

# Decline of a Native Mussel Masked by Sibling Species Invasion

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**Abstract:** *The European blue mussel (Mytilus galloprovincialis) has extensively invaded southern California, whereas a native species, M. trossulus, is abundant in northern California and further north. In this study, a portion of the 16S ribosomal RNA gene was sequenced from mussels collected in southern and central California in the nineteenth century. I aligned these sequences with 22 sequences representing modern M. galloprovincialis, M. edulis, and M. trossulus. Phylogenetic analysis using parsimony identified the nineteenth-century mussels as M. trossulus. Because mussel populations have been continuously present in this region since the time these mussels were collected, these data indicate that M. trossulus were present during the initial invasion of M. galloprovincialis, but, due to the morphological similarity of the two species, declined without notice.*

Disminuciones de un Mejillón Nativo Encubierta por la Invasión de una Especie Hermana

**Resumen:** *El mejillón azul europeo (Mytilus galloprovincialis) ha invadido extensivamente el sur de California mientras que la especie nativa M. trossulus es abundante en el norte. En este estudio, una porción del gen ARN 16S ribosomal fue secuenciada de los mejillones colectados en el sur y centro de California en el siglo XIX. Estas secuencias fueron alineadas con 22 secuencias representativas de M. galloprovincialis, M. edulis y M. trossulus modernas. El análisis filogenético de parsimonia identificó a los mejillones del siglo XIX como M. trossulus. Debido a que las poblaciones de mejillones han estado continuamente presentes en esta región, estos datos indican que M. trossulus estuvo presente durante la invasión inicial de M. galloprovincialis, pero debido a la similitud entre estas dos especies, M. trossulus disminuyó sin ser notado.*

## Introduction

Marine biological invasions are known to alter nearshore benthic and pelagic communities (Carlton 1989; Carlton & Geller 1993; Geller 1996; Cohen & Carlton 1998). There is at present, however, no evidence that modern marine invasions have led to regional or global extinctions of native species, which sharply contrasts with the record for terrestrial systems (Mooney & Drake 1986; Drake et al. 1989; Kaufman & Mallory 1993). In fact, modern marine extinctions are almost unknown (Carl-

ton 1993). I present data suggesting that invading sibling species may obscure the regional decline of some marine species.

In southern California the most abundant mussel in protected embayments is the invasive European blue mussel, *Mytilus galloprovincialis*, whereas a native species, *M. trossulus*, is abundant only north of San Francisco Bay (McDonald & Koehn 1988; Sarver & Foltz 1993; Suchanek et al. 1997). These blue mussels, along with *M. edulis*, comprise a complex of morphologically similar species (McDonald & Koehn 1988). Three histories can be suggested: (1) no blue mussels were present in southern California prior to the invasion of *M. galloprovincialis*, (2) *M. trossulus* were once present in southern California but declined prior to the invasion of *M. galloprovincialis*, and (3) *M. trossulus* were common when *M. galloprovincialis* invaded southern California and declined thereafter. The presence of a fossil

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and archeological record for a bay mussel in southern California (Carlton 1979) refutes the first scenario.

Collections and reports of living mussels in southern and central California in this and the last century indicate there have been temporally continuous populations of mussels (Johnson & Snook 1927; Keep 1935; Ricketts & Calvin 1939; MacGinitie & MacGinitie 1949; Carlton 1979). Therefore, if nineteenth-century mussels in southern and central California were native, then no temporal gap would have existed between the decline of native mussels and the twentieth-century arrival of *M. galloprovincialis*. Alternatively, if nineteenth-century mussels were invaders, then such a gap may have occurred earlier. Because morphology is not useful for identification of mussels, DNA sequence analysis is the only feasible means to identify archived mussels.

## Methods

Dry tissues of mussels collected around 1900 from Santa Catalina Island (Los Angeles County Museum of Natural History A-52) or collected in 1871 and 1884 from Monterey Bay (U.S. National Museum of Natural History 2326 and 23403) were homogenized in 500  $\mu$ L CTAB extraction buffer (50 mM Tris-HCl [pH 8.0], 0.7 M NaCl, 10 mM EDTA, 1% CTAB [hexadecyltrimethylammonium bromide], 0.1% 2-mercaptoethanol) and incubated at 65° C for 3 hours. This homogenate was then twice extracted with one volume of 24:1 chloroform:isoamyl-alcohol, and nucleic acids were precipitated with one volume of ice-cold 100% propanol. The DNA was pelleted in a microcentrifuge, washed with 70% ethanol, dried under vacuum, and resuspended in 100  $\mu$ L TE (10 mM Tris and 1 mM EDTA, pH = 8.0).

