A Mitochondrial DNA Discontinuity in the Mussel Mytilus galloprovincialis Lmk: Pleistocene Vicariance Biogeography and Secondary Intergradation

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Studies of clinal variation have helped greatly in the elucidation of the mechanisms of both adaptation and speciation (Endler 1977; Barton and Hewitt 1989). Clines have been explained by selection acting within a continuous population (primary intergradation) or, more frequently, as the result of contact between populations differentiated in allopatry (secondary intergradation) (Endler 1977; Barton and Hewitt 1989). However, a frequent problem in interpreting empirical studies of allozyme or morphological clines is that both primary and secondary intergradation may generate similar distributions of characters (Endler 1977; Barton and Hewitt 1989).

Theoretical and experimental studies suggest that the interpretation of clinal variation is aided by historical considerations (see, e.g., Thorpe 1984; Gonzalez-Villasenor and Powers 1990; Gallant et al. 1993). Mitochondrial DNA analysis provides a historical perspective because it permits the construction of a phylogenetic tree of haplotypes (Avise 1994). If this tree has a deep phylogenetic split, which separates haplotypes occurring at high frequency on either side of the cline, secondary contact is favored, particularly if the phylogenetic split is more ancient than any present-day barrier to gene flow (Gonzalez-Villasenor and Powers 1990; Avise 1994). Alternatively, low haplotype divergence would suggest primary intergradation and a lack of genetic isolation.

The mussel Mytilus galloprovincialis provides an interesting model system for studies of mtDNA phylogeography. In Europe, its range extends from the Black Sea and Mediterranean to the British Isles (Gosling 1992a), a geographical region strongly affected by well-documented Pleistocene glaciations and contemporary oceanographic boundaries (Pielou 1979; Tintore et al. 1988). Mussels, in common with many marine organisms, have a pelagic larval stage that is believed to have a powerful homogenizing effect on genetic differentiation (Gosling 1992b). Despite this, an abrupt multilocus allozyme cline delimiting two groups of populations, each with high internal homogeneity, has been reported in southeast Spain (Quesada 1993). Two factors make it difficult to determine whether this cline is due to primary or secondary intergradation (Quesada et al. 1995). First, the change occurs within an ecological transition zone with habitats sufficiently divergent to lead to the observed cline through disruptive selection. Gametic disequilibrium between selected and neutral loci could reinforce the pattern of coincident clines. Second, southeast Spain is a major marine biogeographical border, where many differentiated populations could presumably meet.

In this study we analyze mtDNA differentiation in order to test the hypotheses of primary versus secondary intergradation for the origin of the cline in southeast Spain and to assess the role of Pleistocene vicariance events and current oceanographic boundaries in determining genetic differentiation and geographical distribution in a marine species with pelagic larval dispersal.

Adult mussels of M. galloprovincialis were collected from five selected localities (fig. 1): Rock, Gijon, Almeria, Cullera, and Chioggia in 1992–93. Mitochondrial DNA was purified from individual animals and digested with the restriction enzymes EcoRI, BstEII, and XbaI. DNA fragments were separated on 1% agarose gels and Southern blotted onto nylon membrane (BioRad Zeta-probe). Digoxigenin labeling of an entire cloned F mtDNA genome (Edwards and Skibinski 1987; Fisher and Skibinski 1990) and hybridization were carried out using colorimetric protocols and kits supplied by Boehringer-Mannheim (Fisher and Skibinski 1990). The nomenclature for restriction patterns follows that of Edwards and Skibinski (1987).

An abrupt cline or discontinuity in mtDNA composite haplotype frequencies occurs between the Almeria and Cullera populations (fig. 1). The three populations to the west (Almeria, Gijon, Rock) are homogeneous in haplotype frequency (P = 0.22, using the simulation method of Roff and Bentzen 1989), as are the two populations to the east (Cullera, Chioggia; P = 0.12). The pooled western and pooled eastern samples differ significantly in haplotype frequencies (P = 0.0001). The cline in mtDNA haplotype frequencies is coincident with the previously reported allozyme cline (Quesada 1993). The cline corresponds in position with the Almeria-Oran oceanographic front, a well-defined hydrographic boundary, where many differentiated populations could presumably meet.

Key words: Mytilus, secondary intergradation, mitochondrial DNA, clinal variation.

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boundary between Atlantic and Mediterranean surface water (Tintore et al. 1988). This front is associated with dramatic temperature (1.4°C) and salinity (2 psu) gradients over a distance of 2 km and with strong water currents (40 cm/s average speed) that flow in a south-easterly direction toward north Africa (Tintore et al. 1988; Arnone et al. 1990). The combined influence of these frontal water currents and associated ecological gradients on larval dispersal is probably a major factor responsible for the current position and maintenance of the cline.

