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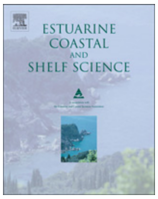

Genetic heterogeneity in populations of the Mediterranean shore crab, *Carcinus aestuarii* (Decapoda, Portunidae), from the Venice Lagoon

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ABSTRACT

Heterogeneity in genetic composition among recruits, mostly due to a large variance in reproductive success mediated by oceanographic processes, has been reported for marine species but is less understood in coastal lagoons' organisms. Temporal genetic variation in natural populations of the Mediterranean shore crab *Carcinus aestuarii* was quantified over a multi-year sample. A total of 486 adult crabs were collected at eight different sites of the Venice Lagoon during the period 2005–2007 and screened for genetic variation using 11 microsatellite loci. Two additional samples ($N = 115$) from neighbouring sites, located approximately 100 km North and South to the Venice Lagoon, were included for the sake of comparison. Our results show significant differences in allelic frequencies at the micro-geographic scale of the Venice Lagoon, observed between sites of collection, typologies of habitat, and areas with different class of ecological risk or pattern of hemocyanin expression. However, this pattern was not constant between years, with significant differences observed mainly in 2005 and 2006, but not in 2007. Our results indicate significant temporal differences suggesting the existence of dynamic processes that act on the genetic pool of this species. Although natural selection and gene flow might play a role, we suggest that genetic drift linked to large variation in the reproductive success of individuals is the most probable scenario to explain the local genetic patterns of differentiation in the Mediterranean shore crab. Our study, by providing the first evidence for the existence of genetic differences in this species at the micro-geographic scale, suggests that a better comprehension of the link between reproduction, recruitment and oceanography is critical to understand how colonization and maintenance of genetic variation is achieved in ephemeral and vulnerable environments such as coastal lagoons.

1. Introduction

Although the dispersal potential of larval stages of marine organisms has long been acknowledged, its impact on population dynamics and evolution is still the focus of recent studies (Caley et al., 1996; Kinlan and Gaines, 2003; Palumbi, 2003). For decades, the genetic structure of marine organisms has been thought to be homogeneous due to their extended egg/larval dispersal capabilities and to the lack of obvious barriers to gene flow in the marine environment (Vermeij, 1987; Ward et al., 1994; Hauser and Carvalho, 2008). A large number of marine organisms exhibits large population sizes, external fertilization, high fecundity, an extensive pelagic larval phase, and in many cases a benthic adult stage. These characteristics lead to an expectation of low genetic divergence, due to extensive gene flow during the larval stage, whereas the lower mobility at the adult stage may eventually favour local adaptation (Ward et al., 1994). Yet, this view has been challenged now that complex genetic structures have been discovered in the sea, with the most noticeable examples demonstrating population subdivisions in marine fishes on a limited geographic scale ranging from few to few hundred kilometres (Ruzzante et al., 1998; Knutsen et al., 2003; Taylor and Hellberg, 2003; Nielsen et al., 2004; Olsen et al., 2008).

For highly dispersive marine invertebrates, several surveys have shown the existence of significant differences at the small spatial scale (Watts et al., 1990; Hedgecock, 1994; Edmands et al., 1996; David et al.,

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1997; Moberg and Burton, 2000) often referred as "chaotic genetic patchiness", unpatterned genetic heterogeneity among local populations (Johnson and Black, 1982). For instance, studies on the distribution of genetic variability of the black-lipped pearl oyster (*Pinctada margaritifera*) from the Central Pacific (Arnaud-Haond et al., 2008) suggested a natural restriction to gene flow at the large scale (more than 1000 km), partly obscured by recent spat translocations (Arnaud-Haond et al., 2004), and a substantial homogeneity at a medium scale (>10–100 km); in contrast, at the intra-lagoon scale, a stochastic dynamic of spat recruitment (Friedman et al., 1998) resulted in "chaotic" genetic differences in both space and time (Arnaud-Haond et al., 2008). This trend seems to be rather common. Planktonic dispersal itself, although causing uniformity on the medium or large scale, can determine locally a stochastic variation in the numbers and genotypes of recruits, thus producing fine-scale genetic patchiness (Johnson and Black, 1982).

On the other hand, consistent genetic differences at micro-geographic scale attributable to natural selection has been observed using allozymes in marine invertebrates (Koehn et al., 1980; Hilbish and Koehn, 1985). In these cases, the occurrence of differential selection in space and time favouring genetic differentiation of recruited cohorts has been found to produce clines in genetic polymorphisms or correlations with varying environmental factors (see Crawford and Powers, 1989; Oleksiak et al., 2002 for fish). In addition to direct identification of selective effects that requires a strong genetic linkage between the markers used and some of the fitness component (Arnaud-Haond et al., 2008), indirect effects of selection on multilocus genetic diversity has been reported using microsatellites (Domínguez-Domínguez et al., 2005; Maes et al., 2005; Fratini et al., 2008). In fact, under the "genetic erosion" hypothesis, toxicant exposure may alter the genetic composition of a population favouring more tolerant genotypes, it may alter migration patterns and/or cause genetic bottlenecks (Van Straalen and Timmermans, 2002) thus resulting in a decrease in multilocus genetic variability.

