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Life history and ecological genetics of the colonial ascidian *Botryllus schlosseri*

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ABSTRACT

The colonial ascidian *Botryllus schlosseri* is a cosmopolitan, marine filter feeder, introduced as a laboratory research organism in the 1950s. Currently, it is widely used in many laboratories to investigate a variety of biological questions. Recently, it has become a species of concern, as it is an invasive species in many coastal environments. Here, we review studies on the geographical distribution of the species, sexual and asexual reproduction in the field, tolerance to temperature, salinity and anthropogenic activity, polychromatism, enzymatic polymorphism, and the genetic basis of pigmentation. Studying the relationship between genetic polymorphism and the adaptation of *B. schlosseri* to environmental stress is a challenge of future research and will improve our understanding of its evolutionary success and invasive potential.

Contents

1. Introduction

Botryllus schlosseri is a colonial ascidian (Tunicata), widely distributed in shallow subarctic and temperate waters of both the hemispheres (Ben-Shlomo et al., 2001, 2010; Reem et al., 2013a,b). Colonies live in shallow waters of the littoral zone, including the intertidal zone or adjacent subtidal zone, up to a depth of 200 m. The species prefers sheltered areas of harbors and marinas (Hiscock, 2005) on either natural or artificial substrates, including rocks, algae, eelgrass, bivalves, solitary tunicates, floating docks, wharf pilings, and aquaculture plants. In macrofouling biocoenoses of hard substrata, *B. schlosseri* strongly competes with other benthic filter feeders, such as barnacles and mussels, for space and food (i.e., suspended phytoplankton, zooplankton and organic matter).

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Due to its fast-growing colonies, adaptive abilities and high genetic diversity (Reem et al., 2013b), *B. schlosseri* is an invasive species able to exploit new environments, potentially displacing native species and disrupting community dynamics (Harms and Anger, 1983; Schmidt and Warner, 1986). Once it becomes established, it is very difficult to eradicate; thus, presenting a concern for the aquaculture industry (Carver et al., 2006; Arens et al., 2011). The species is now considered cosmopolitan, living in eurythermal and mildly euryhaline conditions of all epicontinental waters except the Antarctic Circle, and it is classified as "globally secure" or without conservation status. The success of such species as invader is mainly related to its post-larval mode of dispersal by rafting of colony fragments on macrophytes rather than by spreading through larval swimming and its ability to adapt to new habitats. (Worcester, 1994).

Although the species was firstly described by Schlosser (1756) and Pallas (1766) in the Cornwall coasts, its native range is thought to include the Mediterranean and Black seas (Berrill, 1950). This assumption is supported by genetic studies based on the analyses of microsatellite loci, revealing allelic diversity between Mediterranean and Atlantic coast populations of Europe related to recent post-glacial invasions (Ben-Shlomo et al., 2006). A high level of polymorphism (more than 60 alleles for a single locus) was found in Mediterranean populations (Paz, 1999; Paz et al., 2003); this level decreases to 17 alleles at the same locus in Monterey Bay California (Stoner et al., 1997) and to between 4 and 20 alleles for 5 loci in New Zealand (Ben-Shlomo et al., 2001). These studies support the idea that *B. schlosseri* is of Mediterranean origin and suggest that a limited number of different individual colonies founded each of the introduced populations worldwide, as reported in Fig. 1A. According to this view, the species spread to the whole European coasts, probably because of post-glacial period dispersal, extending to Spain, Portugal, France, Great Britain, the Netherlands, Denmark, Germany, Sweden, Norway and the Faroe Islands (Ben-Shlomo et al., 2001, 2006; Cohen, 2005). From Europe, it was presumably introduced into several regions worldwide, likely as fouling organism on ship hulls or floating debris; these regions include the Mediterranean part of the Suez Canal (reported since 1869, down to Lake Timsah), the east continental shelf of North America (reported since 1841), the Gulf of Mexico (reported since 1887), India (reported since 2006), Far East (Japan, Korea, Hong Kong with first sporadic reports in 1929 and 1952), the Great Barrier Reef and southern Australia (reported since 1905), New Zealand (reported since 1922), Tasmania (reported since 1928), the west coast of North America (reported since the mid-1940s), the west coast of South America (reported since 1948), South Africa (reported since 1955), and the east coast of South America (reported since 1964) (Rinkevich et al., 1998a; NIMPIS, 2002; Paz et al., 2003; Carver et al., 2006; Reem et al., 2013a). The current trend of invasiveness appears to be both the Northern latitudes (Iceland form North Europe and Alaska from the U.S. north–west coast) and the tropic-equatorial ones (west coast of Central and Southern America, the latter from populations of New Zealand). Scanty information can be obtained about the recent spotted colonisation of the west coast of India, probably from various populations of the south and east coasts of Africa.