A total of 27 restriction sites were inferred from fragment patterns. Nucleotide sequence divergence (p) between haplotypes was estimated using the site approach of Nei and Tajima (1981) and a rooted phylogenetic tree constructed using the neighbor-joining method (fig. 2). A phylogeographic break is evident, with haplotypes at higher frequency in eastern and western populations separated on two main branches. If the true tree lacks phylogeographical structure, the probability of obtaining the observed perfect separation of the 12 east and nine west haplotypes by chance is about 10^{-5}. The probability remains low even if some of the haplotypes are wrongly classified as east or west. The sequence divergence between western and eastern lineages, corrected for within-region variation (Nei and Li 1979), is p = 0.040.

A plausible vicariance explanation for the divergence of eastern and western populations involves Pleistocene events. Assuming a conventional rate of mtDNA sequence divergence of 2% per million years (Brown et al. 1979), the divergence between western and eastern populations dates back as long as 2 million years (Myr). This corresponds well with the time of wide glacial/interglacial fluctuations in temperature and sea level that affected the entire region during the Pleistocene (2.0–1.8 Myr before present) (Pielou 1979; Peres 1985). In this period, fragmentation of species ranges through the narrow Straits of Gibraltar and Sicily (over 320 m in depth) was regularly facilitated during glacial epochs by the lowering of sea level by as much as 150 m (Thiede 1978; Pielou 1979; Nilsson 1983). Furthermore, both biological and geological evidence exists for refugia recurring in the eastern Mediterranean for populations formerly connected with the Atlantic coast (Pielou 1979; Peres 1985). In the last ice age (18,000 yr before present), the temperature gradient between western and warmer eastern Mediterranean basins (10°C) was substantially greater than today (1°C–2°C) (CLIMAP 1976; Thiede 1978), and water exchange between the Atlantic and Mediterranean was very much modified (Thiede 1978; Loubere 1982). Thus, M. galloprovincialis populations will have experienced many opportunities for allopatric divergence as a result of vicariance events during the last 2 Myr.

If the position and nature of oceanographic and ecological barriers have fluctuated considerably during the Pleistocene, the barrier to gene flow associated with the present-day cline clearly cannot be the one associated with the more ancient genetic divergence between eastern and western populations. This conclusion is rein-

![FIG. 1.—Geographical variation of mtDNA composite haplotype frequencies for restriction enzymes EcoRI, BstEII, and XbaI. Sampling locations: Rock (sample size = 17), Gijon (14), Almeria (21), Cullera (21), and Chioggia (14).](image)

![FIG. 2.—Neighbor-joining phylogenetic tree based on nucleotide sequence divergence estimates. The length of the tree is 0.268, and it is rooted at the midpoint. The haplotypes are numbered as in fig. 1 and labeled West (higher frequency to the west of the phylogeographic breakpoint) or East (higher frequency to the east of the breakpoint). Haplotypes observed both west and east of the breakpoint are labeled with an asterisk (*).](image)
forced by evidence that the stability and position of the Almeria-Oran front is critically affected by small contemporary climatic oscillations (Tintore et al. 1988; Heburn and La Violette 1990). These considerations lend strong support to a secondary (postglacial) contact origin for the parallel allozyme and mtDNA cline in southeast Spain. This conclusion is robust to an error in the estimated mtDNA divergence time of at least an order of magnitude.

Assuming that haplotypes have a unique mutational origin, the detection of some haplotypes in both eastern and western populations (haplotypes 8–13, fig. 2) can only be explained by gene flow between the east and west. Because the migration must postdate the mutational origin, recent gene flow is implicated for these young haplotypes. Furthermore, haplotypes occurring in both eastern and western populations have not spread beyond the populations flanking the phylogeographic breakpoint (fig. 1). These observations are also most easily explained by recent contact and limited gene flow between both groups of populations.

Recent studies of the covariation of allozyme and DNA markers have yielded promising results in the struggle to understand patterns of genetic variation in natural populations. For example, in the oyster Crassostrea, a concordant discontinuity for nuclear DNA and mtDNA at the location of a hydrographic boundary, but geographical homogeneity of allozyme frequencies, was seen as evidence for balancing selection on allozymes (Karl and Avise 1992). In the copepod Tigriopus, a concordant discontinuity for nuclear DNA and mtDNA at the position of a zoogeographical boundary and a steep allozyme cline at a different geographical location was also interpreted as possible evidence for selection on allozymes (Burton and Lee 1994). The results of the present study taken in combination with those of Quesada (1993) provide evidence of coincident discontinuities for mtDNA and allozymes in Mediterranean M. galloprovincialis. This pattern of variation thus differs from that observed in Crassostrea and Tigriopus and is more consistent with neutrality of both mtDNA and allozymes. It is clear that different molecular markers might produce similar or contrasting patterns of variation in different species. This should be borne in mind in studies of clinal variation and the adaptive significance of natural polymorphisms.

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LITERATURE CITED


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