
Fitness landscapes support the dominance theory of post-zygotic isolation in the mussels *Mytilus edulis* and *M. galloprovincialis*

Nicolas Bierne^{1*}, François Bonhomme¹, Pierre Boudry², Marta Szulkin¹, Patrice David³

¹Adaptation UMR5171 CNRS-UMII-IFREMER, Station Méditerranéenne de l'Environnement Littoral Laboratoire Génome, Populations, Interactions 1 Quai de la Daurade, 34200 Sète, France

²IFREMER Laboratoire de Génétique et Pathologie 17390 La Tremblade, France

³CEFE—CNRS 34293 Montpellier Cedex 5, France

*: Corresponding author : Tel: 33 4 67 46 33 75 ; Fax: 33 4 67 46 33 99 ; E-mail: n-bierne@univ-montp2.fr

Abstract: We studied the genetic basis of post-zygotic isolation in the marine mussels *Mytilus edulis* and *Mytilus galloprovincialis*. Evidence was obtained for a high number of recessive Dobzhansky–Muller substitutions in the genome of these two mussel taxa. We analysed the segregation of unlinked diagnostic markers in the progeny of two backcrosses and an F2 cross, 36h and 200 days after fertilization. Directional selection favouring *M. galloprovincialis* genotypes was observed in both kinds of cross. In the F2, epistatic interactions between each pair of chromosome fragments mapped by the markers were identified in addition. Our results imply that homozygous–homozygous interactions are required for breakdown of coadaptation, in accordance with the dominance theory of post-zygotic isolation. Endogenous post-zygotic selection distributed over many loci throughout the genome provides the missing factor explaining the astonishing persistence and strength of barriers to neutral introgression in such a dispersive taxon as *Mytilus*.

Keywords: Hybrid zone, hybrid breakdown, negative epistasis, post-zygotic isolation, Dobzhansky-Muller incompatibilities, *Mytilus edulis*, *Mytilus galloprovincialis*.

1. INTRODUCTION

The genetic basis of post-zygotic isolation is of long standing interest in the study of speciation (Coyne & Orr 1998; Orr & Presgraves 2000; Gavrilets 2004). There is now overwhelming evidence that hybrid fitness depression results from the accumulation of alleles that enhance fitness in their own genetic background but lower fitness in the genetic background of other species. Combination of alleles at two or more loci involved in negative epistatic interactions in hybrid genotypes are known as Dobzhansky-Muller (DM) incompatibilities in recognition of the pioneering work of Dobzhansky (1937) and Muller(1942). The beauty of the DM model is that the accumulation of alleles involved in DM incompatibilities (hereafter referred to as DM substitutions) allows reproductive isolation to evolve without populations having to cross adaptive valleys (Orr 1995; Orr & Turelli 2001; Gavrilets 2004). Only recently, though, were some attempts made to refine the description of the genetic basis of DM incompatibilities (Turelli & Orr 2000).

The motivation for a more detailed description of DM incompatibilities mainly comes from two standard observations: (i) in species with sex chromosomes, hybridization typically causes problems to the heterogametic sex *-i.e.* Haldane's rule (Haldane 1922; Laurie 1997), and (ii) hybrid breakdown is less pronounced in F1 than in successive F2 or backcross generations (Dobzhansky 1937; Edmands 1999). Both phenomena could be accounted for by the simple hypothesis that DM substitutions are preponderantly recessive in their effects on hybrid fitness so that they are expressed when hemizygous in the F1 or when homozygous in successive generations (Muller 1940; Turelli & Orr 2000). If this is confirmed, such a result would have important implications for the probability and dynamics of speciation (Gavrilets 2004). The validation of the dominance theory of post-zygotic isolation (*sensu* Turelli & Orr 1995) requires more direct investigations of the genetic architecture of DM incompatibilities. Although numerous investigations have provided support to the dominance theory in

Drosophila (reviewed by Turelli & Orr 2000), few tests have been attempted in other organisms so far. These studies investigated major genes with strong effect (e.g. sterility or inviability), involved in the reproductive isolation of genetically well delimited species that do not hybridize in nature (Orr & Presgraves 2000). In addition, most of the examples available to date relate to X-linked genes for which the evolution should differ from autosomes (Charlesworth *et al.* 1987). To further refine our knowledge of DM incompatibilities and test the universality of the dominance theory, data are needed on randomly found autosomal loci, with moderate effect on fitness and involved in the reproductive isolation of more weakly isolated taxa. Although the hybrid zone literature has long emphasized the importance of epistasis (Barton & Hewitt 1985), the detailed genetic architecture of reproductive isolation has only been investigated in a few cases (Rieseberg & Buerkle 2002).

