Marine Biology (2004) 145: 713–725
DOI 10.1007/s00227-004-1363-1

RESEARCH ARTICLE

J. Toro · D. J. Innes · R. J. Thompson

Genetic variation among life-history stages of mussels in a *Mytilus edulis–M. trossulus* hybrid zone

Received: 11 August 2003 / Accepted: 9 March 2004 / Published online: 7 May 2004
© Springer-Verlag 2004

Abstract The mussel species *Mytilus edulis* L. and *M. trossulus* Gould coexist and hybridize throughout a large area that includes the north coast of Maine and Atlantic Canada. Previous studies provided genetic evidence for limited hybridization between the two species for mussels >15 mm. The present study used two genetic markers (*ITS*, *Glu-5′*) to examine the genetic composition of early life-history stages by sampling veliger and pediveliger larvae, juveniles (<2.0 to 15.0 mm shell length) and adults (>15 mm shell length) in Trinity Bay, Newfoundland, during three consecutive years (1995–1997) to determine if differential mortality limits the relative abundance of hybrids. The relative frequency of the two species and the different hybrid genotypes was similar among the larvae, juveniles and small adult mussels. The double hybrid genotype (F_1-like) was the rarest genotype observed. There was no evidence for differential mortality during the early life-history stages, and factors limiting production of hybrids appear to operate before the late larval stage. The observed frequency of hybrids is probably due to a combination of pre- and postzygotic reproductive isolating mechanisms operating early in the mussel's life history. *M. trossulus* dominated the early life-history stages, possibly due to a higher population density and a greater reproductive output than *M. edulis*. Differential mortality may explain the observed decrease in frequency of *M. trossulus* and increase in frequency of *M. edulis* with increasing shell length. A similar frequency of hybrid mussels from larvae to the size class of 55 mm shell length may indicate a rate of mortality intermediate between the two parental species. The *M. edulis–M. trossulus* hybrid zone appears to be maintained by reproductive isolating mechanisms limiting the production of hybrids and life-history differences that allow the two species to coexist.

Communicated by J.P. Grassle, New Brunswick

J. Toro
Instituto de Biología Marina "Dr. Jürgen Winter",
Universidad Austral de Chile, Casilla 567,
Valdivia, Chile

D. J. Innes (✉)
Department of Biology, Memorial University,
St. John's, Newfoundland, A1B 3X9, Canada
E-mail: dinnes@mun.ca
Fax: +1-709-7373018

R. J. Thompson
Ocean Sciences Centre, Memorial University,
St. John's, Newfoundland, A1C 5S7, Canada

Introduction

Hybrid zones have attracted a great deal of interest because they represent the interaction between adaptation, reproductive isolation and speciation (Barton and Hewitt 1989; Harrison 1993; Arnold 1997). Much insight into the speciation process can be gained by studying the origin, maintenance and fate of hybrid zones. Natural hybridization involves incomplete reproductive isolation, and hybrid zones have therefore been particularly useful for understanding the evolution of pre- and postzygotic isolating mechanisms that form the basis for reproductive isolation and speciation (Orr and Presgraves 2000; Marshall et al. 2002). Hybridization may represent a transitory phase leading to an increase in reproductive isolation between species and a reduction in the rate of hybrid formation. Low survival and/or fertility of hybrids may select for increased prezygotic isolating mechanisms, a process known as reinforcement (Marshall et al. 2002). However, if hybrids are viable and fertile, then hybrid zones represent a means through which genes can introgress between species. Although its importance is unknown, introgression may be a source of novel genetic variation for adaptation (Arnold and Hodges 1995; Arnold 1997).

Hybrid zones often occur as narrow regions where two allopatric species come into contact and interbreed. Much research has investigated the width, location and stability of hybrid zones, as well as clinal variation of phenotypic and genetic characteristics as a means of

understanding factors that maintain hybrid zones (Barton and Hewitt 1989). Some hybridizing species occur as a mosaic of parental species and hybrids coexisting over large areas consisting of patchily distributed habitats (Rand and Harrison 1989; Ross and Harrison 2002). A mosaic hybrid zone may result in increased opportunities for hybridization and introgression, because geographic proximity increases contact between species and hybrids. Regardless of the geographic structure of a hybrid zone, recent studies have focused on assessing the relative importance of endogenous genetic and exogenous ecological factors in maintaining hybrid zones (Arnold and Hodges 1995; Arnold 1997).

The "Tension Zone" model proposes that hybrids have consistently low fitness due to endogenous genetic incompatibilities and that hybrid zones are maintained by a balance between the formation of hybrids and selection against them (Barton and Hewitt 1989). However, this simple model has been criticized because there are many examples of hybrid genotypes with intermediate fitness, equal fitness or even greater fitness than the parental genotypes (Arnold and Hodges 1995). There are also examples in which the relative fitness of parental and hybrid genotypes depends on the environment (Arnold and Hodges 1995; Arnold 1997). Thus the maintenance of hybrid zones probably results from an interaction between endogenous and exogenous factors. An alternative model to the tension zone model suggests that genetic incompatibility can select against certain hybrid genotypes, while other hybrid genotypes are favored under specific environmental conditions (Arnold 1997).

An understanding of the structure of hybrid zones requires the determination of the relative fitness of parents and hybrids under a variety of natural conditions. Additional information can be obtained from experiments in which fitness differences are estimated from laboratory-produced hybrids. More commonly, basic information on the genetic structure of a hybrid zone is obtained from the distribution of genetic variation between parental species and their natural hybrids as defined by genetic markers. Such information often provides the first indication that hybridization is occurring between two species (Boecklen and Howard 1997). Furthermore, determining genetic variation with several genetic markers allows hybrid zones to be characterized on a continuum from unimodal to bimodal based on the frequency distribution of parental and various types of mixed hybrid genotypes (Jiggins and Mallet 2000). Unimodal hybrid zones have a greater frequency of individuals with mixed hybrid genotypes compared with parental genotypes and are associated with a lack of prezygotic isolation mechanisms. In contrast, bimodal hybrid zones contain more parental than mixed hybrid genotypes and often result from strong prezygotic isolation mechanisms, primarily through assortative mating, although both unimodal and bimodal hybrid zones exhibit similar levels of postzygotic genetic incompatibility, suggesting that postzygotic mechanisms alone cannot generate bimodal hybrid zones (Jiggins and Mallet 2000). These observations further suggest that assortative mating combined with selection against certain hybrid genotypes may be the primary mechanism for maintaining bimodal hybrid zones (Jiggins and Mallet 2000).

Species of mussels in the *Mytilus edulis* complex are often found sympatrically, and there is always hybridization between them (Gosling 1992). Some of these contact zones are located on the Pacific coast of North America, between *M. galloprovincialis* and *M. trossulus* (McDonald and Koehn 1988; Heath et al. 1995; Rawson et al. 1999), in southwest England and France, between *M. edulis* and *M. galloprovincialis* (Skibinski et al. 1978; Edwards and Skibinski 1987; Gardner et al. 1993; Gardner 1994; Daguin et al. 2001; Bierne et al. 2002b, 2003a; Hilbish et al. 2002), and in the Baltic Sea, between *M. edulis* and *M. trossulus* (Väinölä and Hvilsom 1991; Riginos et al. 2002). In the western Atlantic, there is evidence that interbreeding between *M. edulis* and *M. trossulus* is limited in natural populations (Koehn et al. 1984; Varvio et al. 1988; Bates and Innes 1995; Saavedra et al. 1996; Comesaña et al. 1999; Rawson et al. 2001; Toro et al. 2002), despite their sympatric occurrence and the successful production of viable hybrids in laboratory crosses (Zouros et al.1992, 1994).

The *M. edulis–M. trossulus* hybrid zone of eastern North America extends from northern Newfoundland to northern Maine (Koehn et al. 1984; Saavedra et al. 1996; Comesaña et al. 1999; Penney and Hart 1999; Rawson et al. 2001; M. Miranda, unpublished data). Most sites consist of various mixtures of the two species and their hybrids, producing a bimodal distribution of parental and mixed hybrid genotypes (Bates and Innes 1995; Saavedra et al. 1996; Comesaña et al. 1999; Rawson et al. 2001). Although some areas appear to be dominated by one species or the other on both a macro- and micro-geographic scale, no environmental factors have been identified that could definitively explain the observed distribution. However, variations in salinity, temperature and wave exposure may play a role in some stages of the life history (Bates and Innes 1995; Gardner and Thompson 2001; Rawson et al. 2001; Qiu et al. 2002).

