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Do larval types affect genetic connectivity at sea? Testing hypothesis in two sibling marine gastropods with contrasting larval development

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ABSTRACT

In marine environments, connectivity among populations of benthic invertebrates is provided primarily by dispersion of larvae, with the duration of pelagic larval phase (PLD) supposed to represent one of the major factor affecting connectivity. In marine gastropods, PLD is linked to specific larval development types, which may be entirely intracapsular (thus lacking a pelagic dispersal), or include a short pelagic lecithotrophic or a long planktotrophic phase.

In the present study, we investigated two sibling species of the cosmopolitan neogastropod genus *Columbella* (commonly known as dove shells): *Columbella adansoni* Menke, 1853, from the Macaronesian Atlantic archipelagos, with planktotrophic development, and *Columbella rustica* Linnaeus, 1758, from the Mediterranean Sea, with intracapsular development.

We expected to find differences between these two sister species, in terms of phylogeographic structure, levels of genetic diversification and spatial distribution of genetic diversity, if PLD was actually a relevant factor affecting connectivity.

By analysing the sequence variation at the cytochrome *c* oxidase subunit I (COI) in 167 specimens of the two species, collected over a comparable geographic range, we found that *Columbella adansoni,* the species with planktotrophic development, and thus longer PLD, showed no phylogeographic structure, lower levels of genetic diversity, interpopulational variance lower than the intrapopulational one and no spatial structure in the distribution of the genetic diversity; *Columbella rustica*, the species with intracapsular development, thus with evidently lower dispersal abilities, showed a clear phylogeographic structure, higher levels of genetic diversity, high interpopulational and low intrapopulational variance, and a clear signature of global spatial structure in the distribution of the genetic diversity.

Thus, in this study, two sibling species differing almost only in their larval ecology (and PLD), when compared for their genetic variation showed patterns supporting the hypothesis that PLD is a major factor affecting genetic connectivity.

Therefore, it seems reasonable to expect that the ecological attributes of the marine communities - also in terms of the variation in larval ecology of the species involved – are taken into the due consideration in conservation actions, like the design of marine protected areas networks.

1. Introduction

Population connectivity is a key feature of organisms, influencing their genetic variability, persistence, genetic structure and range expansion, and as such has increasingly been investigated in the last years in different taxa (Cowen et al., 2000; Hellberg, 2009; Hastings and Botsford, 2006; Lowe and Allendorf, 2010; Weber et al., 2015). Clarifying the extent at which populations are connected allows the understanding of evolutionary and ecological processes shaping the distribution of individuals through their range, disentangling the effects of historical patterns and local adaptations (Laine, 2005; Sanford and Kelly, 2011). Additionally, connectivity studies are crucial to implement effective conservation and management strategies both in terrestrial and in marine environments (Webster et al., 2002; Palumbi, 2003; Shanks et al.,

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2003; Crooks and Sanjayan, 2006; Jones et al., 2007; Allendorf et al., 2010; Rabinowitz and Zeller, 2010; Funk et al., 2012).

In marine benthic invertebrates, dispersal is generally addressed by the earliest life history stages, while the adult stage is only slightly mobile, or even sessile (Knowlton and Jackson, 1993; Cowen and Sponaugle, 2009; Ellingson and Krug, 2015). Major factors affecting connectivity include both extrinsic (habitat characteristics and currents) and intrinsic factors, such as larval mortality, settlement competency features, and the duration of pelagic larval phase (PLD, the length of time that larva spends in water column before settling). The latter parameter is the most frequently used proxy of dispersal, since direct measurement of dispersal can be difficult in marine invertebrates. Early studies highlighted the presence of a correlation (with some exceptions) between PLD and dispersal distance (Shanks et al., 2003; Shanks, 2009; Siegel et al., 2003), although PLD is often assessed in laboratory settings that may not accurately represents actual conditions that larvae experiment in their natural environment (e.g.: Tyler and Young, 1999; Selkoe and Toonen, 2011; Villanueva et al., 2016).

The prediction that species with planktonic larvae displaying a longer PLD and larger dispersal kernels should also possess a lower level of genetic structure when compared with species lacking a dispersal phase (e.g. aplanktonic larvae, brooding) is supported by a number of studies (Berger, 1973; Duffy, 1993; Hunt, 1993; Hellberg, 1996; Hoskin, 1997; Arndt and Smith, 1998; Collin, 2001; Dawson et al., 2002; Teske et al., 2007; Sherman et al., 2008; Lee and Boulding, 2009; Steele et al., 2009; Hoffman et al., 2011; Guzmán et al., 2011; Tarnowska et al., 2012; Barbosa et al., 2013; Hoareau et al., 2013; Riginos et al., 2014). Anyway, the suitability of PLD as a good predictor of genetic connectivity has been questioned in a number of other cases, especially for species with a long PLD (Shanks, 2009), highlighting that other factors may have a major impact on connectivity, including habitat differences (Ayre et al., 2009) and past biogeographical events (Edmands, 2001; Marko, 2004).

