Biol. 182:219–227.

Toro, J. E., R. J. Thompson, and D. J. Innes. 2002. Reproductive isolation and reproductive output in two sympatric mussel species (*Mytilus edulis, M. trossulus*) and their hybrids from Newfoundland. Mar. Biol. 141:897–909.

Vacquier, V. D., W. J. Swanson, and M. E. Hellberg. 1995. What have we learned about sea-urchin sperm bindin. Dev. Growth Diff. 37:1–10.

Vacquier, V. D., W. J. Swanson, and Y. H. Lee. 1997. Positive Darwinian selection on two homologous fertilization proteins: what is the selective pressure driving their divergence?. J. Mol. Evol. 44:S15–S22.

Venier, P., A. Pallavicini, B. De Nardi, and G. Lanfranchi. 2003. Towards a catalogue of genes transcribed in multiple tissues of *Mytilus galloprovincialis*. Gene 314:29–40.

Yang, Z. H. 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. Comput. Appl. Biosci. 13:555–556.

Yang, Z., W. Swanson, and V. Vacquier. 2000. Maximum-likelihood analysis of molecular adaptation in abalone sperm lysin reveals variable selective pressures among lineages and sites. Mol. Biol. Evol. 17:1455.

Zhang, J. 2006. Parallel adaptive origins of digestive RNases in Asian and African leaf monkeys. Nat. Genet. 38:819.

Zigler, K. S., and H. A. Lessios. 2003. Evolution of bindin in the pantropical sea urchin *Tripneustes*: comparisons to bindin of other genera. Mol. Biol. Evol. 20:220–231.

Associate Editor: R. Harrison

Supplementary Material

The following supplementary material is available for this article:

Table S1. Individual genotypes at Lysin-M7, GLU and Efbis loci.

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1558-5646.2007.00073.x
(This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.