Information from four diagnostic nuclear genetic markers and mtDNA variation in samples from Trinity Bay, Newfoundland, collected during July 1997 showed that *M. trossulus* dominated the smaller size classes (15–20 mm SL), while *M. edulis* dominated the larger size classes (> 40 mm SL) (Comesaña et al. 1999). The relative frequency of hybrids was independent of shell length (SL), and most of the hybrids were backcross genotypes. Only five of the 400 individuals sampled had a genotype that could be classified as F_1. The low frequency of F_1 individuals is consistent with strong prezygotic isolation (Jiggins and Mallet 2000), and differences in the gametogenic cycles of the two species may contribute to reproductive isolation (Toro et al. 2002). The Toro et al. (2002) study, which was also done

at the same sites in Trinity Bay, Newfoundland, in 1996 showed that *M. edulis* spawned over 2–3 weeks in July, while *M. trossulus* and hybrids spawned in late summer to early autumn. However, postzygotic genetic incompatibility may also play a role in limiting the occurrence of F_1 genotypes, particularly during the early life-history stages. It is possible that genetic incompatibility may be expressed at metamorphosis, a critical stage at which larvae settle from the plankton.

Recently developed genetic markers allow the genotyping of individual mussel larvae, extending genetic information on species composition and hybridization to the earlier life-history stages (Toro 1998; Martel et al. 2000; Bierne et al. 2003b; Gilg and Hilbish 2003a, 2003b; Wood et al. 2003). The specific question addressed in the present study was: does differential mortality during the early life-history stages (larvae and juveniles) explain the high frequency of *M. trossulus* and low frequency of *M. edulis* and hybrids observed in the small adult size classes (15–20 mm SL) by Comesaña et al. (1999)?

Materials and methods

Study sites and sampling

Adult mussels (*Mytilus edulis* Linnaeus, 1758; *M. trossulus* Gould, 1850) were collected subtidally by SCUBA at a depth of approximately 1.5 m below mean low tide at two locations, Chance Cove and Bellevue, in Trinity Bay (47.62°N; 53.75°W) on the east coast of Newfoundland, in October 1995, 1996 and 1997. Dalley et al. (2002) provide a summary of oceanographic conditions in Trinity Bay. At each location, two sites were sampled: one exposed to wave action (Chance Cove exposed, CE; Bellevue exposed, BE) and another protected and sheltered from wave action (Chance Cove protected, CP; Bellevue protected, BP) (see Fig. 1 in Comesaña et al. 1999). Exposed and protected sites at each location are separated by about 2 km and the Bellevue and Chance Cove locations are separated by about 16 km. Haphazard samples of approximately 200 adult mussels (15.0–98.6 mm SL) were taken from each mussel bed at each site. The mussels were brought alive to the laboratory, shell length was measured with an electronic caliper (±0.01 mm), and the mussels were dissected. A small piece of mantle edge tissue (approximately 200 mg) was removed, placed in a 1.5-ml Eppendorf tube, fixed with 95% ethanol and stored at −20°C until DNA extraction.

Mussel juveniles (<2.0 to 15.0 mm SL) were collected from brown filamentous algae and from within mussel clumps at each of the sites described above during October and December 1995, September, October, and December 1996, and September and December 1997. The samples were placed in 95% ethanol and refrigerated (4°C) until DNA extraction. Larvae were qualitatively sampled in plankton tows (20 μm mesh) at the same sites sampled for adult mussels at Bellevue and Chance Cove, Newfoundland, during 1996 (19 July, 9 August, 3 and 19 September, 11 October), 1997 (2 September) and 1998 (1 August). Plankton samples were fixed with 95% ethanol and stored at 4°C until DNA extraction.

DNA extraction

Approximately 50–100 mg of mantle-edge tissue from each mussel (juvenile or adult) was coarsely chopped and digested overnight in 500 μl lysis buffer (50 mM Tris-HCl, pH 8.0, 1.0% SDS; 25 mM EDTA) with 200 μg proteinase K (Sigma) at 37°C. The digest was then extracted twice with 500 μl phenol–chloroform–isoamyl alcohol (24:24:1) followed by precipitation in 95% ethanol at −20°C. The extracted DNA was resuspended in 200 μl ultra-pure sterile distilled water.

For mussels of 2–5 mm SL, the whole flesh was removed from the shell and used for DNA extraction. For very small individuals (<2 mm SL), the whole mussel was used. DNA was extracted from individual larvae collected from plankton tows. For each sample, the largest larvae were selected to ensure that sufficient DNA could be extracted. *Mytilus* spp. larvae were the dominant bivalve species in the samples, and the species-specific primers ensured that only *Mytilus* spp. larval DNA was amplified. The DNA extraction procedure for larvae and newly settled juveniles was similar to that described for the mantle tissue, except that the shell length of each individual was first measured with a graduated eyepiece fitted to a stereomicroscope at ×40 magnification and the individual was isolated using a Pasteur pipette. Each individual was then placed in a separate 1.5-ml Eppendorf tube and washed twice in 0.5 ml distilled water before DNA extraction. In addition, 60 D-stage larvae from each of the two parent species and hybrids from laboratory cultures were analyzed in order to confirm species-specific genetic markers. For both genetic markers, the cultured larvae produced genotype patterns identical to samples from adult tissue for each species and hybrids.

Genetic markers

Genetic information was collected for two nuclear-DNA species-specific markers using the polymerase chain reaction (PCR). *ITS* and *Glu-5'* are co-dominant genetic markers producing two specific *M. edulis* and *M. trossulus* patterns and distinct patterns for hybrids. The *ITS* marker, developed by Heath et al. (1995), is based on the internal transcribed spacer (*ITS*) regions between the 18S and 28S nuclear-rDNA coding region. The primers used were *ITS*1 (5′-GTTTCCGTAGGTGAACCTG-3′) and *ITS*2 (5′-CTCGTCTGATCTGAGGTCG-3′), with an expected PCR gene fragment size of 1250 bp. Standard PCR amplifications were carried out in 25-μl reaction mixtures (500-μl thin-walled PCR microtubes,

Gordon Technologies) containing 2 μl DNA template (1:10 dilution), 0.2 mM each of the four deoxyribonucleotide triphosphates (dNTPs) (Sigma), 2.0 mM $MgCl_2$, primers at 0.4 mM, 1 U of *Taq* DNA polymerase (Promega), the manufacturer-supplied PCR buffer and sterile distilled water. The reaction mixtures were overlaid with a drop of mineral oil (Sigma) to prevent evaporation, and were then placed in a programmable thermocycler (MJ Research). The thermocycler protocol consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 20 s, 50°C for 20 s and 72°C for 2 min. Rigorous precautions were taken to prevent template contamination during the PCR procedure: positive displacement pipettes and autoclaved, sterile tips, tubes and reagents were used, and a negative control without the DNA template was run with every batch of samples.

Of each amplified PCR product, 5 μl was digested for 12 h at 37°C with 0.5 U of the restriction enzyme HhaI in a total volume of 15 μl, including 3 μl of buffer supplied by the manufacturer (Pharmacia) and 6.5 μl of ultra-pure distilled water. Enzyme inactivation was accomplished at 65°C for 20 min in a water bath, and the sample was allowed to cool for 20 min at room temperature. The digested products were fractionated electrophoretically in 3% agarose gels (2% Sigma and 1% NuSieve GTG agarose) with 0.5× Tris–borate–EDTA (TBE) buffer for 30 min at 112 V. A negative control with no PCR product was run with every batch of samples. A 1-kb DNA ladder (Gibco BRL) was run on each gel; the DNA fragments were visualized by placing the gels in ethidium bromide (0.5 $\mu g\ ml^{-1}$) and the genotype determined from Polaroid images taken under UV light. A negative control without a DNA template was run with every batch of samples.

A second nuclear-DNA marker, *Glu-5′*, developed by Rawson et al. (1996), targets the gene encoding the mussel polyphenolic adhesive protein produced by the pedal gland. The forward primer used was JH-5 (5′-GTAGGAACAAAGCATGAACCA-3′) and the reverse primer was JH54 (5′-GGGGGGATAAGTTTTCTTAGG-3′). Standard PCR amplifications were carried out in 25-μl reaction mixtures containing approximately 50 ng DNA template, 2.5 nmol dNTPs, 2.0 mM $MgCl_2$, 50 pmol of each primer, 1 U of Tfl DNA polymerase (Promega), the manufacturer-supplied PCR buffer and sterile distilled water. The reaction mixtures were overlaid with a drop of mineral oil (Sigma) to prevent evaporation, and were then placed in the thermocycler. The thermocycler protocol consisted of an initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 20 s, 53 °C for 20 s and 72°C for 45 s. PCR products were then separated on 3% agarose gels, stained with ethidium bromide and photographed under UV light.