Here, we have conducted a blindfold search for DM incompatibilities in the mussels *Mytilus edulis* and *M. galloprovincialis*. These two mussel taxa form a well-known mosaic hybrid zone along the Western European coast (Skibinski *et al.* 1983; Coustau *et al.* 1991a; Daguin *et al.* 2001; Bierne *et al.* 2003c). Some pre-zygotic isolation mechanisms have been documented in previous studies such as habitat preference (Gosling & McGrath 1990; Bierne *et al.* 2003b), spawning asynchrony (Gardner & Skibinski 1990; Secor *et al.* 2001; Bierne *et al.* 2003b) and assortative fertilization (Bierne *et al.* 2002a). However, we argued that these mechanisms were insufficient alone to explain the observed strength of the barrier to introgression of neutral markers (Bierne *et al.* 2002a,b). As some hybrid genotypes are produced in visible numbers, the theory would predict that neutral introgression should proceed quickly unless some categories of hybrids exhibit reduced fitness (Barton 2001). Yet, the existence of intrinsic hybrid breakdown still remains controversial in *Mytilus* (e.g. Wilhelm & Hilbish 1998). Surprisingly, the fitness of hybrids has mostly been investigated

using correlations between genotypes at marker loci and phenotypes in natural populations (Gardner 1994b) while experimental crossing has seldom been used (but see Beaumont *et al.* 1993; 2004) and has never been conducted to the F2. However the variable degree of local introgression in natural populations poses the problem that so-called "hybrid individuals" are complex genetic mosaics in which neutral loci may have loose or unknown linkage disequilibrium with potentially selected loci (Bierne *et al.* 2003c). In contrast, a high proportion of useful and perfectly defined hybrid genotypes can be produced in the lab. We have conducted an analysis of the fitness of genotypes at three unlinked chromosomal regions marked by neutral loci in backcrosses and F2s. Following previous works (reviewed in Bierne *et al.* 2003a), we by-passed the difficulty of measuring fitness in hatchery experiments by the use of molecular markers and a diachronic sampling scheme. We obtained support for the dominance theory and a high number of recessive DM substitutions in the genome of the two mussel taxa.

2. MATERIALS AND METHODS

(a) Crosses, larval rearing, sampling and genotyping

F1 hybrids obtained from a previous experiment (Bierne *et al.* 2002a), *Mytilus edulis* from Grand-Fort-Philippe (North Sea, France) and *M. galloprovincialis* adults from Sète (Mediterranean Sea, France) were individually induced to spawn as previously described (Bierne *et al.* 2002a). Five females and five males *M. galloprovincialis*, one F1 female and five F1 males emitted gametes. Unfortunately, *M. edulis* individuals did not spawn, probably because they were insufficiently mature. Gametes were sieved, counted, pooled according to sex and genotype, and fertilization was performed as in Bierne *et al.* (2002a) following four kinds of treatments: an F2 cross between the oocytes of the F1 female and the pooled sperm of the five F1 males; two backcrosses on *M. galloprovincialis*, between oocytes of the F1 female and the pooled sperm of the five *M. galloprovincialis* males (hereafter BC1) and between pooled oocytes of the five *M. galloprovincialis* females and the pooled sperm of the five F1 males (hereafter BC2); and finally, a control cross between *M. galloprovincialis* males and females.

In contrast to the previous F1 generation, larval rearing was not performed in our laboratory in Sète, on the Mediterranean Sea, but in the experimental IFREMER hatchery of La Tremblade (Charente Maritime, France) on the French Atlantic coast, a region where natural populations are predominantly *M. edulis* (Bierne *et al.* 2003c). The salinity of the sea water was 27‰. Otherwise we followed the same protocol as previously described (Bierne *et al.* 2002a).

Samples were taken 36h after fertilization and at the juvenile stage, 200 days after fertilization (D200). DNA extraction of individual D-larvae and juveniles was performed according to our standard protocols (Bierne *et al.* 2003). Sample sizes were ~100 for the F2 cross and ~50 for backcrosses. The sampling effort was stronger in the F2 treatment because

