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Gene flow and demographic history of the mangrove crab *Neosarmatium meinerti*: A case study from the western Indian Ocean

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ABSTRACT

Most marine organisms are characterized by at least one planktonic phase during their life history, potentially allowing interconnection of populations separated by several hundred kilometers. For many years, the idea that marine species are genetically homogenous throughout their range of distribution, due to passive larval transport, has been a paradigm. Nowadays, a growing number of studies underline the existence of boundaries in the marine realm and highlight how larval dispersal is a complex process depending on biotic as well as abiotic factors. Marine fragmented habitats, such as atolls, mangroves and estuaries, are optimal systems for investigating the marine dispersion process under a metapopulation approach, since populations can be geographically defined a priori as opposed to those occupying open marine environments. Within this frame, the present paper investigates the population genetic structure and the demographic history of the mangrove crab *Neosarmatium meinerti* within the western Indian Ocean by partial sequences of the mitochondrial DNA cytochrome oxidase subunit I. A total of 167 specimens were sampled from six mangrove sites distributed along the East African coast, from Kenya to South Africa, also including a mangrove forest located on Mahé Island, Seychelles. A sharp genetic break between the mainland and the Seychelles is recorded, revealing the existence of two historically distinct groups that can be defined as independent evolutionary units. Gene flow along the East African coast appears to be high enough to form a single metapopulation, probably by means of stepping stone populations. Otherwise, this mainland metapopulation is currently under expansion through a gradual moving front from the subtropical toward the equatorial populations.

1. Introduction

The majority of marine invertebrate species are characterized by at least one widely potentially dispersive phase during their life history, such as gametes, eggs, larvae or spores, generically called propagules (Fairweather, 1991). These planktonic stages are released into the open sea where, after a developmental period that can vary from hours to months or even years (Levin, 2006), they settle in the proper habitat where they can grow to adulthood. Thus, the dispersal stages have the ability to potentially disperse across hundreds of kilometers by means of ocean currents. Larvae of coastal species, for instance, have been observed in mid-ocean plankton, suggesting to have crossed long distances (Palumbi, 2001, 2003). For many years these findings, together with the lack of evident geographical barriers in the marine realm, supported the idea that marine species are demographically open, with high level of population exchange (Becker et al., 2007) and genetically homogenous throughout their geographical range (Cowen et al., 2000). On the other hand, in recent years several studies employing genetic techniques, chemical tags and/or biological and physical models, revealed many examples of marine species with reduced connectivity among populations (Knowlton, 2000; DiBacco et al., 2006) and highlighted how larval dispersal is a complex process depending on several biotic and abiotic factors (Bilton et al., 2002).

Many marine habitats, such as atolls, coral reefs and lagoons, are naturally fragmented and patchy, consequently allowing their populations to be defined a priori as opposed to those occupying open marine environments. This allows us to approach the study of connectivity among populations using a metapopulation-based approach (DiBacco et al., 2006). With this approach, "*landscapes are viewed as a network of habitat patches or fragments in which species occur as discrete local populations connected by the passive and active migration of individuals*" (DiBacco et al., 2006, p. 184). Thus, the exchange of larvae among populations may be described by two alternative models: the Island Model (Wright, 1940), in which defined populations of the same size exchange individuals with

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equal probability, and the Stepping Stone Model (Kimura and Weiss, 1964), in which the genetic exchange is supposed to happen exclusively between adjacent demes, and consequently the connection among populations would be proportional to their geographic distance (Palumbi, 2003).

Mangrove forests are another example of a fragmented habitat. Along the East African coast mangroves are on average separated by about 150 km of stretches of sandy or rocky shores and are populated by several crab and mollusc macrobenthonic species strictly associated with this environment (Cannicci et al., 2008). For these various intertidal and semi-terrestrial species a double problem arises: how to optimize the possibilities of dispersal along the coast and how to identify and colonize the most suitable habitat. Most mangrove crabs release larvae in synchrony with spring tides (Skov et al., 2005), probably to be transported by oceanic waters and thus improving their dispersal capabilities (Wooldridge, 1991; Papadopoulos et al., 2002). In the open oceanic waters larvae pass through a determined number of stages, all of them characterized by a different pattern of vertical migration (called ontogenetic migration: Shanks, 1995; Queiroga and Blanton, 2005) in the water column. The vertical migration, depending on the larval stage, should guide both the export of larvae to the open oceanic waters as well as the recolonization of the adequate habitat for the settlement (Shanks, 1995; Queiroga and Blanton, 2005). The last larval stage, the megalopa, returns to the mangroves probably guided by chemical and physical cues (the "return migration": Shanks, 1995; Queiroga and Blanton, 2005).