Genetic variation for *ITS* and *Glu-5′* was assessed for larvae sampled in July and August 1996, for juveniles at the BP site in 1996 and 1997 and for adults from all four sites in 1995. For the remaining samples only the *Glu-5′* marker was used to reduce costs, since the *Glu-5′* marker is adequate for classifying individuals into species and hybrids for the analysis of variation in *Mytilus* spp. population genetic structure (Hilbish et al. 2002; Gilg and Hilbish 2003a, 2003b). Although classification based on one or a few markers could be misleading if introgression were extensive, it appears that hybridization in the *M. edulis–M. trossulus* hybrid zone is sufficiently limited that classification based on these diagnostic markers is adequate for comparing groups identified as different species and hybrids (Bates and Innes 1995; Saavedra et al. 1996; Comesaña et al. 1999; Rawson et al. 2001). The nomenclature used in the present study therefore identifies *M. edulis*, *M. trossulus* and hybrids based on the genetic markers but with the caveat that any introgression will reduce the distinction of phenotypic characters among the designated groups.

Statistical analysis

Variation in relative frequency of *M. edulis*, *M. trossulus* and individuals of mixed genotype (hybrids) was tested with R×C *G*-tests (Sokal and Rohlf 1981) or R×C randomization chi-squared tests with the MONTE program (McElroy et al. 1991), based on the algorithm of Roff and Bentzen (1989). Tests of fit to Hardy–Weinberg equilibrium (HWE) for individual loci and tests for linkage disequilibrium between two loci were carried out with GENEPOP (Raymond and Rousset 1995) using a web-based version of the program (http://wbiomed.curtin.edu.au/genepop/). The Analyse program (http://helios.bto.ed.ac.uk/evolgen/Mac/Analyse/index.html) developed by N.H. Barton and S.J.E. Baird was used to estimate the inbreeding coefficient (F_{is}) and the standardized linkage disequilibrium (R) using maximum-likelihood methods. Variation in mussel size was analyzed with ANOVA and Tukey's multiple comparison using the statistics program "R" (Ihaka and Gentleman 1996). Whenever multiple samples involving different sites, years, or months were tested, a sequential Bonferroni procedure (Rice 1989) was used to correct multiple tests for type-I error based on a critical probability value of 0.05.

Results

Larvae

Based on the *Glu-5′* marker, the relative frequency of the *Mytilus edulis*, *M. trossulus* and hybrid larvae (Fig. 1) showed no significant variation among the four sites for five of the six sample dates (19 July 1996 $X^2=12.33$, $df=6$, $P=0.05$; 9 August 1996 $X^2=3.67$, $df=6$, $P=0.726$; 19 September 1996 $X^2=15.84$, $df=6$, $P=0.016$; 2 September 1997 $X^2=12.97$, $df=6$, $P=0.041$; 1 August 1998 $X^2=2.14$, $df=6$, $P=0.909$). Only the 3 September 1996 ($X^2=26.15$, $df=6$,

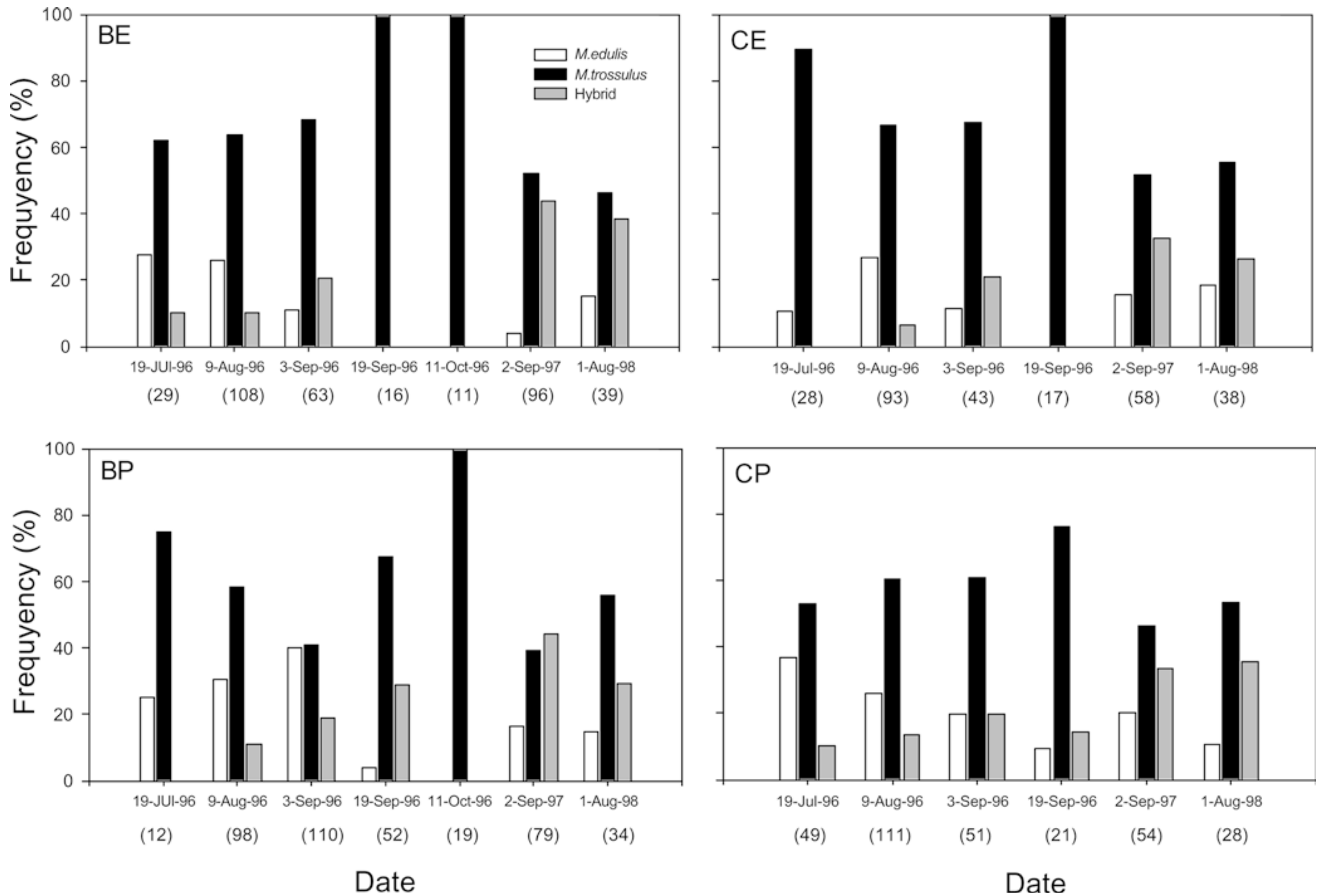

Fig. 1 *Mytilus* spp. Relative frequency (%) of *M. edulis*, *M. trossulus* and hybrid larvae (based on the *Glu-5′* marker) for samples from four sites (*BE*, *BP* Bellevue exposed and protected; *CE*, *CP* Chance Cove exposed and protected) in Trinity Bay, Newfoundland. The CE and CP sites were not sampled on 11 October 1996. Sample size for each date *in parentheses*

$P=0.0001$) sample showed a significantly different relative frequency of the three taxa among the sites following the Bonferroni procedure. The BP site differed from the other three sites on this date due to a lower frequency of *M. trossulus* and a higher frequency of *M. edulis* larvae (Fig. 1). There was, however, significant variation in relative frequency of the three taxa among sample dates within all four sites (BE $X^2=72.01$, $df=12$, $P<0.0001$; BP $X^2=78.36$, $df=12$, $P<0.0001$; CE $X^2=43.02$, $df=10$, $P<0.0001$; CP $X^2=24.89$, $df=10$, $P=0.006$), a similar temporal pattern of variation being observed in each (Fig. 1). *M. trossulus* larvae occurred with the highest frequency in all samples, and only *M. trossulus* was detected in the late season 1996 samples, except for the CP site, where larvae of both species and hybrids were also present. Although *M. edulis* larvae showed a greater frequency than hybrids in July and August 1996, hybrids showed a greater frequency in September 1997 and August 1998 (Fig. 1).

Twenty-two samples of larvae were tested for fit to HWE for the *Glu-5′* marker data given in Fig. 1 (samples containing only *M. trossulus* were excluded). For 11 of the samples the deviation from HWE was significant ($P<0.05$), with a deficiency of heterozygotes (hybrids). Samples from all four sites in July and August 1996 also showed a highly significant ($P<0.001$) deficiency of heterozygotes (hybrids) for the *ITS* marker, and there was highly significant linkage disequilibrium between the two markers in all samples ($P<0.0001$).