The 16S mitochondrial rDNA was amplified with the polymerase chain reaction using 0.5  $\mu$ L of total DNA preparations as template in 25  $\mu$ L PCR mixes. Each reaction mix consisted of 10 mM Tris (pH = 8.3), 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 0.01% Triton-X 100, 0.01% gelatin, 0.01% NP-40, 200  $\mu$ M of each dNTP, 1 unit Taq DNA polymerase (Perkin-Elmer), and 25 pmoles of each primer. Reaction mixes were subjected to 30 cycles of 1 minute at 94° C, 1 minute at 52° C, and 1 minute at 72° C on an automated thermocycler (Perkin-Elmer). Negative controls lacked template. The primers used were MYT16SA-RI and MYT16SB as described in Geller and Powers (1994). A second round of amplification with a nested biotinylated primer (MYT16SAX 5' CGA AGA AGG TGC TGT GTC T[A/G]A 3') and MYT16SB, was necessary to increase yield for sequencing. The first-round negative control was used as template for a second round negative control, along with another control lacking any added template. Single-stranded PCR products were isolated with strepavidin-coated magnetic beads according to the manufacturer's protocol (Dynal) and sequenced with the Sequen-

nase kit (Amersham) and  $\alpha$ -<sup>35</sup>S dATP. Twenty-two sequences from GenBank (U22864-U22885, Rawson & Hilbish 1995) were aligned by means of Clustal W (Thompson et al. 1994), and three new sequences (Catalina 1900, 131 bp, GenBank U40235; Monterey 1884, 98 bp, U40236; Monterey 1871, 84 bp U40237) were added to the alignment by eye.

