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Effect of pollution on genetic diversity in the bay mussel *Mytilus galloprovincialis* and the acorn barnacle *Balanus glandula*

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Abstract

To test if environmental contamination acts as a selection force affecting genetic diversity at the population level, two intertidal invertebrate species, *Mytilus galloprovincialis* and *Balanus glandula*, were collected from seven different bay sites in southern California. Collections were made at three relatively pristine 'clean' sites and four 'impacted' sites exposed to heavy industrial or boating activity, and which had previously been identified as having measurable levels of pollution. Genetic diversity at each site was assessed by comparing fragment polymorphisms generated from genomic DNA by randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). All populations retained a large amount of genetic diversity and were genetically similar to each other. However, several different measures of diversity indicated that, for most primers, the populations of both species from impacted sites had lower genetic diversity compared to those populations from clean sites. Individuals at impacted sites were more likely to share the same haplotypes than were those from clean sites. Few bands seen in the clean sites were absent from the impacted sites or vice versa, but a number of bands in the clean site populations were significantly less common in the impacted populations, while a few bands uncommon in clean site populations were more common at impacted sites. Together, these results suggest that pollution at the impacted sites may reduce genetic diversity among the resident invertebrate populations.

Keywords: Genetic diversity; *Mytilus galloprovincialis*; *Balanus glandula*; RAPD-PCR; Pollution; UPGMA

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Traditional approaches to the assessment of the impact of pollution on aquatic communities have generally been based on ecological assessments such as measures of changes in biomass, of diversity, and of the species assemblages present. However, pollution may affect animals in many ways that fall short of the outright elimination of the species (Reish, Oshida, Mearns & Ginn, 1993). Certain individuals within a population may be more vulnerable than others to this incipient toxicity due to their specific phenotypes, and disappear from the population long before others are affected. Sublethal levels of pollutants may thus be associated with the loss of genetic diversity within a population subjected to pollution even though the population as a whole, at least over the short term, is able to survive and thrive (Street & Montagna, 1996).

To test whether such an effect could be measured in existing populations, we compared genetic diversity among populations of two different intertidal species at seven bay sites within the Southern California Bight (SCB). Four of the sites, Port Hueneme, inner Los Angeles Harbor, Long Beach Harbor, and Balboa Island in Newport Bay, were in areas of heavy industrial or boating activity had been identified by the Bay Protection and Toxic Cleanup Program (California State Water Resources Control Board et al., 1998a, b) as having substantial levels of pollutants in the sediment. Pollutants included a variety of heavy metals, pesticides, polycyclic aromatic hydrocarbons, and tributyltin. Sediment pollutants serve as a historically integrated proxy for pollutants occurring in the water column. We also confirmed the existence of deleterious genetic impact on the test species at these sites by means of the single-cell gel electrophoresis 'comet assay' (Tice, Andrews, Hirai & Singh, 1990). These sites we designated as 'impacted' sites. Three other sites, Catalina Harbor, the outer harbor at Dana Point, and the jetty in lower Newport Bay, had less boating and industrial activity and were designated as 'clean' sites. Lower but still detectable levels of the same pollutants existed at all of these sites which had been tested.

The species tested were the bay mussel *Mytilus galloprovincialis* and the acorn barnacle *Balanus glandula*. Both species are found intertidally in abundance within bays of the SCB. They are both sessile and filter feed and are therefore thoroughly exposed to the local water characteristics of their site as adults. Both have long-lived pelagic larvae (Anderson, 1994; Bayne, 1976) which, given the tidal flushing and water circulation patterns of the SCB, should allow rapid dispersal of larvae throughout the bight, so that the entire bight should contain a single deme for each species. Genetic diversity, at least at the time of larval settlement, should therefore be similar throughout the bight and differences among adult populations would reflect post-settlement selection.

Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) annealing (temperature 36°C) was used in this study to assess genetic diversity in each population. This method can be used to examine the entire genome by producing a number of amplified DNA fragments with no need for sequencing specific fragments (Adamkewicz & Harasewych, 1994; Yu & Pauls, 1992). Two primers, Operon OPG-08 and OPG-06, were employed to amplify DNA from gills of the mussel and four primers, OPG-12, OPG-11, OPG-10, and OPG-05, were used to

amplify DNA isolated from the entire body (except cirri) of the barnacle. Samples were run in triplicate, and bands clearly expressed in at least two replicates were scored and used to create genetic profiles of each individual.

Several different techniques were employed to assess genetic diversity within and among the populations based on the PCR band profiles. Arlequin version 1.1 was used to measure gene diversity indices. Rarefaction analysis, as used in ecological studies of species diversity, was used to compare diversity of haplotypes among the clean and impacted populations, and analysis of variance and chi-square analysis were used to compare band frequencies. Since absolute frequencies of different bands varied widely they were rank-transformed before comparing frequencies among populations. The genetic similarity of the seven populations to each other based on RAPD band frequencies was assessed by unweighted pair-group method using arithmetic average (UPGMA, SPSS version 8.0). A total of 196 barnacles and 176 mussels were analyzed from the clean sites, and 273 barnacles and 223 mussels from the impacted sites.

A very consistent pattern emerged from comparisons of genetic diversity between the clean and impacted sites (Table 1). Though the diversity indices varied among primers, species, and sites, they were nearly always lower at impacted sites than at clean sites. Rank transformation of the indices, which removed the effect of variability among primers, showed that the differences between clean and impacted sites were significant. Similarly, rarefaction analysis consistently showed lower diversity at the impacted sites. No specific bands were being completely lost from the impacted sites but that a number of bands common at clean sites were significantly less common at impacted sites, while a few bands uncommon at clean sites were significantly more common at impacted sites.