more genotypes are produced (for example 9 bi-locus genotypes are produced in the F2 while only 4 are produced in a backcross). Three length-polymorphic DNA loci were used, *Glu-5'* (Rawson *et al.* 1996), *mac-1* (Ohresser *et al.* 1997) and *EFbis* (Bierne *et al.* 2002a) using the primer sequences and the fluorescent dye 5' end-labeled-primer technique described in Bierne *et al.* (2003c). Overall we screened 1230 single-locus genotypes. Although several alleles were observed at loci *mac-1* and *EFbis*, they were here used as bi-allelic loci – alleles at a single locus were pooled into species-specific compound alleles according to their frequencies in reference samples from each species (Bierne *et al.* 2003c). Synthetic alleles characteristic of *M. galloprovincialis* populations were called “G” and synthetic alleles characteristic of *M. edulis* populations were called “E”. A non-negligible level of introgression is observed in natural populations at these loci (Bierne *et al.* 2003c), however they appeared to be diagnostic of the ancestral *M. edulis* and *M. galloprovincialis* genomes in the present experiment – *i.e.* we were lucky enough that parents did not carry introgressed alleles so that *M. galloprovincialis* parents were tri-homozygotes for G alleles, *M. edulis* parents were tri-homozygotes for E alleles and F1 parents were tri-heterozygotes. The estimated population frequencies of alleles observed in parents are given in Table S1 of the electronic Appendix (available on The Royal Society’s Publications Web site). The segregations we observed at the early larval stage (see below) allowed us to consider these three loci as unlinked.

(b) Statistical analyses

Population sizes of larval stocks were estimated every two days during the 19 days of larval growth for each treatment. This allowed us to estimate exponential larval mortality rates, β , from a loglinear regression according to the following model: $N(t) = \alpha \text{Exp}(-\beta t)$, where $N(t)$ is the stock size at time t .

Mendelian proportions and homogeneity of genotypic frequencies among loci, time samples and crosses were tested using Fisher's exact tests in the GENEPOP software (Raymond and Rousset 1995). Pairwise associations of alleles across loci have been estimated and tested with the maximum likelihood method of Barton (2000). Multilocus genotype proportions have also been analyzed with the help of a simple hybrid index that was the number of G alleles per individual. Significance levels of tests were adjusted for multiple testing according to a sequential Bonferroni procedure (Rice 1989), but probabilities below the 5% threshold (before adjustment) are mentioned.

We drew fitness landscapes. We here define a fitness landscape to be the simple relationship between genotype and fitness and we do not consider landscapes that depict the relationship between some variables of the genetic structure of a population and its mean fitness (a definition for which some authors reserve the term "adaptive" landscape, see Gravilets 2004). As a surrogate of fitness we used survival rates estimated from the evolution of genotype frequencies between the two time-samples, 36 hours and 200 days after fertilization. Fitnesses were standardized by the fittest genotype –*i.e.* the fittest genotype had a fitness of one. An averaged fitness landscape was obtained by averaging fitness across landscapes and by considering symmetrical genotypes (e.g. EEGG and GGEE) as identical.

(c) A simple model

We investigated the consequences of the observed fitness landscape on the evolution of a hybrid zone using a simple model. Our basic question was whether epistatic interactions were sufficiently strong to prevent introgression and generate a stable hybrid zone. We considered a diploid model with two unlinked loci with two alleles G and E. Fitnesses of the nine genotypes were specified at the beginning of the simulation. We modeled a secondary contact of populations initially fixed for alternative alleles using a classical one-dimensional stepping-

stone model (Feldman & Christiansen, 1974). Demes on the left side of the chain were fixed for the GGGG genotype and demes on the right side were fixed for the EEEE genotype. The migration rate between demes was m (half in either direction). Migration was followed by reproduction and selection. The model is deterministic –genotypic frequencies in each deme at a given generation are deduced from the frequencies of the previous generation after accounting for migration, recombination and selection (see e.g. Slatkin 1975). We registered allele frequencies in order to depict cline shapes and linkage disequilibria that inform us about the strength of the barrier to gene flow. A Borland Delphi 4.0 program is available from the authors upon request.

3. RESULTS

(a) Larval mortalities

Exponential larval mortality rates (β) and the proportion of dead larvae after 19 days of larval growth are presented in Table 1. The exponential model fitted well with the data and provides a synthetic measure of the dynamics of larval mortality (in other words, we did not observe sudden peaks in mortality rates during the larval stage). Overall we observed high larval mortalities which are common features in hatchery experiments (e.g. Beaumont *et al.* 1993). Nonetheless, mortality was far more pronounced in the tank where the F2s were grown than in any other tanks. Unfortunately, in the absence of replicates we cannot disentangle an always possible tank effect. Indeed, between-tanks variance in mortality is frequently high during larval rearing. However, previous experiments in the hatchery of La Tremblade have sometimes managed to eliminate tank effects (Ernande *et al.* 2003). In the present study, the observed variance in mortality rates was low, with very similar results obtained for every non-F2 treatments (Table 1). Maternal effects could also explain the results of the F2 cross. However, a maternal effect is not corroborated by the results obtained with the BC1 cross that share the same F1 female but exhibited a similar mortality rate to other non-F2 treatments. Although the higher mortality of F2 larvae may not be taken at face value, there is no evidence to suggest this was artifactual. Furthermore, this result is in agreement with the fact that differential larval mortalities were observed between time-samples in the F2 but not in backcrosses (see below).