There were no significant differences in the frequency of the nine two-locus genotypes between the July and August 1996 samples at each of the four sites (BE $X^2=3.12$, $df=7$, $P>0.05$; BP $X^{2=}5.56$, $df=8$, $P>0.05$; CE $X^2=12.11$, $df=6$, $P>0.05$; CP $X^2=16.42$, $df=8$, $P=0.03$); therefore, data from the two months were pooled. There was a significant difference in the frequencies of the nine two-locus genotypes among the four sites ($X^2=46.94$, $df=24$, $P=0.003$), but the sites all showed the same general pattern (Table 1). *M. trossulus* larvae occurred with greatest frequency, followed by *M. edulis*, and the seven classes of mixed genotypes were least frequent. Mixed genotypes occurred with low frequency, particularly those consisting of two alleles from one species and two from the other (e/e t/t, t/t e/e). Individuals heterozygous at both loci (F_1-like: e/t e/t) occurred with the lowest frequency (Table 1), and there was a highly significant deviation ($G=46.21$, $df=6$, $P<0.0001$) from equal frequency among the seven mixed genotypes. However, when the F_1-like class was removed, no significant difference ($G=8.29$, $df=5$,

Table 1 *Mytilus* spp. Number of larvae for each of nine two-locus genotypes (*ITS, Glu-5′*) sampled from four Trinity Bay sites (abbreviations see Fig. 1) in 1996. Mean shell length for largest larvae selected from July and August samples. Genotype classes with different *superscripts* (1, 2) have a significantly different mean length within each month based on a multiple comparisons analysis

Genotype		Site						Mean length, μm (SE)	
ITS	*Glu-5′*	BE	BP	CE	CP	Total	Percent	July	August
e/e	e/e	19	21	22	36	98	18.6	139.6 (2.92)	237.4 (1.99)
e/e	e/t	10	3	5	8	26	4.9	150.0 (10.00)	249.6 (3.47)[1]
e/t	e/e	12	8	6	9	35	6.6	134.0 (9.27)	244.0 (2.90)
e/e	t/t	2	5	7	5	19	3.6	140.0 (4.08)	241.3 (5.84)
e/t	e/t	0	2	0	1	3	0.6	140.0 (–)	230.0 (10.00)
t/t	e/e	5	4	0	2	11	2.1	126.7 (3.33)	226.3 (7.78)
e/t	t/t	4	4	4	18	30	5.7	144.0 (8.72)	249.6 (3.29)[1]
t/t	e/t	4	6	1	11	22	4.2	147.5 (4.79)	251.1 (4.84)[1]
t/t	t/t	81	57	76	70	284	53.8	130.4 (1.62)	232.6 (1.41)[2]
Total		137	110	121	160	528			

$P > 0.05$) from equal frequency was detected among the remaining six mixed genotype classes.

Larvae sampled in July were smaller than those sampled in August (Table 1). Although there was significant variation overall ($F = 2.52$, $df = 8, 109$, $P < 0.05$) in larval size among the nine genotype classes in July, a multiple comparisons analysis (Tukey) among all pairs of genotypes did not detect any significant differences. There was also significant ($F = 6.06$, $df = 8, 401$, $P < 0.001$) variation in larval length among the nine genotypic classes in August. A multiple comparisons analysis showed significant differences in shell length only between the smaller t/t t/t genotype and the larger e/e e/t, e/t t/t and t/t e/t genotypes.

Juveniles

The relative frequency of the two species and hybrids (based on the *Glu-5′* marker) showed no significant heterogeneity among seven length classes (2–15 mm SL) for each of three years (1995, 1996, 1997), except for the BP site (1995 $X^2 = 45.09$, $df = 12$, $P < 0.0001$; 1996 $X^2 = 45.00$, $df = 12$, $P < 0.0001$; 1997 $X^2 = 60.00$, $df = 12$, $P < 0.0001$) and the 1995 CP sample ($X^2 = 48.99$, $df = 12$, $P < 0.0001$). In general, *M. trossulus* juveniles were much more frequent than *M. edulis* or hybrid juveniles and for most size classes of juveniles at all of the sites averaged over the three years (Fig. 2). However, samples from the BP and CP sites showed a more complicated pattern, with a decline in relative frequency for *M. trossulus* and an increase in *M. edulis* with increasing size class (Fig. 2).

All 12 samples of juveniles (3 years and 4 sites) showed significant ($P < 0.001$) deviation from HWE, with a deficiency of heterozygotes (hybrids) for the *Glu-5′* marker. Samples from 1996 and 1997 from the BP site also showed a highly significant ($P < 0.001$) deficiency of heterozygotes for *ITS*, and there was a highly significant linkage disequilibrium ($P < 0.001$) between the two loci in both years.

No difference in relative frequency of the nine two-locus genotypes was observed between 1996 and 1997 ($G = 5.29$, $df = 8$, $P > 0.05$), and the two years were therefore pooled (Table 2). As in the larvae, mixed genotypes were much less frequent than pure *M. trossulus* or *M. edulis* genotypes, and F_1-like individuals were rare (Table 2). There was a marginally significant difference ($G = 12.98$, $df = 6$, $P = 0.043$) from equal frequency among the seven mixed genotype classes, and no significant difference ($G = 5.78$, $df = 5$, $P > 0.05$) from equal frequency among the six mixed genotypes after the F_1-like class was removed.

There was significant variation ($F = 7.37$, $df = 8, 321$, $P < 0.001$) in shell length among the nine genotype classes (Table 2). A multiple comparisons analysis revealed that the only significant comparison was that the e/e e/e genotype class was larger than the t/t t/t genotype class.

Adults

The change in frequency of *M. edulis*, *M. trossulus* and hybrids (based on the *Glu-5′* marker) with increasing size (averaged over 1995, 1996, 1997) showed a similar pattern at the four sites (Fig. 2). There was a general decline in frequency of *M. trossulus* and an increase in frequency of *M. edulis* with increasing size. Hybrids showed a low frequency in all size classes at all sites and were absent from the larger size classes. The fit to HWE for the *Glu-5′* marker was tested for the smallest adult size class (15–20 mm SL) for each of the samples from the four sites in each year (1995, 1996, 1997). Only two (CP 1995, CP 1997) of the 12 tests showed a significant deviation from HWE with a deficiency of heterozygotes (hybrids). Tests of the *ITS* locus for fit to HWE for the 15–25 mm size class (1995 data) showed a significant ($P < 0.001$) deficiency of heterozygotes for three (BE, BP, CE) of the four sites. There was also highly significant ($P < 0.001$) linkage disequilibrium between the two loci.

Of the five size classes of adult mussels (Table 3), only the 15–25 mm size class showed a significant difference ($X^2 = 51.6$, $df = 24$, $P < 0.001$) in relative frequency of the nine two-locus genotypes among the four sites. For this size class the sites differed in relative frequency of