Table 1
Comparison of measures of diversity between clean and impacted sites[a]

		Clean	Impacted	Significance
Molecular diversity index ($\hat{\pi}$)	Mussel	0.295	0.231	*
	Barnacle	0.343	0.289	0.09
Rank of molecular diversity index	Mussel	5.17	3.13	0.065
	Barnacle	5.25	3.06	0.01
Standard diversity index ($\hat{H}$)	Mussel	0.97	0.91	*
	Barnacle	0.99	0.98	NS
Rank of standard diversity index	Mussel	5.00	3.25	*
	Barnacle	4.71	3.47	NS
Diversity by rarefaction analysis	Mussel	Higher	Lower	
	Barnacle	Higher	Lower	
Proportion of shared haplotypes	Mussel	0.067	0.110	0.004
	Barnacle	0.034	0.066	0.059

[a] Indices are the average calculated for the 'clean' and 'impacted' categories and for all the primers used. Ranks are based upon the seven sites. Significance levels are based on analysis of variance. An asterisk indicates was significant for some but not all primers. NS, not significant.

A clear pattern also emerged from comparisons of genetic distance among the populations. UPGMA showed the impacted sites to be more genetically similar to each other than they were to the clean sites (Fig. 1), while clean sites were much more different from one another. Similarly, the proportion of shared haplotypes among the impacted sites was significantly higher than that among the clean sites. Even impacted sites widely separated geographically were much more similar to one another than they were to nearby clean sites.

Taken together, these data show that even though the mussel and barnacle populations are thriving at the impacted sites, these populations have undergone a significant loss of genetic diversity with a number of genetic patterns becoming less common and a few becoming more common. No doubt this effect is most prominent at specific loci, but it is pervasive enough to be detected by RAPD, which surveys the entire genome. Though dispersed throughout the SCB, populations at these impacted sites are becoming more genetically similar to one another than they are even to clean sites located nearby. In the process, a hidden loss of genetic diversity is

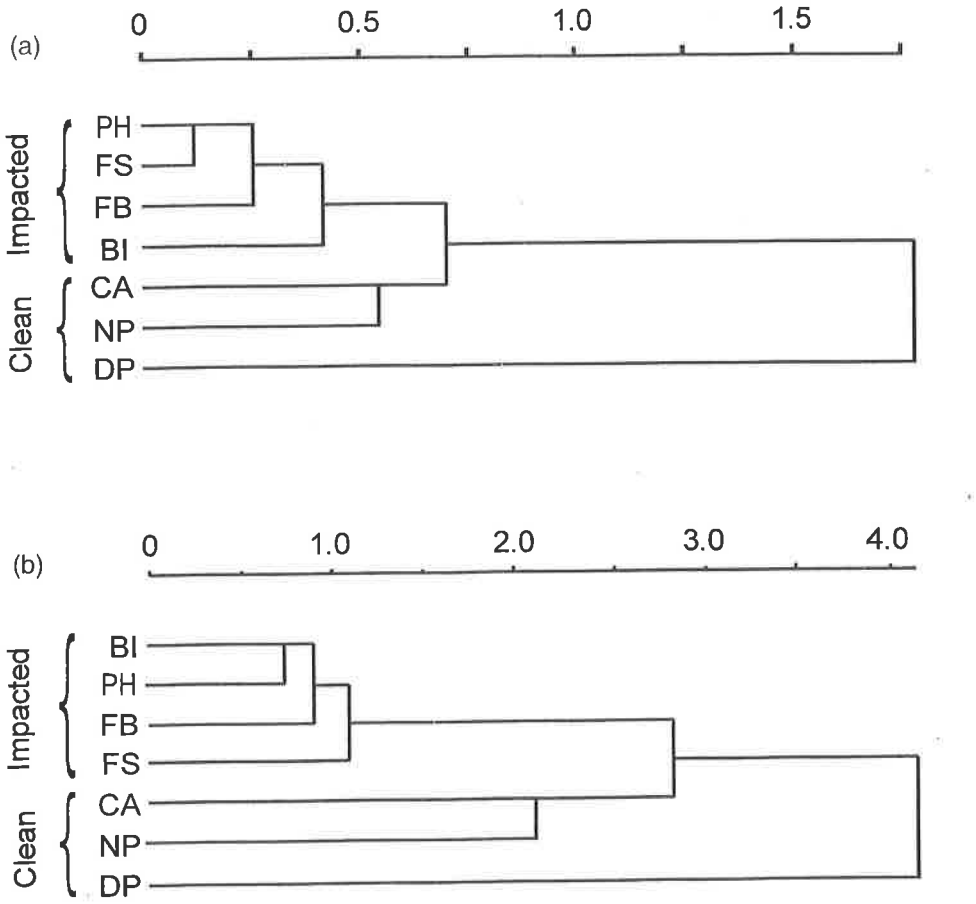

Fig. 1. Unweighted pair-group method using arithmetic average (UPGMA) dendrogram of similarity among sites, based on band frequencies at each site: (a) mussel; (b) barnacle. For both species, the four impacted sites were all similar to each other while the clean sites were different from one another and from the clean sites. PH, Port Hueneme; FS, Fire Station #49, inner Los Angeles Harbor; FB, Fire Station #20, inner Long Beach Harbor; BI, Balboa Island, Newport Bay; CA, Catalina Island; NP, near mouth of Newport Bay; DP, outer harbor at Dana Point.

taking place, which may decrease the population's ability to respond to future environmental change.

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