[Table 1]

(b) Genotype frequencies in backcrosses

Backcrosses correspond to the case where one type of parents is heterozygous EG and the other type is homozygous GG, at the three loci. Two single-locus genotypes are expected in

the progeny, EG and GG, in a 1:1 ratio. Numbers of genotypes in each sample are reported in Table S2 of the electronic Appendix (available on The Royal Society's Publications Web site). No significant departure from the Mendelian expectation was observed at the single-locus level within samples. As homogeneity in genotype frequencies was never rejected among samples (after correction for multiple testing but often at the 5% level, Table S2), we were able to pool the results across sampling times, loci and treatments (BC1 and BC2). We found a significant overall tendency for an excess of GG over EG genotypes (55% of GG). This was more pronounced in BC2 (58% of GG) than in BC1 (52% of GG). Note that we always needed to pool time-samples to obtain significant deviations while heterogeneities between time-samples were never significant. Multilocus genotype frequencies did not depart from those expected under free recombination and association tests were never significant (data not shown). The single locus trend for an advantage of G alleles was observed at the multilocus level. This is illustrated in figure 1 where the hybrid index distribution is compared to the Mendelian expectation with no linkage. In figure 1, we also have plotted the multilocus expectation deduced from the single locus frequencies with the assumption of no linkage in order to illustrate the absence of disequilibrium.

[Figure 1]

(c) Genotype frequencies in the F2s

F2s correspond to the case where both parents are EG heterozygotes. Three single-locus genotypes are expected in the progeny –EE, EG and GG– in a 1:2:1 ratio. 36 hours after fertilization, single locus genotype frequencies did not depart significantly from the Mendelian expectation (Table 2). Homogeneity in genotype frequencies among loci was not rejected which allowed us to pool results across loci. Genotype frequencies pooled across loci agreed well with the Mendelian expectation (0.94:2.13:0.94). In addition, association tests

were not significant (not shown) suggesting once again that our loci segregated independently.

Two hundred days after fertilization, genotype frequencies at locus *EFbis* departed significantly from the Mendelian expectation (Table 2). Pooling the results across loci, genotype frequencies significantly departed from the Mendelian expectation. A significant heterogeneity in genotype frequencies was observed at locus *EFbis* and in the pooled dataset. The single locus trend was visible at the multilocus level and is illustrated in Figure 2. Heterogeneity of multilocus genotypic frequencies among time samples was significant ($p < 0.001$), primarily as a consequence of directional selection favoring genotypes with the highest hybrid indexes (i.e. genotypes with a high number of G alleles, see Figure 2). In addition, strong and significant pairwise associations across loci appeared at this stage (Table 3), suggesting epistatic interactions between the chromosome fragments mapped by our markers. Interestingly, selection did not result in significant heterozygote deficiencies (Table 3). Note that contrary to backcrosses we detected differences in genotype frequencies between the early larval stage and the juvenile stage (e.g. Figure 2) which provide unequivocal evidence of differential mortalities.

[Figure 2]

(d) Bi-locus fitness landscapes

Our sample size did not allow drawing tri-locus fitness landscapes with confidence and we here present the three bi-locus fitness landscapes (Figure 3). As already observed with the hybrid index distribution, we observed a tendency for an advantage towards genotypes with more G alleles. However, epistatic interactions were visible in addition to directional selection. The least fit genotype always was an EE homozygote at one locus and a GG homozygote at the other. The two homozygous-homozygous recombined genotypes, EEGG

and GGEE, were always less fit than the double heterozygote (EGEG) while these three genotypes have the same hybrid index. Finally, at least one of the homozygous-homozygous hybrid genotypes was less fit than the parental *edulis* genotype (EEEE), although a simple additive effect of the dose of G alleles would predict the reverse.

[Figure 3]

The genotype space produced in a F2 is different than in a backcross: in a backcross genomes are mosaics of GG homozygous and EG heterozygous chromosome portions while in F2s some chromosome chunks are EE homozygous. A closer look at the averaged bi-locus landscape (Figure 3) would provide explanation to why selection was not as easily detected in backcrosses as it was in the F2s. Although in a different genetic background (without EE homozygous portions), only the four top-right genotypes of the landscape were produced in backcrosses –their fitnesses were not very different when compared to the fitnesses of the other five genotypes additionally produced in the F2s.