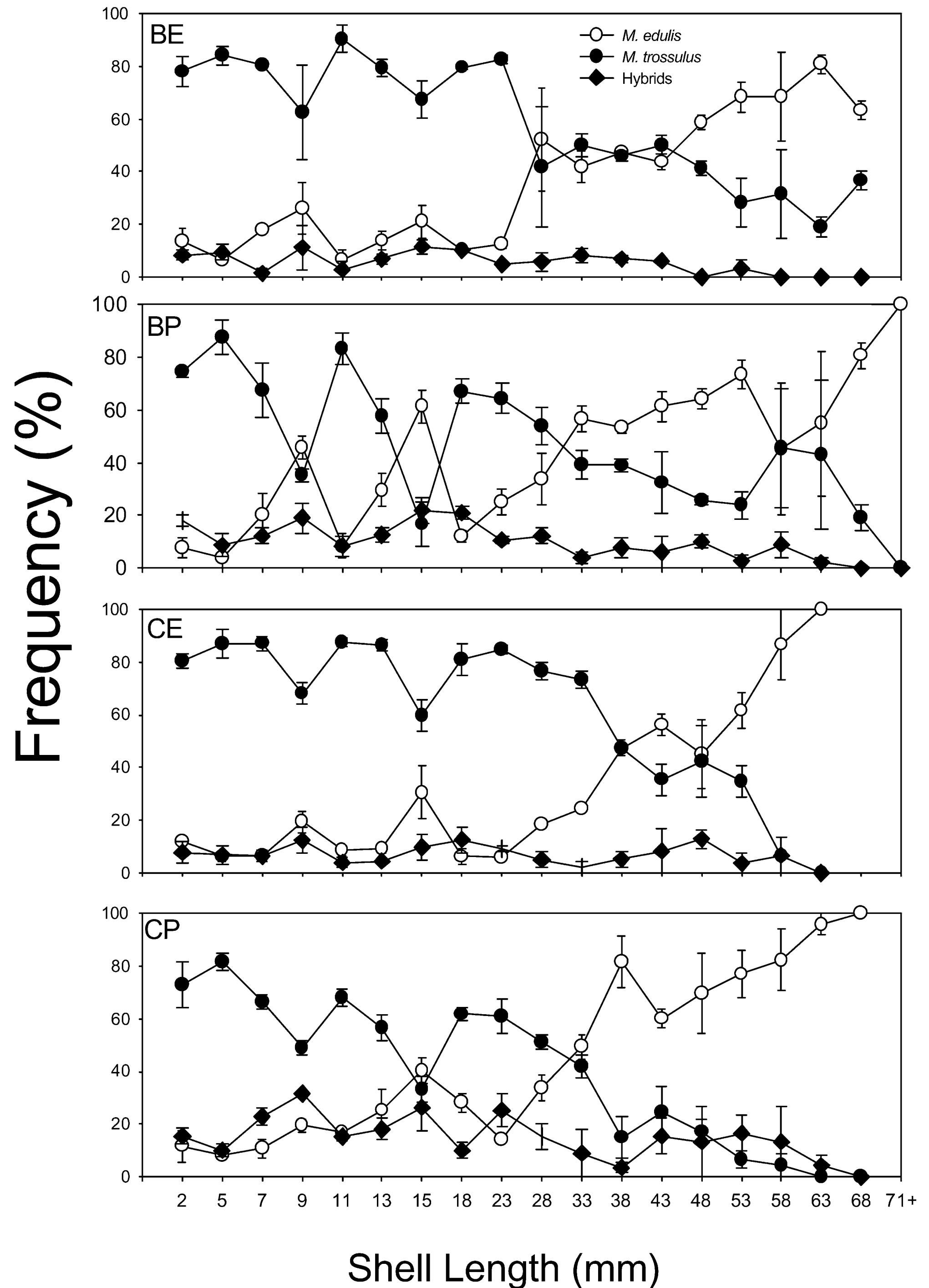

Fig. 2 *Mytilus* spp. Mean (± SE) relative frequency (%) (average of 1995, 1996, 1997 samples) of *M. edulis*, *M. trossulus* and hybrids (based on the *Glu-5′* marker) in different size classes for samples from four sites (abbreviations see Fig. 1) in Trinity Bay, Newfoundland. Juvenile size classes are 2–15 mm SL (2-mm size class intervals), and adult size classes are 18–71+ mm SL (5-mm size class intervals). Average sample size for each year: BE (377), BP (406), CE (358), CP (406)

M. trossulus, but no significant difference ($X^2 = 22.06$, $df = 21$, $P = 0.23$) was found among the sites when the *M. trossulus* genotype was removed. Pooling among the four sites, the nine two-locus genotype frequencies showed significant variation ($X^2 = 155.1$, $df = 32$, $P < 0.001$) among the five size classes (Table 3). The smallest size class was dominated by *M. trossulus*, and the largest by *M. edulis*. Individuals of mixed genotype occurred at low frequency in all size classes, the F_1-like hybrid occurring with the lowest frequency (Table 3).

Comparison among life-history stages

There was significant heterogeneity ($X^2 = 277.8$, $df = 54$, $P < 0.0001$) in the frequency of the nine genotype classes among larvae, juveniles and the five adult size classes. Significant heterogeneity ($X^2 = 67.9$, $df = 36$, $P = 0.0014$) was also detected for the frequency of the seven mixed genotype classes among larvae, juveniles and the five adult size classes. However, a comparison of relative frequency of the nine genotypes and the seven mixed

Table 2 *Mytilus* spp. Number of juveniles and mean shell length for nine two-locus genotypes (*ITS, Glu-5′*) at the BP site sampled in two years (September, October, December 1996; September, December 1997). Genotype classes with different superscripts (1, 2) have a significantly different mean length based on a multiple comparisons analysis

Genotype		1996	1997	Total	Percent	Mean length (mm)	SE
ITS	*Glu-5′*						
e/e	e/e	21	38	59	17.9	10.3[1]	0.41
e/e	e/t	6	5	11	3.3	9.5	0.90
e/t	e/e	5	5	10	3.0	10.0	1.09
e/e	t/t	5	4	9	2.7	9.5	1.25
e/t	e/t	1	2	3	0.9	10.8	2.22
t/t	e/e	3	4	7	2.1	10.3	0.96
e/t	t/t	10	8	18	5.5	8.8	0.88
t/t	e/t	5	6	11	3.3	9.3	0.91
t/t	t/t	74	128	202	61.2	7.0[2]	0.25
Total		130	200	330			

Table 3 *Mytilis* spp. Number of adults occurring in five shell-length classes for nine two-locus genotypes (*ITS, Glu-5′*) pooled from four Trinity Bay sites in October 1995

Genotype		Shell length (mm)					Total	Percent
ITS	*Glu-5′*	15–25	26–35	36–45	46–55	56 +		
e/e	e/e	12	34	55	49	52	202	37.2
e/e	e/t	3	4	11	11	6	35	6.5
e/t	e/e	7	8	5	1	1	22	4.0
e/e	t/t	4	5	2	2	1	14	2.6
e/t	e/t	4	0	1	2	0	7	1.3
t/t	e/e	6	5	5	4	0	20	3.7
e/t	t/t	11	5	3	1	0	20	3.7
t/t	e/t	13	10	1	3	1	28	5.2
t/t	t/t	83	47	34	19	12	195	35.9
Total		143	118	117	92	73	543	

Table 4 *Mytilus* spp. Comparison between successive life-history stages for differences in the relative frequency of nine two-locus (*Glu-5′*–ITS) genotypes and seven mixed (hybrid) genotypes. $^*P < 0.05$

Life-history comparison	Nine two-locus genotypes (X^2,*df* = 8)	Seven mixed genotypes (X^2,*df* = 6)
Larvae vs. Juveniles	9.4	4.0
Juveniles vs. 15–25 mm	18.9	5.5
15–25 mm vs. 26–35 mm	25.4*	5.7
26–35 mm vs. 36–45 mm	21.2*	7.7
36–45 mm vs. 46–55 mm	6.8	4.8
46–55 mm vs. 56 + mm	9.4	3.8

genotypes between successive life-history stages showed that only two comparisons between intermediate size classes (15–25 vs. 26–35 mm and 26–35 vs. 36–45 mm) were significantly different (Table 4). The relative frequency of the seven mixed genotype classes as a pooled group of hybrids showed no significant variation ($X^2 = 11.15$, $df = 5$, $P = 0.05$) among larvae, juveniles, or the first four adult size classes and only significant variation ($X^2 = 21.96$, $df = 6$, $P = 0.0016$) when the > 56 mm size class was included (Fig. 3A).

Estimates of the overall inbreeding coefficients (F_{is}), based on *ITS* and *Glu-5′*, were significantly different from zero for each life-history stage (Fig. 3B). There was significant heterogeneity (log-likelihood = 14.18, $df = 6$, $P = 0.013$) among the life-history stages for the estimated inbreeding coefficients due to a decrease observed in F_{is} for the 15–25 mm size class. In addition, linkage disequilibrium, as measured by the standard disequilibrium coefficient *R*, was also significantly different from zero for the life-history stages (Fig. 3B) and was homogeneous among the different life-history stages (log-likelihood = 5.98, $df = 6$, $P = 0.425$).

Discussion

The present study extends information on the genetic composition of mussels in the adult stages reported in a previous study (Comesaña et al. 1999) to earlier life-history stages (pre-settlement larvae and juveniles) at the same sites in Trinity Bay, Newfoundland. The high relative frequency of *Mytilus trossulus* and lower frequency of *M. edulis* and hybrids observed in small adult mussels (15–20 mm SL) by Comesaña et al. (1999) was also found for the same size class over three consecutive years (1995–1997) in the present study, and was also recorded for the late-larval stage and juveniles at the same sites. *M. trossulus* larvae were much more frequent than *M. edulis* and hybrid larvae in all samples, and *M. trossulus* was the only species observed in four samples of larvae taken later in the year (September, October). The high relative frequency of *M. trossulus* larvae may be related to differences between the species in population density, reproductive output and spawning time. For example, at many sites