The aim of the present study was to describe the population genetic structure of the mangrove crab *Neosarmatium meinerti* (Decapoda: Brachyura: Sesarmidae) and to depict its demographic history within the western Indian Ocean (WIO). We also aimed to shed light on the historical and ecological factors affecting the gene flow, both in terms of direction and entity. *N. meinerti* occupies the rearward belt of mangrove forests and is widespread throughout the Indo-Pacific Ocean (Davie, 1994), but genetically highly structured (Ragionieri et al., 2009). Mangrove crabs such as *N. meinerti* that occupy the middle-upper belt have a seasonally defined breeding season, conversely to those species living in the lower intertidal belt which are continuous breeders (Emmerson, 1994). Moreover, the *N. meinerti* breeding period is also affected by local conditions: in Kenya and Tanzania ovigerous females release larvae at spring tide every month during the Northwestern Monsoon (between February and March) (Skov et al., 2005), while in South Africa the spawning happens during the summer, thus a little earlier and for a shorter period than in Kenya and Tanzania, when the temperature is warmer (Emmerson, 1994). After the hatching, larvae have a supposed lasting period in the plankton of about four weeks (Pereyra Lago, 1989), thus being able to potentially interconnect populations separated by hundreds of kilometers.

To achieve our goals, we investigated the pattern of genetic variation in the mitochondrial cytochrome oxidase subunit 1 (mtDNA CoxI) in six populations of the East African coast and in one population from Seychelles Islands. Two main scenarios can be supposed: (1) *Neosarmatium meinerti* populations of the WIO are a single metapopulation with high level of genetic exchange, or alternatively (2) some barriers to dispersal occur, inducing an interruption of gene flow between the mainland and island populations and/or along the continental margin. Different separation scenarios are possible. On the one hand, the Seychelles Islands are separated from the East African coast by more than 1500 km of open sea, a geographic gap that could exceed the distance the larvae may cover (see Palumbi, 2003). On the other hand, a separation among tropical (Kenya) and subtropical populations (South Africa) may be induced by local scale currents and ebbs as well as by larval and adult local adaptations to sharp temperature gradients, as recorded along the South African coast for the estuarine mud prawn *Upogebia Africana* by Teske et al. (2008). Moreover, in a metapopulation which is latitudinally widespread over a wide range, connectivity should be interrupted due to reproductive isolation among populations under different seasonal conditions.

2. Materials and methods

2.1. Main circulation of western Indian Ocean

The main WIO circulation is characterized by the South Equatorial Current (SEC) which flow from the eastern Indian Ocean toward the western Indian Ocean at around 10° of latitude south before splitting into two main currents when reaching the eastern coast of Africa (Fig. 1). One of these currents flows southward and includes the Mozambique Currents and Madagascar Currents, while the other one flows northward comprising the East Africa Coastal Current (EACC). It should separate populations of Tanzania and Kenya from the populations of Mozambique and South Africa. Even the monsoon plays an important role with its seasonal changing of the Somali Current direction. During the Southeast Monsoon, the EACC extends further becoming the Somali Current and later joining the Indian Monsoon Currents, while during the Northeast Monsoon the northerly flow of the EACC is reduced and turns eastward becoming the Equatorial Counter Current, together with the Somali Current which flows southward (Fig. 1).

2.2. Sample collections and DNA extraction

A total of 167 specimens of *Neosarmatium meinerti* were collected along the East African coast from Kenya (Lamu $n = 18$, Mida Creek $n = 26$, Gazi Bay $n = 27$), Tanzania (Dar es Salaam $n = 26$), Mozambique (Inhaca Island $n = 28$) and South Africa (Durban $n = 22$) (Fig. 1). In addition, we sampled a population from the Seychelles (Mahé Island $n = 20$) (Fig. 1). From each specimen, muscle tissue was removed from a walking leg and immediately placed in a vial with absolute ethanol. Subsequently, the specimens were released into their natural environment. The genomic DNA was extracted using the Puregene Kit (Gentra System) and then resuspended in TE or distilled water and stored at −20 °C.

2.3. DNA amplification

A fragment of 658 base pairs (bp) of the cytochrome oxidase subunit 1 gene (CoxI) was amplified by polymerase chain reaction (PCR) using the following primers: COL6b 5′-aca aat cat aaa gat aty gg-3′ (Schubart and Huber, 2006) and HCO2198 5′-taa act tca ggg tga cca aaa aat ca-3′ (Folmer et al., 1994). The PCR amplification was performed in a Perkin Elmer 9600 thermal cycler with the following PCR conditions: 40 cycles with 45 s at 94 °C for denaturation, 1 min at 48 °C for annealing, 1 min at 72 °C for extension, preceded by 10 min at 94 °C for initial denaturation and 10 min at 72 °C for final extension. Subsequently, PCR products were visualized on agarose gels, purified by precipitation with Sure Clean (Bioline) and then resuspended in water. The sequence reactions were performed with the Big Dye terminator mix (Big Dye Terminator® V 1.1 Cycle Sequencing kit; Applied Biosystems) followed by electrophoreses in an ABI Prism automated sequencer (ABI Prism™ 310 Genetic Analyzer; Applied Biosystems). The sequences were corrected manually with Flinch TV 1.4.0 (Geospiza®) and 620 bp aligned manually with Bioedit (Hall, 1999).