(e) Modeling a hybrid zone with the observed fitness landscape

We used the averaged pairwise fitness landscape (see Figure 3) to run our two-locus model. Our model is of course too simple to depict the complexity of the *Mytilus* hybrid zone. Among many other things, it only considers a pair of loci while epistatic interactions potentially rely on more loci and each locus cumulates indirect selective effects from other loci in multilocus clines (Barton 1983). Furthermore, fitness landscapes of figure 3 are rough estimates for which a surrogate of fitness is measured (larval survival), in a single artificial environment and the pattern obscured by unknown recombination rates between markers and fitness loci. However, such a model is still useful to make some basic observations. First, the resulting hybrid zone is not stable: we observed a wave of advance of G alleles resulting in their ultimate fixation in all demes. Second, the consequences of epistasis can be evaluated by

comparing the results based on the observed fitness landscape to those obtained under a fully additive model. In the additive model, the fitness was defined as $W=1-Hs$, where H is the hybrid index of the genotype (0 to 4) and s is the selective coefficient. We used $s=0.175$ so that the fitness of the EEEE matched the observed value of 0.3. As shown in Figure 4, the additive model generates somewhat less steep and less symmetric clines and a slightly faster wave of advance (3% faster, not shown). The main difference between additive and non-additive models lies with linkage disequilibria, which are much smaller in the additive model and even slightly negative in the tail of the cline. In contrast, linkage disequilibria are strong and always positive with the observed fitness landscape, implying limited recombination between parental genomes and a strong barrier to neutral gene flow.

[Figure 4]

4. DISCUSSION

We have studied the segregation of three marker loci in second generations of hybridization between *Mytilus edulis* and *M. galloprovincialis*. We did not detect evidence of physical linkage between the three loci which segregated independently in the early larval stage. We found evidence of two sorts of selection: (i) directional selection favoring *M. galloprovincialis* genotypes and (ii) dominance by dominance epistatic interactions expressed in multi-homozygous recombinant genotypes.

Our results add to the already long list of studies that found a fitness advantage of *M. galloprovincialis* genotypes over *M. edulis* ones (Skibinski 1983; Coustau *et al.* 1991b; Gardner 1994a; Hilbish *et al.* 1994; review in Gardner 1994b). Interestingly, the conditions under which a reciprocal advantage of *M. edulis* genotypes could be expressed remain to be found. In contrast with our previous experiment (Bierne *et al.* 2002a), our rearing facilities in La Tremblade should *a priori* have favored *M. edulis* genotypes according to the genetic composition of natural populations surrounding the hatchery and the relatively low salinity of the water (27‰). As a consequence, the hypothesis of an intrinsic advantage to *M. galloprovincialis* in a broad set of environmental conditions should be seriously considered.

The second type of selection was epistatic. Strong and significant pairwise associations among loci appeared after 200 days of larval and juvenile life in the F2s. Sampling the early larval stage allowed us to tell apart the effect of epistatic selection from physical linkage in the interpretation of our results, a distinction that was not possible previously with allozyme markers (Hilbish *et al.* 1994). Bi-locus fitness landscapes presented in Figure 3 visibly depart from a simple additive model as the least fit genotype was always either one or the other homozygous-homozygous hybrid genotypes (EEGG and GGEE), despite their having one half of their alleles of the G type. Our markers are thus linked to recessive DM substitutions. The predominantly recessive nature of DM substitutions is also supported in our experiment

by the stronger larval mortalities observed in the F2s while other treatments, including backcrosses, presented consistently lower mortalities (Table 1). We acknowledge however that this latter result cannot be rigorously disentangled from a tank effect.

The ease of detecting pairs of DM substitutions with only three markers and for each of the three pairs of marker is very surprising. This result suggests that a high number of recessive DM substitutions have accumulated in the genome of these two mussel taxa. Indeed, our design was unlikely to detect incompatibilities if they were due to only two or a few loci because the three markers together mark a small proportion of the genome –knowing that the haploid chromosome number is 14 in *Mytilus* (Ahmed & Sparks 1970) and considering 1-3 chiasmata per bivalent, roughly 2-5% of the genome is marked by each locus in the F2 generation. Our data suggest that any marker is likely to be linked to a DM substitution, which means there is at least one DM locus per chromosome. Furthermore, any pair of chromosomes seems to contain a pair of interacting DM loci. This suggests that either more than two loci on different chromosomes are involved in multi-way epistatic interactions, or many DM loci are present on each chromosome, each being involved in a pairwise interaction.

