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Intron-length polymorphism at the actin gene locus *mac-1:* a genetic marker for population studies in the marine mussels *Mytilus galloprovincialis* Lmk. and *M. edulis* L.

Marc Ohresser
Laboratoire Génome et Populations, UPR CNRS 9060, Génétique des Populations marines, Station Méditerranéenne de l'Environnement Littoral, Quai de la Daurade, 34200 Sète, France

Philippe Borsa
ORSTOM-Zoologie, B.P. 5045, 34032 Montpellier cedex 1, France, and Laboratoire Génome et Populations, UPR CNRS 9060, Station Méditerranéenne de l'Environnement Littoral, Quai de la Daurade, 34200 Sète, France

Claude Delsert*
IFREMER, Laboratoire de Genétique, Aquaculture et Pathologie, BP 133, 17390 La Tremblade, France, and

tions. The frequencies of the two other allelomorphs significantly differed between *M. galloprovincialis* and *M. edulis* populations. The comparison of six *mac-1* intron sequences over 277 bp showed at once that allelomorphs encompassed alleles differing from one another by substantial numbers of mutations, and that identical alleles were present in both *M. galloprovincialis* and *M. edulis* individuals, a probable result of the recent introgression between the two species.

Introduction

Genetic studies of natural populations usually involve large sets of individual genotypic data, either

morphism as a genetic marker, provided the intron is limited in size. First, degenerate oligonucleotides have to be deduced from the amino acid sequences encoded by the two exons flanking the targeted intron and used for PCR amplification. Then, following sequence determination of the amplified DNA fragment, new specific primers are designed. This approach (Lessa, 1992) has already been successfully used, in particular, to provide molecular mark-

insert in their first intron. PCR amplification was done on the DNA of individual *M. galloprovincialis,* using primers designed in the regions flanking the insert. Analysis of the products of this amplification by electrophoresis on agarose gel revealed the existence of three size-classes of alleles. The distribution of individual phenotype frequencies in *M. galloprovincialis* and *M. edulis* natural populations did not depart from the expectations of the Hardy-

A

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mac-1b1    CATATTGGAACTTCAAGTCGTCTAGCGTAGTACTTAAATTGATTAAAGTTCTTAAAAGTT
mac-1c1                                    ............................
mac-1a1    ...........A................................................ +240
           1——————————————>

mac-1b1    TTATTGAGACATAAA-TTATGAATAACATACATAAATTCGATTTATTTTATCaatctgct
mac-1c1    ...............-............-----------------------------...
mac-1a1    ...........AC..A............................................ +300

mac-1b1    TATA-TGTATAATTATATTTCATGCACTTTTTGAATAAAATTTTGTTTAAACTAAACTaa
mac-1c1    ....T.......................................................
mac-1a1    ------------------------------------------------------------

mac-1b1    tctgctATGGTACCCAGTACAACACAAGATGTAAATTGTTGAAACTATTCTATATGGAGA
mac-1c1    .................................................A..........
mac-1a1    ------...........................................A.......... +354

mac-1b1    TATCAATGTAGTCTAAATACTTCCACGACAATAAGCAAATCATAACGTTGAAA--TACTA
mac-1c1    ..........A..........................................AA.....
mac-1a1    ........................................T............AA..... +414

mac-1b1    CATATATTTTCTCACAAAACTAGACTACAATTTTCGTTTTTCGTCTTATTTCTCGAATTC
mac-1c1    ....--....................................................
mac-1a1    .....T...................................................... +474
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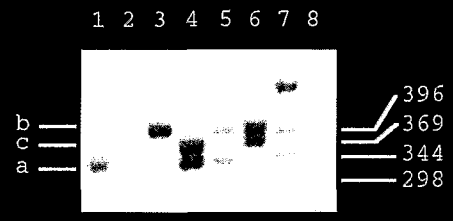

Figure 2. Agarose gel separation of *mac-1* allelomorphs after PCR-amplification using primers 1 and 2 (see Fig. 1) on genomic DNA of individual *Mytilus galloprovincialis.* Letters a, b and c indicate the relative electrophoretic locations of Allelomorphs *mac-1a, mac-1b* and *mac-1c,* respectively. Lanes 1 to 6 are for individuals with *mac-1 aa, cc, bb, ac, ab* and *bc* inferred genotypes, respectively. Numbers in bp refer to the 123 bp DNA ladder (Lane 7) and the 1 kb DNA ladder (Lane 8).

could therefore be observed among alleles between and within allelomorphs. Large differences occurred between *mac-1a* alleles from Aiguillon and Sète, while *mac-1c* alleles from each of these two localities were identical. All results are summarized in Figure 3.

Variability within and between populations

Electrophoresis on agarose gel of the PCR products

delian and selectively neutral, and that the samples represent panmictic or nearly panmictic populations.

The overall θ value between samples from different regions was highly significant, reflecting strong differences between populations (Table 2, "Europe"). A small fraction of this structure may be explained by differences between subpopulations as inferred from the θ value estimated at the regional scale (Table 2, "Charente"). Within-subpopulation structure was not evident, but the moderately strong (yet not significant) *f*value in the Polzeath Bay sample may reflect some level of heterozygote deficit in the population (Table 2).

Discussion

The length polymorphism reported here provides a new nuclear marker to discriminate between *Mytilus galloprovincialis* and *M. edulis* populations. This is an interesting feature in view of the thorny systematics of the genus *Mytilus* (Skinbinski and Beardmore, 1979; Varvio et al., 1988; Beaumont et al., 1989), of the process of introgression in areas where the two species hybridize (Skibinski et al., 1978; Skibinski and Beardmore, 1979; Skibinski et

[redacted]

mac-1a	−324	0.83	0.95	0.12	0.53
mac-1b	−388	0.00	0.00	0.27	0.09
mac-1c	−360	0.17	0.05	0.61	0.38
(*N*)		(15)	(33)	(59)	(59)
*h**		0.29	0.10	0.15	0.58

N, sample size; *h**, gene diversity, with correction for individuals' sampling bias.

Table 2. *Mytilus galloprovincialis* and *M. edulis*. Summary statistics on genetic structure at Locus *mac-1*, at two geographic scales.

Geographic scale, population	(*N*)	*f*	θ
Charente			0.072*
Boyard	(15)	−0.167	
Aiguillon	(33)	−0.033	
Europe			0.386***
Charente	(48)	−0.080	
Sète	(59)	0.004	
Polzeath	(59)	0.171	

presumably deleted the 5′ octonucleotide, which led to *mac-1c*. PCR amplification using primers located in more conserved regions of *mac-1* intron 1 allowed the characterization of the three allelomorphs *mac-1a*, *-1b* and *-1c*. Some preliminary results on other noncoding regions of the *M. galloprovincialis* genome showed that length polymorphism is probably common in the noncoding DNA of this species, and can lead to the development of population genetic markers with similar properties (M. Ohresser and C. Delsert, unpublished results).

Population genetic studies of marine mussels,

be desirable, though, for a more powerful test), then these could be best explained by some admixture of *M. galloprovincialis* with individuals bearing *M. edulis mac-1* genes. Some observations may support this hypothesis: the occurrence of typical *M. edulis*

lished results), of *mac-1a*$_1$ and *mac-1b*$_1$ differed by a 65-bp insertion, by single-base substitutions, and by a few short gaps in the intron.

To determine whether these genes were allelic, the DNA content of individual mussels was PCR-

[redacted]

Lessa, E.P. (1992). Rapid surveying of DNA sequence variation

tween heterozygosity and evolutionary rate of proteins.