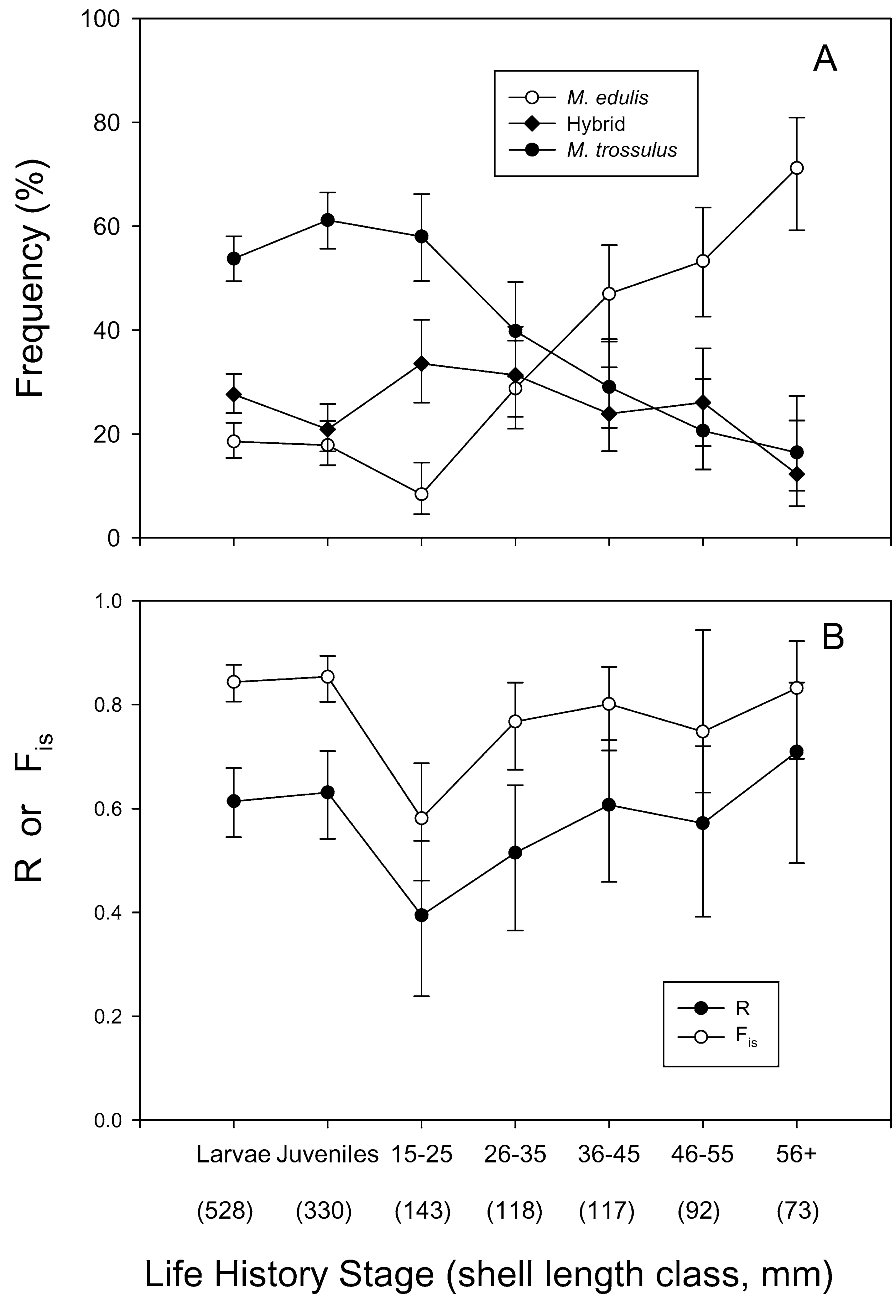

Fig. 3A, B *Mytilus* spp. **A** Frequency (*error bars*: 95% confidence intervals) of *M. edulis*, *M. trossulus* and hybrids for larvae, juvenile and five adult classes of shell length based on two genetic markers (*ITS*, *Glu-5′*). **B** Linkage disequilibrium (*R*) and inbreeding coefficient (F_{is}) (*error bars*: two-unit support limits). Sample size *in parentheses*

M. trossulus dominates the smaller adult size classes (15–30 mm SL) (Comesaña et al. 1999; M. Miranda, unpublished data), which occur at higher density than the larger size classes (Bates 1992). The high density of *M. trossulus*, combined with a greater investment in reproduction than *M. edulis* for individuals of the same size (Toro et al. 2002), probably results in a larger number of *M. trossulus* larvae being produced. The occurrence of only *M. trossulus* larvae in some samples late in the year may be a consequence of the later spawning of *M. trossulus* compared with *M. edulis* at these Trinity Bay sites (Toro et al. 2002). There was a tendency for *M. trossulus* larvae and juveniles to have a smaller shell length than *M. edulis* and hybrids. Although differences in shell length may be due to differences in growth rate, the observed later spawning of *M. trossulus* (Toro et al. 2002) could also explain the smaller size of the larvae.

Juveniles ranging from <2 to 15 mm SL were also predominantly composed of *M. trossulus*, although this pattern differed at two sites such that *M. trossulus* juveniles were less frequent and *M. edulis* more frequent in the larger juvenile size classes. Despite this site difference, there was a consistent pattern in which *M. trossulus* occurred with a high relative frequency and *M. edulis* and hybrid genotypes occurred with much lower frequency among the pre-settlement larvae, juveniles and the 15–30 mm SL adult size class.

Hybrids were uncommon in the present study and, according to the single *Glu-5′* marker, showed little change in relative frequency among the larvae, juveniles and the various size classes of adult mussels (> 15 mm SL). Two markers (*ITS*, *Glu-5′*) identified more hybrid individuals, but the seven hybrid genotype classes thus obtained also showed little change in relative frequency among the larvae, juveniles and the different size classes of juvenile and adult mussels. Furthermore, hybrids for both *ITS* and *Glu-5′* occurred with the lowest frequency for all life-history stages, which is consistent with our previous observations on mussels > 15 mm SL (Comesaña et al. 1999). Although hybrids for both markers may not necessarily be true F_1 hybrids, Comesaña et al. (1999) observed that all F_1-like genotypes, identified using four genetic markers, also had the mtDNA genotype expected for F_1 hybrids. If some of the mussels classified as F_1-like in the present study are actually other variants of mixed genotypes (F_2, etc.), this would support the hypothesis that F_1 hybrids are rare in the *M. edulis*–*M. trossulus* hybrid zone, as was found by Saavedra et al. (1996) and Comesaña et al. (1999). Hybrid individuals in the larger size classes (> 36 mm) were generally biased towards *M. edulis*.

No change in relative frequency was observed for the hybrid genotypes among the different size classes. This contrasts with the two species, which showed very large shifts in relative frequency with increasing size, as documented previously (Comesaña et al. 1999). The observed pattern of *M. trossulus* declining and *M. edulis* increasing in relative frequency with increasing size class could be due to a greater mortality rate in *M. trossulus* than in *M. edulis*. *M. trossulus* appears to have a thinner shell than *M. edulis*, a weaker adductor muscle and lower byssus production (J. Lowen and M. Miranda, unpublished observations). These differences suggest that *M. trossulus* is more susceptible to predation and has compensated for an increased probability of mortality by increasing investment in reproduction relative to *M. edulis* (Toro et al. 2002). The hypothesis that *M. trossulus* is more susceptible to predation than *M. edulis* is currently being tested.

It cannot be assumed that larvae and juveniles sampled at a particular site were produced by adult mussels at the same site. Based on laboratory studies (Bierne et al 2002a), mussel larvae cultured at 18°C settle after approximately 35 days and therefore have the potential for long-distance dispersal in the plankton. The genetic composition of larvae and juveniles reflects the genetic composition of the source population and may include mixtures of larvae from several different populations (Pedersen et al. 2000). However, recruits may be derived from the local population if the pattern of ocean currents limits dispersal (Gilg and Hilbish 2003a). McQuaid and Phillips (2000) observed that most recruitment of the invasive *M. galloprovincialis* in South African waters occurred within < 5 km of the parent population. It is not known whether local recruitment occurs at the Trinity Bay sites sampled in the present study. The sites lie at the head of a large enclosed bay, so some self-recruitment may be possible. A study of oceanographic conditions in Trinity Bay suggested that weak gyres might retain certain larvae within the bay (Dalley et al. 2002). Regardless of the source population, the genetic composition of larvae and juveniles sampled in three consecutive years consistently showed a high frequency of *M. trossulus* at all four sites sampled. Although there is some variation in the relative frequency of the two species at different sites along the coast of Newfoundland, most sites contain a similar mixture of both species (Bates and Innes 1995; Innes et al. 1999; Penney and Hart 1999). Therefore, the species and hybrid composition of the source population for recruiting larvae and juveniles is not likely to differ very much from that of adult mussels from the Trinity Bay sites. Pedersen et al. (2000) also found that *M. trossulus* dominated newly settled mussels at a site in Nova Scotia. They observed some heterogeneity in the genetic composition of recruits that could be attributed to variation in the relative proportion of the two species. The high frequency of *M. trossulus* in the recruits was probably attributable to the greater frequency of this species in the adult populations in the area (Pedersen et al. 2000).