The recessivity of DM incompatibilities between the two mussel taxa is in accordance with Turelli and Orr's dominance theory of post-zygotic isolation. An important drawback of this theory is that we still have little explanation to why DM substitutions should be recessive (Orr & Presgraves 2000). Interestingly, Kondrashov *et al.* (2002) and Welch (2004) have recently argued that DM incompatibilities may be originally mildly deleterious mutations that became positively-selected as a consequence of a substitution at another locus. DM alleles could thus be recruited from the standing pool of segregating deleterious mutations, most of which are usually recessive. Here, attention might be called to the fact that marine bivalves have one of the highest deleterious mutation loads ever reported in the animal kingdom to

date (Bierne *et al.* 1998; Launey & Hedgecock 2001). Note, however, that the recessive effects of DM substitutions on hybrid fitness do not necessarily mean that they were recessive in the genetic context corresponding to their fixation.

How do the two types of selection, directional and epistatic, combine in the mussel hybrid zone? More precisely, are epistatic interactions sufficiently strong to generate a stable hybrid zone? This is a difficult question to answer. Our simple two-locus model suggests that the magnitude of epistasis detected in our experiment is not sufficient to prevent the replacement of *M. edulis* by *M. galloprovincialis*. Unless *M. edulis* compensates its weakness by an advantage in another, still unexplored, component of fitness, the *Mytilus* hybrid zone is thus expected to move. It does not mean that the movement should be visible at a time scale of years or even decades. Furthermore, in the mosaic zone of *Mytilus* the movement would not necessarily be a wave of advance but could proceed by jumps owing to stochastic dispersal and/or selection in a heterogeneous environment. Evidences for moving hybrid zones usually are indirect (Moran 1981, Rohwer *et al.* 2001, Cruzan 2005) and exploit the theoretical prediction that waves of advance are expected to leave a footprint in the form of asymmetrical introgression (Barton & Hewitt 1985). We here suggest that a strong barrier to neutral gene flow generated by the superposition of epistasis on directional selection may partly mask the effect. A closer look at allele frequencies observed in natural populations at the three markers used would however reveal a slight tendency for asymmetrical introgression (Bierne *et al.* 2003) which would be in accordance with a northward progression of the mussel hybrid zone.

To finish with, we need to find explanations to why endogenous hybrid depression has not easily been detected in *Mytilus* previously. In our view, this results from the fact that the fitness of hybrids has previously been investigated through correlations between phenotypes and genotypes at marker loci in natural populations. These studies may miss important patterns for several reasons. (i) For a genetic barrier to neutral gene flow to be effective,

hybrids need to be rare in natural populations (Barton & Bengtsson 1986). (ii) If hybrids are found to be frequent they probably are irrelevant introgressed genotypes –*i.e.* repeated interbreeding may have locally decreased the linkage disequilibrium between marker loci and selected loci to the point that patterns of selection on the latter are hardly visible on the former. (iii) In mosaic hybrid zones, local parental populations are more introgressed at neutral loci than external populations; however the latter have traditionally been used as references, which may lead to misidentification of parental genotypes as recently recombined hybrids (Bierne et al. 2002b). (iv) Only adult patterns of mortality and growth have been investigated while most of the developmental processes and mortality take place during the larval phase and metamorphosis. On the other hand, artificial crossing allowed us to produce a high number of well-defined genotypes. Evidence for the existence of endogenous post-zygotic selection distributed on many loci of small effects can provide the missing factor explaining the persistence -at least in the short term- and strength of barriers to neutral introgression in *Mytilus* hybrid zones.

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Figure legend:

Figure 1: Observed tri-locus hybrid index distribution in backcrosses (pooling the two backcrosses, BC1 and BC2, over the two sampling-times, 36h and D200), expected hybrid index distribution from single locus frequencies with the assumption of no linkage and expected hybrid index distribution from single locus Mendelian expectations and no linkage. On the bottom panel are presented the deviations of the observed distribution from the Mendelian expectation with no linkage and the expectation from single locus frequencies and no linkage.

Figure 2: Tri-locus hybrid index distributions in the F2 generation, 36h and 200 days after fertilisation; and deviation from the Mendelian expectation with no linkage in the D200 sample.

Figure 3: Bi-locus fitness landscapes in the F2 generation. The fourth landscape at the bottom of the figure is the averaged pairwise landscape obtained by considering symmetrical genotypes (e.g. EEGG and GGEE) to be identical.