The influence of PLD and dispersal abilities on genetic structure can be easily tested in most gastropods, as developmental type can be inferred from the structure of the protoconch, the shell produced by the embryo and the larva before metamorphosis or hatchling, and commonly retained at the top of the adult shell (Jablonski, 1980; Lima and Lutz, 1990).

In marine gastropods development can, as first described by Thorson (1949), either be entirely intracapsular, or include a pelagic phase during which larvae actively feed on plankton (planktotrophy), barely do so, or rely only on yolk reserves (lecithotrophy). Entirely intracapsular development is realized within the egg capsule, which is generally attached to the sea bottom; the eggs are provided with a large yolk supply and/or individuals may feed on nurse eggs until metamorphosis occurs, hatchling as benthic postlarvae. Yolk supply is also exploited by lecithotrophic planktonic larvae, which hatch as free living and spend a reduced time in the water column. Similarly, planktotrophic larvae hatch as free living, but they are able to actively collect phytoplankton using their velum; the life span of these larvae typically extends over weeks or months, and some cases can exceed several years (Strathmann and Strathmann, 2007).

Among Caenogastropoda, a large number of pairs of sibling species are known, differing only in their larval development (planktotrophic v. lecithotrophic), particularly studied in the Northeastern Atlantic (Oliverio, 1996) but well known on a global scale (Oliverio, 1997a). This offers the possibility to study the bearing of larval development on species otherwise very similar in their biology and ecology. In the present study, we investigated the genetic implications of different larval developments in two sibling species of the cosmopolitan neogastropod genus *Columbella* Lamarck, 1799, currently including 30 recognised species worldwide (Bouchet and Gofas, 2010). This genus has been recently reviewed in the East Atlantic region (Russini et al., 2017) and three species have been clearly identified by molecular data: *Columbella rustica* Linnaeus, 1758, *Columbella adansoni* Menke, 1853, and *Columbella xiphitella* Duclos, 1840. These three species share nearly identical adult shell morphology and anatomical features, occupy the same macrohabitat (all are shallow water, rock dwelling, algae associated, herbivorous), and their ranges do not overlap (Oliverio, 1995; Rolán, 2005; Russini et al., 2017). *C. rustica* is endemic to the Mediterranean Sea and its Atlantic approaches, where it is extremely common in shallow-water rocky habitats; *C. adansoni* inhabits the Macaronesian archipelagos; and *C. xiphitella* lives along East African coast from Ghana to Angola (including Sao Tomé and Principe Islands). According to molecular phylogenetic data, *C. rustica* and *C. adansoni* are sister species, whereas *C. xiphitella* is more distantly related (Russini et al., 2017). Planktotrophic larvae (39–73) hatch from the egg capsules of *C. adansoni* from Canary Islands and Cape Verde Islands (Knudsen, 1950, 1995), whereas the capsules of Mediterranean *C. rustica* have been described to contain 40-60 eggs, most of which are nurse eggs to nourish the 1–12 developing embryos (1–2: Franc, 1943; 6–12: Bacci, 1943). The only morphological features allowing separation of *C. rustica* and *C. adansoni* are, in fact, in their protoconchs. In *C. adansoni* the protoconch is multispiral with an evident 'sinusigera mark' i.e. a thin sigmoid sinus marking the protoconch-teleoconch boundary, clearly indicating a planktotrophic development (same protoconch of *C. xiphitella* for which a similar planktotrophic development can be inferred). The paucispiral protoconch of *C. rustica* possesses a very peculiar appearance, being irregularly cylindrical with a more or less pronounced apical keel and a flat top; its reduced whorl number, bluntness and the absence of a 'sinusigera mark' at the protoconch-teleoconch transition, attest a lecithotrophic development (Oliverio, 1995).

If PLD is a relevant factor affecting connectivity, we expect to find differences between these two sister species, in terms of phylogeographic structure, levels of genetic diversification and spatial distribution of genetic diversity. In particular, the species with planktotrophic development, and thus longer PLD, is expected to show weaker or no phylogeographic structure, lower levels of genetic diversity and no spatial structure in the distribution of the genetic diversity, when compared with the species with lecithotrophic development. The few samples available for the third species, *C. xiphitella*, did not allow their use for the same analyses as in the pair *C.adansoni/C. rustica*; however, they could serve as an optimal outgroup for phylogeographic analyses.

2. Material and methods

2.1. Samples collection and laboratory procedures

We obtained sequences from 99 specimens of *Columbella rustica* from the Mediterranean Sea, and 68 of *C. adansoni* from the Atlantic Ocean, in particular Azores, Madeira, Canary and Cape Verde Islands (Fig. 1). Details of collection localities are reported in Table 1. Sequences of *C. xiphitella* from Gabon were used as outgroup to root trees according to the phylogenetic pattern in Russini et al. (2017).

All specimens were collected in shallow-water rocky bottom, fixed and preserved in 95°–100° ethanol, and vouchers were stored in the Malacological Collection of Department of Biology and Biotechnologies "Charles Darwin" (acronym BAU) at Sapienza University of Rome (Italy). DNA was extracted from a fragment of foot tissue, using a modified phenol-chloroform protocol (Oliverio and Mariottini, 2001). A 658 bp fragment of the mitochondrial COI gene was PCR amplified, using the universal primers LCO1490 and