Our observations of high levels of linkage disequilibrium and a high inbreeding coefficient (heterozygote deficiency) for the genetic markers confirm previous studies showing that *M. edulis* and *M. trossulus* are partially reproductively isolated, with the occurrence of some hybrids in natural populations (Saavedra et al. 1996; Comesaña et al. 1999). A low frequency of hybrids may result from prezygotic factors such as spatial separation of the two species, spawning asynchrony, gamete incompatibility and gamete choice, or postzygotic factors such as genetic incompatibility and mortality at different developmental stages (Palumbi 1994). The relative importance of these factors for reproductive isolation between mussel species has yet to be determined. Metamorphosis from the swimming larva to the settled juveniles represents a major developmental transition in mussels during which any postzygotic genetic incompatibilities in hybrid individuals may be expressed. However, in the present study, no difference in the frequency of hybrids was detected between these two life-history stages for individuals collected from natural populations. Furthermore, laboratory crosses between the two species have produced large numbers of viable F_1 hybrid larvae, juveniles and adults, with little evidence for extensive genetic incompatibility (M. Miranda, unpublished data). A similar low frequency of hybrids observed among larvae and juveniles in nature suggests that factors limiting the production of hybrids operate prior to the late larval stage. Although a degree of spawning asynchrony has been observed between *M. trossulus* and *M. edulis* at the Trinity Bay sites (Toro et al. 2002), it is not clear how much this asynchrony prevents hybrid production, because there is sufficient overlap in the spawning to permit the potential production of some hybrids.

Gamete incompatibility can also be an effective prezygotic reproductive isolating mechanism for coexisting marine invertebrate species that release their gametes into the water column (Levitan 2002). Laboratory fertilization experiments have demonstrated incompatibility between *M. edulis* and *M. trossulus* at the gamete stage (Rawson et al. 2003; M. Miranda, unpublished data), interspecific fertilizations being less frequent than intraspecific fertilizations (Rawson et al. 2003). Furthermore, not all interspecific matings showed the same low fertilization success, and there was evidence for asymmetry in interspecific fertilization success. Fertilization of *M. edulis* eggs by *M. trossulus* sperm was more successful than fertilization of *M. trossulus* eggs by *M. edulis* sperm (Rawson et al. 2003). Nevertheless, the degree of gamete incompatibility is probably sufficient to account for the low frequency of hybrids observed in natural populations of *M. edulis* and *M. trossulus*. Further genetic incompatibility during early embryo and larval development may also contribute to a reduction in the frequency of hybrids (M. Miranda, unpublished data). Our observation of a similar frequency of hybrids in natural populations among the late larval stage and the different adult size classes suggests that hybrids may have a rate of survival intermediate between the two parental species. However, these observations are restricted to samples of the life-history stages and size classes over a short time period (static cohort analysis) rather than following a single cohort (dynamic cohort analysis). Furthermore, changes in relative frequency among genotypes can be explained by a number of possible mortality rates for each genotype. Determination of the survival of a cohort of the two species and hybrids reared in the laboratory as well as deployed to different natural sites is currently in progress (M. Miranda, unpublished data).

The observed higher frequency of mixed hybrid genotypes but lower frequency of F_1-like hybrids suggests that backcross mating between F_1 individuals and one or the other parent species may result in a lower level of gamete and genetic incompatibility. However, the low relative frequency of F_1-like hybrids and higher frequency of other mixed hybrid genotypes could also be explained by spatial and/or temporal segregation of spawning between the two species combined with genetic incompatibility during early development. Subsequent spawning by a small number of F_1 hybrids will generate many more backcross genotypes, because each F_1 individual will release large numbers of gametes that are most likely to encounter gametes from one or the other parent species. Thus, several factors may interact to limit the formation of F_1 hybrids, but once a few viable and fertile F_1 hybrids are produced they can generate more mixed hybrid genotypes. The absence or low frequency of F_1-like individuals in the presence of other hybrid genotypes at higher frequency is commonly seen in hybrid zones (Arnold 1997).

M. edulis and *M. galloprovincialis* hybridize in Europe, and gamete recognition rather than gamete incompatibility may be the important factor limiting this hybridization (Bierne et al. 2002a). Bierne et al. (2002a) carried out fertilization experiments involving mixtures of gametes from the two species, which allowed gamete competition. Mixtures of embryos derived from within and between species crosses were used as controls to test for gamete and genetic incompatibility. A significant deficiency of hybrids was observed only when gamete competition was allowed. The authors concluded that no strong barriers to fertilization exist between the two species, but when gametes of both species are present intra-specific fertilizations occur more frequently than inter-specific fertilizations. There was also some evidence for differential viability among genotypes during the larval and juvenile stages, but the results were equivocal with respect to hybrid viability. Similarly, observations by Beaumont et al. (1993) suggest that the role of post-fertilization incompatibility in the maintenance of the *M. edulis–M. galloprovincialis* hybrid zone requires further study.

A comprehensive understanding of the dynamics and fate of mussel hybrid zones requires information on life-history differences between coexisting species as well as environmental and genetic factors that can explain the observed frequency of hybrids. The structure of the *M. edulis–M. trossulus* and the *M. edulis–M. galloprovincialis* hybrid zones appears to involve two distinct but related processes: those responsible for the production and persistence of hybrids without a merging of the two species and those maintaining the coexistence of two ecologically similar species. *Mytilus* species have a similar habitat, with individuals attached to the substrate, removing food particles from the water column and producing planktonic larvae. We would expect coexisting species to compete for space and food, assuming both factors are limiting. Opportunities for niche diversification to reduce inter-specific competition may include spatial separation through habitat specialization. The *M. edulis–M. galloprovincialis* hybrid zone has been studied more extensively and for a longer period than the *M. edulis–M. trossulus* hybrid zone (Gardner 1994). *M. edulis* and *M. galloprovincialis* differ in habitat distribution as a result of variation in wave exposure and salinity (Bierne et al. 2002b, 2003a). *M. galloprovincialis* has a stronger byssal attachment, and is thus better adapted to habitats with greater wave energy (Gardner and Skibinski 1991; Willis and Skibinski 1992). The consequence is that spatial separation and niche diversification may explain the coexistence of the two species in the same area. The *M. edulis–M. trossulus* hybrid zone exhibits less geographic structure than the *M. edulis–M. galloprovincialis* hybrid zone, and no environmental factors have been identified that favor one species or the other. Similar frequencies of *M. edulis*, *M. trossulus* and hybrids were found at exposed and sheltered sites in Trinity Bay. However, mussels were sampled subtidally, and the habitat differences may not have been great enough to reveal any selective effect of wave exposure. Future sampling should include intertidal sites varying

in wave exposure to test for habitat segregation by *M. edulis* and *M. trossulus*. Life-history differences between *M. edulis* and *M. trossulus*, such as differences in age-specific survival and reproduction, may also explain their coexistence in the absence of any differences in adaptation to specific environmental conditions.

The limited occurrence of hybrids observed in natural populations suggests that *M. edulis* and *M. trossulus* are partially reproductively isolated and that both pre- and postzygotic mechanisms operate. The low frequency of F_1 hybrids observed in natural populations may be due to a combination of spawning asynchrony, gamete incompatibility and genetic incompatibility during early development. Further studies are in progress to investigate the environmental, genetic and life-history factors involved in maintaining the *M. edulis*–*M. trossulus* hybrid zone.

Acknowledgements We thank M. Miranda and two anonymous reviewers for comments on the manuscript. S. Baird provided advice for the Analyse program. J.E.T. was supported by the Universidad Austral de Chile and a PhD fellowship from the CIDA/MUN Marine Science Scholarship Programme. The study was supported by grants from the Natural Sciences and Engineering Research Council (Canada) to D.J.I. and R.J.T.