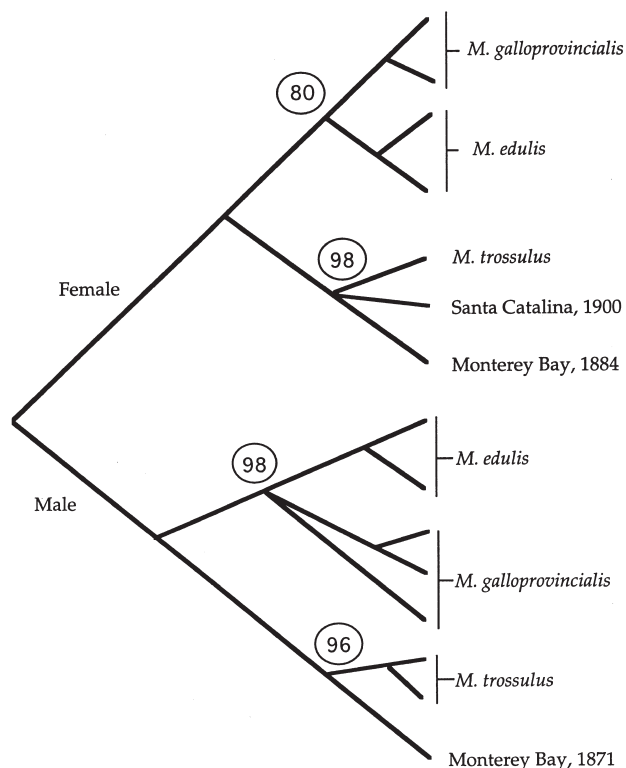
## Results and Discussion

### Phylogenetic Analysis

Partial mitochondrial 16S rRNA gene sequences known to be phylogenetically informative for *Mytilus* spp. (Rawson & Hilbish 1995) were obtained from mussels collected in southern California around 1900 (Santa Catalina Island) and in central California in 1871 and 1884. These were compared to 22 homologous sequences of modern male and female blue mussels (separate male and female mtDNA lineages are found in *Mytilus* spp., Skibinski et al. 1994). A branch-and-bound search for a maximally parsimonious phylogenetic tree using PAUP 3.0s (Swofford 1989) revealed 688 equally parsimonious trees (101 steps). A strict consensus of these trees showed that male and female *M. trossulus* formed monophyletic clades in 100% of trees. Thus, these sequences effectively discriminate *M. trossulus* from the other two species. In 100% of the 688 trees, the sequence from Santa Catalina Island and one sequence from Monterey Bay clustered with female *M. trossulus* sequences, and the other sequence from Monterey Bay clustered with male *M. trossulus* sequences. One measure of reliability of branch placement is to compare alternative trees (Bremer 1988). No sites required fewer steps in an alternative tree in which archival sequences were moved to male or female *M. galloprovincialis* clades, whereas the consensus tree was shorter for 11 sites and was 12 steps shorter. The bootstrap procedure, a more commonly used measure of reliability of branches (Felsenstein 1985), was not tractable with the entire data set due to computer memory constraints. Therefore another branch-and-bound search was bootstrapped with 500 replicates with a reduced data set (Fig. 1). A majority-rule consensus of the 500 trees thus generated provided support levels of 96% (male mussels) and 98% (female mussels) for the phylogenetic placement of the nineteenth-century sequences as *M. trossulus* (Fig. 1).

### Cryptic Invasion of *Mytilus galloprovincialis*

All recent genetic investigations of blue mussels in southern California and Monterey Bay agree that introduced *M. galloprovincialis* are abundant (McDonald & Koehn 1988; Sarver & Foltz 1993; Geller et al. 1994; Suchanek et al. 1997). In contrast, the mtDNA sequences from mus-



**Figure 1.** Phylogenetic placement of sequences obtained from nineteenth-century mussels collected in southern and central California in clades with recently collected *Mytilus trossulus*. Relevant bootstrap values (percent occurrence in 500 replicates) are circled. Genbank accession numbers for sequences used are FMGU22870, FMGU22877, FMEU22866, FMEU22864, FMTU22879, FMT22880, MMEU22865, MMEU22868, MMGU22869, MMGU22873, MGU2878, MMTU22882, and MMTU22883.

sels from three different collections from 1870–1900 were unambiguously that of *M. trossulus* and suggest that the decline of this species in this geographic range has occurred since that time period. Additional samples from other sites and dates would of course provide better spatial and temporal resolution to a reconstruction of these events. Unfortunately, retrospective molecular studies will always be limited to available and adequately preserved material. Nonetheless, these data confirm the absence of a temporal gap between known *M. trossulus* populations and the arrival of *M. galloprovincialis*.

Mussel populations in southern California experienced a period of explosive population growth in the early to middle 1940s, and some workers speculated that these mussels could have been nonnative (reviewed in Carlton 1979). Extensive overgrowth of other benthic organisms was noted at this time: “since the coming of *Mytilus*. . . everything is smothered. . . (in Newport Bay)” (Boerstler to Burch, in Carlton 1979). Circumstantial evi-

dence suggests that a causal relationship between the arrival of *M. galloprovincialis* and the decline of *M. trossulus* is possible. In San Francisco Bay, where *M. trossulus* and *M. galloprovincialis* cooccur, *M. galloprovincialis* is more abundant in sites with warmer water of more constant salinity (Sarver & Foltz 1993); this would have given the invader an advantage in competition for space during its expansion in southern Californian waters. Interference competition is also plausible, as the quote above suggests. For example, *M. galloprovincialis* climbs over and smothers another native mussel, *M. californianus*, in wave-protected areas in southern California (Harger 1968) and may have exhibited similar behavior in patches of *M. trossulus*. *M. galloprovincialis* is also a strong competitor in other regions of the world. Where *M. edulis* and *M. galloprovincialis* coexist in Europe, the latter consistently suffers less predation by predatory snails, exhibits lower incidence of parasite infections, forms stronger attachments, and experiences lower mortality rates (Coustau et al. 1991; Skibinski & Roderick 1991; Willis & Skibinski 1992; Gardner 1994). In South Africa invading *M. galloprovincialis* is outcompeting a native mussel (*Aulacomya ater*) by overgrowth (Hockey & van Erkom Schurink 1992). A test of a hypothesis of competitive displacement would involve experimental transplantation of *M. trossulus* to southern California. The data presented here make this experiment meaningful because, if the decline of *M. trossulus* preceded the arrival of *M. galloprovincialis*, competition as an explanation for the decline is refuted.

I conclude that the decline of *M. trossulus* in southern California went unobserved due to the presence of an invading sibling species, regardless of the proximate cause for the decline. More generally, the prevalence of sibling species in the sea (Knowlton 1993) may cause the number and effects of invasions to be overlooked and make the conservation status of many species difficult to assess.

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