High polymorphism and heterozygote deficiency appear as common attributes of all the investigated populations of *B. schlosseri* (Ben-Shlomo et al., 2001, 2008; Rinkevich et al., 2001; Reem et al., 2013a,b). Recently, further insights into the dispersal potential and the degree of genetic differentiation among populations and subpopulations have been gained using molecular techniques. Yund and O'Neil (2000) noted that genetic differentiation may occur over very short distances (8–21 m) and that the patterns were consistent with inbreeding and genetic drift models.

The eastern and western coasts of North America had different founders over different invasion periods (Stoner et al., 2002). The east coast population is similar to the European one. The California population is younger than the New Zealand population and is the result of different founders, with continuous introductions from either European or Asian populations (Stoner et al., 2002) within a period of time shorter than the introductions to New Zealand. The same events occurred in western coastal populations in South America, which were subjected to introductions from either European or South Pacific populations (Ben-Shlomo et al., 2010). In New Zealand, the allele distribution pattern significantly differs among subpopulations probably due to both limited gene flow and founder effects, resulting in heterozygote deficiency at most loci. The latter may also reflect high levels of natural chimerism as a result of aggregated settlement of sibling larvae (Ben-Shlomo et al., 2001).

Recently, Bock et al. (2012) characterized the phylogenetic and population genetic structure of European and North American populations using cytochrome c oxidase subunit I, nuclear 18S rRNA and 10 polymorphic microsatellite loci. They reported that the species comprises at least five putative, previously unrecognized cryptic species that are morphologically indistinguishable, three of which are likely reproductively isolated from one another. One of these putatively cryptic species is widespread globally, whereas its sibling species are highly restricted geographically, limited to the Mediterranean coast of Spain and the English Channel. The genetic divergence among these lineages represents approximately 4.3–11.0 Myr of evolutionary history, likely starting during the Messinian Salinity Crisis of the Mediterranean Sea which caused habitat fragmentation. Some intra-specific variability in *B. schlosseri* has also been reported using detailed whole-mitogenome comparisons, suggesting on-going speciation events (Griggio et al., 2014).

2. Anatomy of a colony

A colony is a clone and is derived from the metamorphosis of a single, tadpole-like larva into a sessile zooid that founds the new colony; this zooid is known as an oozooid as it is derived from a fertilized egg. Interestingly, the founder oozooid contains the bud that will give rise to a blastozooid (a zooid derived from blastogenesis; i.e., budding). As a blastozooid can form more than a single bud, the colony grows through repeated cycles of budding. Zooids are arranged in star-shaped systems (7–12 blastozooids) (Fig. 1B), with their individual oral siphons opening anteriorly and their atrial siphons converging into a common, cloacal cavity in the center of each system. The cloacal cavity is connected to the environment through the cloacal siphon. Adult zooids bear buds (primary buds) that, in turn, bear budlets or secondary buds. In a colony, individuals are interconnected by a general vascular apparatus (the colonial circulatory system) that crosses the colonial tunic, i.e., the extrazooidal collagenous matrix rich in cellulose, typical of tunicates (Brunetti and Burighel, 1969; Burighel and Brunetti, 1971; Gasparini et al., 2007).

Cyclical generation changes or take-overs occur during the colonial lifespan, wherein the adult generation is replaced by the subsequent one (represented by primary buds) and new budlet primordia appear (Fig. 1C). A blastogenetic cycle is defined as the period of time from one take-over to the next and includes seven main developmental phases (Manni et al., 2007; Gasparini et al., 2014).

The replication and succession of genetically identical individuals provides the colony with strong regulatory ability demonstrating the adaptive value of both the colony lifestyle and the population dynamics of the species (Sabbadin, 1979). This homeostasis represents the main feature of colonial life, based on a somatic plasticity that allows the continual repetition of blasto-