References

Arnold ML (1997) Natural hybridization and evolution. Oxford University Press, Oxford

Arnold ML, Hodges SA (1995) Are natural hybrids fit or unfit relative to their parents? Trends Ecol Evol 10:67–71

Barton NH, Hewitt GM (1989) Adaptation, speciation and hybrid zones. Nature 341:497–503

Bates JA (1992) Genetic and morphological variation in the mussel *Mytilus* in Newfoundland. MSc thesis, Memorial University of Newfoundland, St. John's

Bates JA, Innes DJ (1995) Genetic variation among populations of *Mytilus* spp. in eastern Newfoundland. Mar Biol 124:417–424

Beaumont AR, Abdul-Matin AKM, Seed R (1993) Early development, survival and growth in pure and hybrid larvae of *Mytilus edulis* and *Mytilus galloprovincialis*. J Molluscan Stud 59:120–123

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

Bierne N, David P, Langlade A, Bonhomme F (2002b) Can habitat specialisation maintain a mosaic hybrid zone in marine bivalves? Mar Ecol Prog Ser 245:157–170

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

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

Boecklen WJ, Howard DJ (1997) Genetic analysis of hybrid zones: numbers of markers and power of resolution. Ecology 78:2611–2616

Comesaña AS, Toro JE, Innes DJ, Thompson RJ (1999) A molecular approach to the ecology of a mussel (*Mytilus edulis*–*M. trossulus*) hybrid zone on the east coast of Newfoundland, Canada. Mar Biol 133:213–221

Daguin C, Bonhomme F, Borsa P (2001) The zone of sympatry and hybridization of *Mytilus edulis* and *M. galloprovincialis*, as described by intron length polymorphism at locus *mac-1*. Heredity 86:342–354

Dalley EL, Anderson JT, deYoung B (2002) Atmospheric forcing, larval drift, and recruitment of capelin (*Mallotus villosus*). ICES J Mar Sci 59:929–941

Edwards CA, Skibinski DOF (1987) Genetic variation of mitochondrial DNA in mussel (*Mytilus edulis* and *Mytilus galloprovincialis*) populations from southwest England and South Wales. Mar Biol 94:547–556

Gardner JPA (1994) The structure and dynamics of naturally occurring hybrid *Mytilus edulis* Linnaeus, 1758 and *Mytilus galloprovincialis* Lamarck, 1819 (Bivalvia: Mollusca) populations: review and interpretation. Arch Hydrobiol 99[Suppl]:37–71

Gardner JPA, Skibinski DOF (1991) Biological and physical factors influencing genotype-dependent mortality in hybrid mussel populations. Mar Ecol Prog Ser 71:235–243

Gardner JPA, Thompson RJ (2001) The effects of coastal and estuarine conditions on the physiology and survivorship of the mussels *Mytilus edulis*, *M. trossulus* and their hybrids. J Exp Mar Biol Ecol 265:119–140

Gardner JPA, Skibinski DOF, Bajdik CD (1993) Shell growth and mortality differences between the marine mussels *Mytilus edulis* (L.), *Mytilus galloprovincialis* (Lmk.) and their hybrids from two sympatric populations in S.W. England. Biol Bull (Woods Hole) 185:405–416

Gilg MR, Hilbish TJ (2003a) Patterns of larval dispersal and their effect on the maintenance of a blue mussel hybrid zone in southwestern England. Evolution 57:1061–1077

Gilg MR, Hilbish TJ (2003b) Spatio-temporal patterns in the genetic structure of recently settled blue mussels (*Mytilus* spp.) across a hybrid zone. Mar Biol 143:679–690

Gosling EM (1992) Systematics and geographical distribution of *Mytilus*. In: Gosling EM (ed) The mussel *Mytilus*: ecology, physiology, genetics and culture. Elsevier, Amsterdam, pp 1–20

Harrison RG (1993) Hybrid zones and the evolutionary process. Oxford University Press, Oxford

Heath DD, Rawson PD, Hilbish TJ (1995) PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp.) genotypes on the west coast of Canada. Can J Fish Aquat Sci 52:2621–2627

Hilbish TJ, Carson EW, Plante JR, Weaver LA, Gilg MR (2002) Distribution of *Mytilus edulis*, *M. galloprovincialis*, and their hybrids in open-coast populations of mussels in southwestern England. Mar Biol 140:137–142

Ihaka R, Gentleman R (1996) R: a language for data analysis and graphics. J Comput Graph Stat 5:299–314

Innes DJ, Comesaña AS, Toro JE, Thompson RJ (1999) The distribution of *Mytilus edulis* and *M. trossulus* at spat collection sites in Newfoundland. Bull Aquac Assoc Can 99:22–23

Jiggins CD, Mallet J (2000) Bimodal hybrid zones and speciation. Trends Ecol Evol 15:250–255

Koehn RK, Hall JG, Innes DJ, Zera AJ (1984) Genetic differentiation of *Mytilus edulis* in eastern North America. Mar Biol 79:117–126

Levitan DR (2002) The relationship between conspecific fertilization success and reproductive isolation among three congeneric sea urchins. Evolution 56:1599–1609

Marshall JL, Arnold ML, Howard DJ (2002) Reinforcement: the road not taken. Trends Ecol Evol 17:558–563

Martel AL, Auffrey LM, Robles CD, Honda BM (2000) Identification of settling and early postlarval stages of mussels (*Mytilus* spp.) from the Pacific coast of North America, using prodissoconch morphology and genomic DNA. Mar Biol 137:811–818

McDonald JH, Koehn RK (1988) The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific Coast of North America. Mar Biol 99:111–118

McElroy D, Moran P, Bermingham E, Kornfield I (1991) REAP: the Restriction Enzyme Analysis Package, version 4.0. Department of Zoology, University of Maine, Walpole

McQuaid CD, Phillips TE (2000) Limited wind-driven dispersal of intertidal mussel larvae: in situ evidence from the plankton and the spread of the invasive species *Mytilus galloprovincialis* in South Africa. Mar Ecol Prog Ser 201:211–220

Orr HA, Presgraves DC (2000) Speciation by postzygotic isolation: forces, genes and molecules. BioEssays 22:1085–1094
Palumbi SR (1994) Genetic divergence, reproductive isolation and marine speciation. Annu Rev Ecol Syst 25:547–72
Pedersen EM, Hunt HL, Scheibling RE (2000) Temporal genetic heterogeneity within a developing mussel (*Mytilus trossulus* and *M. edulis*) assemblage. J Mar Biol Assoc UK 80:843–854
Penney RW, Hart MJ (1999) Distribution, genetic structure, and morphometry of *Mytilus edulis* and *M. trossulus* within a mixed species zone. J Shellfish Res 18:367–374
Qiu JW, Tremblay R, Bourget E (2002) Ontogenetic changes in hyposaline tolerance in the mussels *Mytilus edulis* and *M. trossulus*: implications for distribution. Mar Ecol Prog Ser 228:143–152
Rand DM, Harrison RG (1989) Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. Evolution 43:432–449
Rawson PD, Joyner KL, Meetze K, Hilbish TJ (1996) Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. Heredity 77:599–607
Rawson PD, Agrawal V, Hilbish TJ (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 PD, Hayhurst S, Vanscoyoc B (2001) Species composition of blue mussel populations in the northeastern Gulf of Maine. J Shellfish Res 20:31–38
Rawson PD, Slaughter C, Yund PO (2003) Patterns of gamete incompatibility between the blue mussels *Mytilus edulis* and *M. trossulus*. Mar Biol 143:317–325
Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249
Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225
Rieseberg LH (1998) Molecular ecology of hybridization. In: Carvalho GR (ed) Advances in molecular ecology. IOS Press, Amsterdam, pp 243–265
Riginos C, Sukhdeo K, Cunningham CW (2002) Evidence for selection at multiple allozyme loci across a mussel hybrid zone. Mol Biol Evol 19:347–351
Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: chi-square and the problem of small samples. Mol Biol Evol 6:539–545
Ross CL, Harrison RG (2002) A fine-scale spatial analysis of the mosaic hybrid zone between *Gryllus firmus* and *Gryllus pennsylvanicus*. Evolution 56:2296–2312
Saavedra C, Stewart DT, Stanwood RR, Zouros E (1996) Species-specific segregation of gender-associated mitochondrial DNA types in an area where two mussel species (*Mytilus edulis* and *M. trossulus*) hybridize. Genetics 143:1359–1367
Skibinski DOF, Ahmad M, Beadmore JA (1978) Genetic evidence of naturally occurring hybrids between *Mytilus edulis* and *Mytilus galloprovincialis*. Evolution 32:354–364
Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman, San Francisco
Toro JE (1998) Molecular identification of four species of mussels from southern Chile by PCR-based nuclear markers: the potential use in studies involving planktonic surveys. J Shellfish Res 17:1203–1205
Toro JE, Thompson RJ, Innes DJ (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
Väinölä R, Hvilsom MM (1991) Genetic divergence and a hybrid zone between Baltic and North Sea *Mytilus* populations (Mytilidae: Mollusca). Biol J Linn Soc 43:127–148
Varvio SL, Koehn RK, Väinölä R (1988) Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. Mar Biol 98:51–60
Willis GL, Skibinski DOF (1992) Variation in strength of attachment to the substrate explains differential mortality in hybrid mussel (*Mytilus galloprovincialis* and *Mytilus edulis*) populations. Mar Biol 112:403–408
Wood AR, Beaumont AR, Skibinski DOF, Turner G (2003) Analysis of a nuclear-DNA marker for species identification of adults and larvae in the *Mytilus edulis* complex. J Molluscan Stud 69:61–66
Zouros E, Freeman KR, Ball A, Pogson GH (1992) Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel *Mytilus*. Nature 359:412–414
Zouros E, Ball AO, Saavedra C, Freeman KR (1994) An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. Proc Natl Acad Sci USA 91:7463–7467