The existence of genetic differentiation or natural selection effects at the small scale, independent of the possible large scale homogeneity of population and gene flow among distant sites, underlines the importance of sampling scale for population and biogeographic studies (David et al., 1997; Benzie, 2000; Arnaud-Haond et al., 2008).

In this study, we focus on the pattern of population differentiation in a high dispersal species, the Mediterranean shore crab (*Carcinus aestuarii*, Decapoda: Portunidae) at the micro-geographic scale. *Carcinus aestuarii* is a euryhaline and eurytherm species occupying estuarine and lagoon waters of the Mediterranean Sea and it is closely related to the Atlantic European green crab *Carcinus maenas*. Due to substantial overlapping of morphological and ecological characters, the taxonomic status of the two species has been disputed (Cohen et al., 1995; Bulnheim and Bahns, 1996; Behrens Yamada, 2001). Recently, genetic studies confirmed the existence of fixed differences between the Atlantic and Mediterranean forms (Geller et al., 1997; Roman and Palumbi, 2004) suggesting their specific status, but the possibility that the two forms hybridize in the contact zone of the Gibraltar strait is still open (Behrens Yamada and Hauck, 2001). Both *C. aestuarii* and *C. maenas* are highly invasive species that can adversely affect marine communities by altering food webs, disturbing habitats, displacing native species, and preying on commercially important clams, mussels, oysters, and juvenile native crabs (Carlton and Cohen, 2003). It has been suggested that the same life-history characteristics that allow for successful invasions – wide environmental tolerance, high fecundity and long larval stages – should lead to weakly or seemingly unstructured populations of the species (Waples, 1998).

Evidence for weak differentiation has been reported in *Carcinus maenas* using the cytochrome *c* oxidase I gene (Roman and Palumbi, 2004; Darling et al., 2008). In particular, after removing samples from offshore locations such as the Faeroe Islands and Iceland in which highly differentiated haplotypes were found, genetic structure was very slight along the European shore. In fact, a small but significant break explaining 1.2–2.9% of the genetic variation, was found between Western and Northern Europe biogeographic regions, whereas differentiation within the two regions was negligible on a scale of several hundreds kilometres. Considerably weaker structure was detected using microsatellites (Darling et al., 2008) with estimates of genetic differences associated with onshore/offshore and European shore locations and biogeographic regions ranging from 1.6% to 1.0% respectively.

Here, we aim at extending these large scale results by investigating the pattern of genetic differentiation between multi-year samples of *Carcinus aestuarii* collected at different sites of the Venice Lagoon (Northern Adriatic Sea, Italy), separated by a maximum of about fifty kilometres. The Lagoon of Venice (45.2°–45.6° N, 12.2°–12.6° E), the largest Italian Lagoon and one of the largest in Europe, is sub-divided in three sub-basins separated by two watersheds along which the intensity of the tidal currents is low. Recent studies show that the lagoon is a complex system, which presents both stable ecological differences between sites than variability, in time and space, of several parameters including temperature, salinity, dissolved nutrients, chlorophyll a, dissolved oxygen, and turbidity (MAV-CVN, 2002a,b). In addition, the impact of the anthropogenic activities affects the various sub-areas to a different extent and adverse effects, such as anoxic crises and pollutant accumulation in the sediment, generally occur in the more confined sub-areas (Wenning et al., 2000; Critto and Marcomini, 2001; Bellucci et al., 2002).

In this context, by collecting *Carcinus aestuarii* genotypes of 11 microsatellite loci, we intend to: (1) Identify the existence of genetic differences among sites to reveal the existence of locally adapted populations. (2) Identify differences between years of collection and partition the amount of genetic differentiation into an inter- and intra-annual component to address the existence of temporal variation in the local genetic pool of the species. (3) Compare samples from areas characterized by different habitat typology (Guerzoni and Tagliapietra, 2006; Tagliapietra et al., 2009b), reflecting the confinement (*sensu* Guélorget and Perthuisot (1983), but see Tagliapietra et al., 2009a) and by different level of pollution (Critto et al., 2005). (4) Compare population samples characterized by different patterns of hemocyanin subunit expression, a phenotypic parameter that depends on chemical and physical factors such as salinity, dissolved oxygen and temperature (Mangum and Rainer, 1988; DeFur et al., 1990; Mangum, 1990) and might then reflect differential exposure to these stressors.

2. Materials and methods

2.1. Sampling

A total of 486 specimens of the Mediterranean shore crab *Carcinus aestuarii* were collected over three years, between 2005 and 2007, at several sites in the Venice Lagoon (Fig. 1, Table 1). For the sake of comparison, we included in the analysis two population samples ($N = 115$) from Marano and Goro, located about 100 km North and South of the Venice Lagoon (Fig. 1, Table 1).

After collection, the crabs were brought to the laboratory and frozen. One cheliped or both were taken and frozen at −80 °C for DNA extraction.

The sampling sites in the Venice Lagoon were characterized by different habitat typologies following the classification specifically