Figure 4: Cline shapes and linkage disequilibria, D , obtained from a simple two-locus one-dimensional stepping-stone model. The black arrow gives the direction of propagation of waves of advance. Bold lines are the results obtained with the averaged pairwise landscape of figure 3. Thin lines are the results obtained with an additive model with a selection coefficient of 0.175.

Short title for page headings: Hybrid breakdown in mussels.

Table 1: Exponential larval mortality rates (β) and proportion of dead larvae after 19 days of larval growth.

	Tank 1: F2	Tank 2: BC1	Tank 3: BC2	Tank 4: Control
	♀F1 x ♂F1	♀F1 x ♂G	♀G x ♂F1	♀G x ♂G
$\beta \pm 95\% \text{ CI}$	0.17 \pm 0.04	0.06 \pm 0.02	0.06 \pm 0.03	0.08 \pm 0.01
Mortality at day 19	93%	67%	70%	75%

Table 2: Genotype numbers, tests of the Mendelian expectation and heterogeneity tests in the F2. Significant tests at the experimentwise level are in bold.

	<i>Glu-5'</i>	<i>EFbis</i>	<i>mac-1</i>	Σ locus
	EE:EG:GG	EE:EG:GG	EE:EG:GG	
F2-36h	23:41:30 ^{NS}	24:48:22 ^{NS}	19:61:14 ^{*,a}	66:150:66 ^{NS}
F2-D200	11:50:24 ^{*,a}	08:46:31^{**}	13:51:21 ^{NS}	32:147:66 ^{***}
Heterogeneity	NS	*	NS	**
Σ F2	34:91:54 ^{NS}	-	32:112:35^{**}	-

^{NS}: p>0.05; * : 0.01<p<0.05; ** : 0.001<p<0.01; *** : p<0.001; ^a: not significant at the experimentwise level; -: not feasible.

Table 3: Pairwise associations of alleles within loci (heterozygote deficiency) and between loci (linkage disequilibrium), and confidence interval, 200 days after fertilization in the F2.

	<i>mac-1</i> / <i>EFbis</i>	<i>Glu-5'</i> / <i>EFbis</i>	<i>Glu-5'</i> / <i>mac-1</i>	tri-locus
Heterozygote deficiency	-0.03	-0.02	-0.03	-0.02
[95% CI]	[-0.05 ; 0]	[-0.06 ; 0]	[-0.06 ; 0]	[-0.05 ; 0]
Linkage disequilibrium	0.11	0.07	0.07	0.06
[95% CI]	[0.06 ; 0.12]	[0.01 ; 0.1]	[0 ; 0.1]	[0.04 ; 0.09]

Figure 1

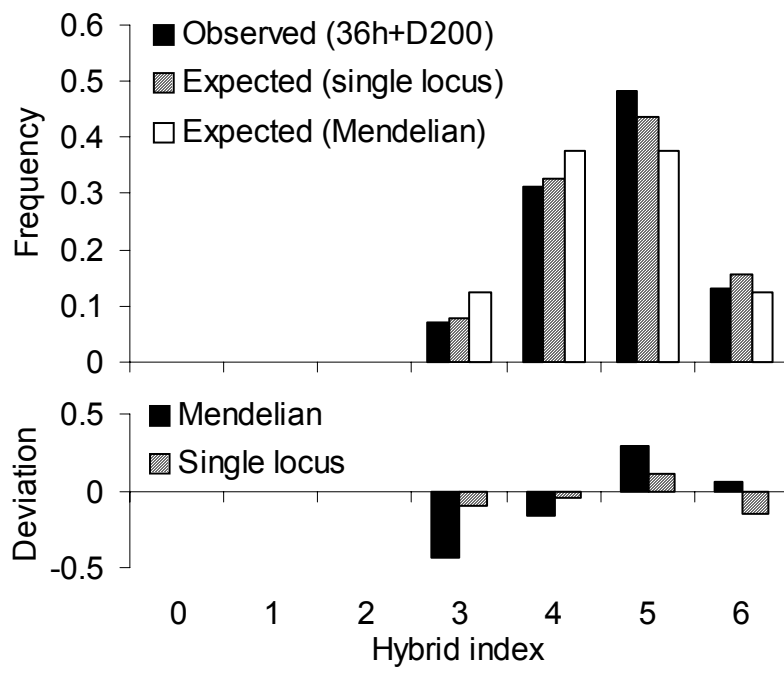


Figure 2

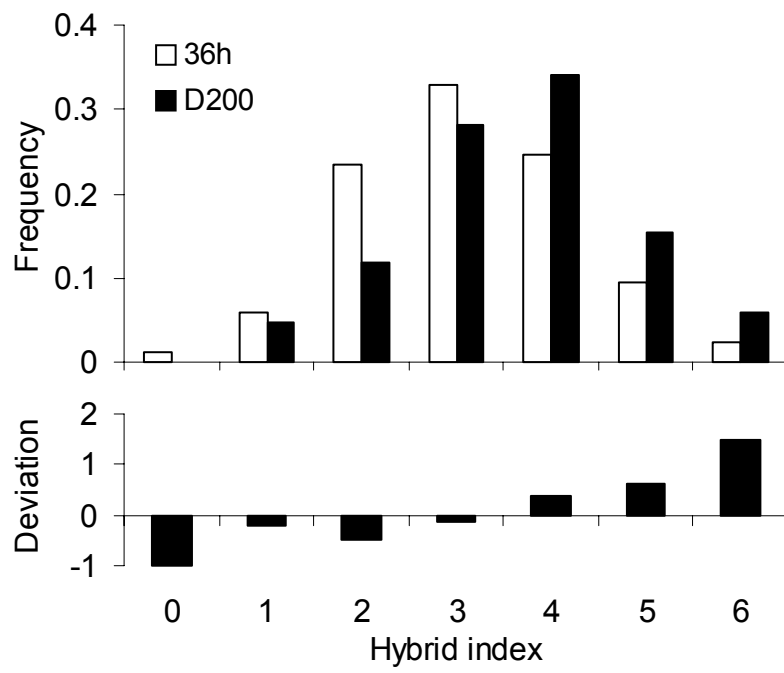
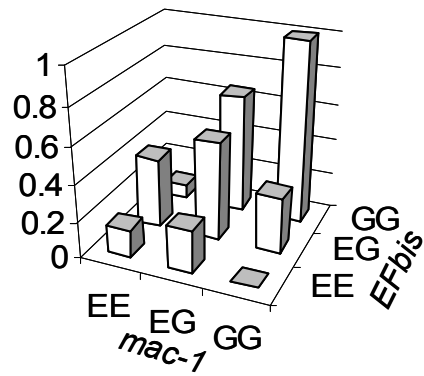
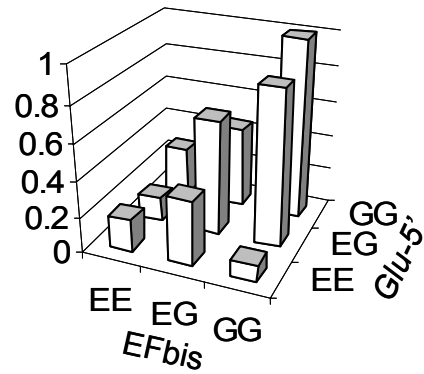


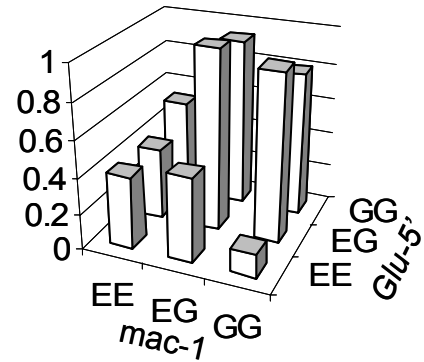
Figure 3



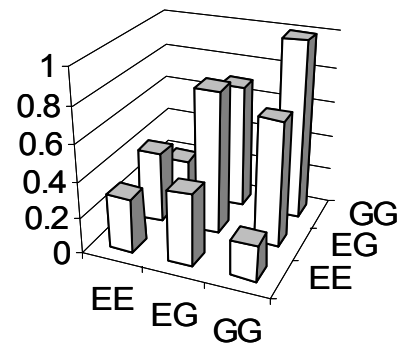
		<i>mac-1</i>		
		EE	EG	GG
<i>EFbis</i>	GG	0.1	0.7	1
	EG	0.4	0.5	0.3
	EE	0.2	0.2	0



		<i>EFbis</i>		
		EE	EG	GG
<i>Glu-5'</i>	GG	0.3	0.5	1
	EG	0.1	0.6	0.9
	EE	0.2	0.4	0.1



		<i>mac-1</i>		
		EE	EG	GG
<i>Glu-5'</i>	GG	0.5	0.9	0.8
	EG	0.4	1	0.9
	EE	0.4	0.5	0.1



		<i>Locus 1</i>		
		EE	EG	GG
<i>Locus 2</i>	GG	0.2	0.7	1
	EG	0.4	0.8	0.7
	EE	0.3	0.4	0.2

Figure 4

