

First report of chimerism in Mediterranean red coral (*Corallium rubrum*)

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Abstract

We reported and quantified the phenomenon of chimerism in Mediterranean red coral (*Corallium rubrum*) in semi-natural conditions. A total of 1688 larvae were maintained in close circuit in the presence of a suitable settlement surface (marble tiles). Post settlement survival and chimera formation were monitored for 1 year. When polyps settled close enough to each other, a high frequency of chimerism was observed (32%). After 1 year, only 33% of chimeric individuals survived but they were 40% bigger than non-chimeric individuals, suggesting that chimerism could confer a competitive advantage linked to an increased growth rate.

Keywords: *Corallium rubrum*; chimerism; settlement; growth rate; octocoral.

Introduction

In biology, the term chimerism refers to the fusion of individuals of the same species, (see Flake *et al.*, 1986; Tilney-Bassett, 1986), more precisely it is the co-habitation of more than one genotype originated by different fecundation processes within the same individual (solitary or colonial). Despite that the phenomenon of chimerism has been described in at least 9 phyla (Buss, 1982), including protists, animals (vertebrates and invertebrates, marine and terrestrial), plants, and fungi, it is more frequent in sessile colonial marine organisms with a planktonic dispersal phase (Rinkevich & Weissman, 1987). There is still limited research about the evolutionary and ecological value of chimerism, with some studies proposing its positive adaptive value (e.g., Hughes & Jackson, 1985; Amar *et al.*, 2008; Rinkevich *et al.*, 2016) and others highlighting its long-term costs (e.g., Rinkevich & Weissman, 1987).

Chimerism has been studied both in broadcast spawning (Puill-Stephan *et al.*, 2012) and in brooding corals (Rinkevich *et al.*, 2019 and references therein). The formation of chimeras involving adult coral colonies is quite rare, while it has been shown that it is more common during a limited period in young stages (window in ontogeny, Barki *et al.*, 2002), especially during larval settlement, when fusion between larvae is more likely to happen (Hidaka, 1985; Amar *et al.*, 2008).

Mediterranean red coral (*Corallium rubrum*) is endemic to the Mediterranean Sea, where it occurs between 10- and 800-meters depth. However, it has also been reported in nearby areas of the Strait of Gibraltar (Zibrowius *et al.*, 1984). Its high economic value due to its use in jewelry, brought several populations to overexploitation (Tsounis *et al.*, 2010). It is an internal brooding species that releases larvae once a year (Santangelo *et al.*, 2003; Bramanti *et al.*, 2003; 2005). Low larval settlement rates and post-settlement survival represent a bottleneck in the population dynamics of this species (Zelli *et al.*, 2020). In particular, slow growth rates result in prolonged vulnerability of young settlers that, due to their small size, are exposed to predation and affected by sedimentation (Cau *et al.*, 2016).

The early life-history traits of this species are still poorly known, and therefore, it is important to investigate the processes regulating settlement and post-settlement mortality (Santangelo *et al.*, 2012) to achieve effective management of local populations and to test strategies for restoration.

In the framework of a settlement and growth experiment of *C. rubrum* on artificial substrates, we observed several larval aggregations, some of which resulted in the formation of chimeric individuals. In the present paper, we quantitatively described the phenomenon and tested the hypothesis that chimera formation results in a significant increase in size.

Materials and Methods

Larval settlement and recruit maintenance

C. rubrum larvae were maintained in a closed circuit, oxygenated, and temperature-controlled aquaria (see Zelli *et al.*, 2020 for details) and offered two marble tiles (10 x 10 cm) as a settlement substrate (Bramanti *et al.*, 2003). In September 2019, once larvae settled and metamorphosed into polyps, the tiles were transferred to an open-circuit oxygenated, and temperature-controlled 3.5 L aquarium. To monitor the mortality and growth of young polyps, each recruit was photographed at different ages: 0 months (September 2019, t_1), 3 months (December 2019, t_2), and 12 months (September 2020, t_3).

Nearest neighbor distance

Images were calibrated and the coordinates corresponding to the polyps' location on the tiles were recorded to draw a map of recruits' distribution, using ImageJ software (Schneider *et al.*, 2012). The distance of each recruit from its nearest neighbor (NND) was computed with the *nnDist* R software function. A NND threshold of 1.0 mm was chosen to discriminate between aggregated and non-aggregated polyps. The choice of NND threshold was based on the assumption that to have a chance make contact and develop chimeras, the distance between two individuals should be less than the size of an individual. As the average diameter of new settlers was 0.75 mm, 1.0 mm represented a distance of 0.25 mm between the hedges of the 2 closest settlers. Moreover, 1 mm was the value used in previous works (Amar *et al.* 2008; Shefy *et al.* 2020). Therefore, the NND threshold of 1 mm was used to classify the settled polyps into two categories (*has.close* function, R software): close contact polyps ($\text{NND} \leq 1.0$ mm), and not close contact polyps ($\text{NND} > 1.0$ mm, Fig. 1a).

Size

Basal diameter is widely accepted as a reliable size descriptor for *C. rubrum* (Marschal *et al.*, 2004). To measure the diameter, perpendicular pictures of the tiles were taken by a Sony DFW-X700 camera assembled on an Olympus SZ61 stereomicroscope at 2X magnification. Due to the asymmetry of the young colonies, the diameter was calculated by averaging the highest and lowest diameter values measured using the image analysis software Visilog6. Student's T-tests were applied to test the differences between the diameter of 3 months (December 2019, t_2) and 1 year old (September 2020, t_3) chimeric and non-chimeric individuals. The normal distribution of the data was verified through a Shapiro-Wilk normality test and visual analysis of the Q-Q-plot, whereas homoscedasticity was tested by the *var.test* function (R software).

Results

Of the 1688 larvae released in the experimental aquaria, 342 settled on the marble tiles and metamorphosed into a polyp, resulting in a 20% settlement success rate.

The distance of settled larvae from the nearest neighbor varied between 0.6 and 11.17 mm with an average

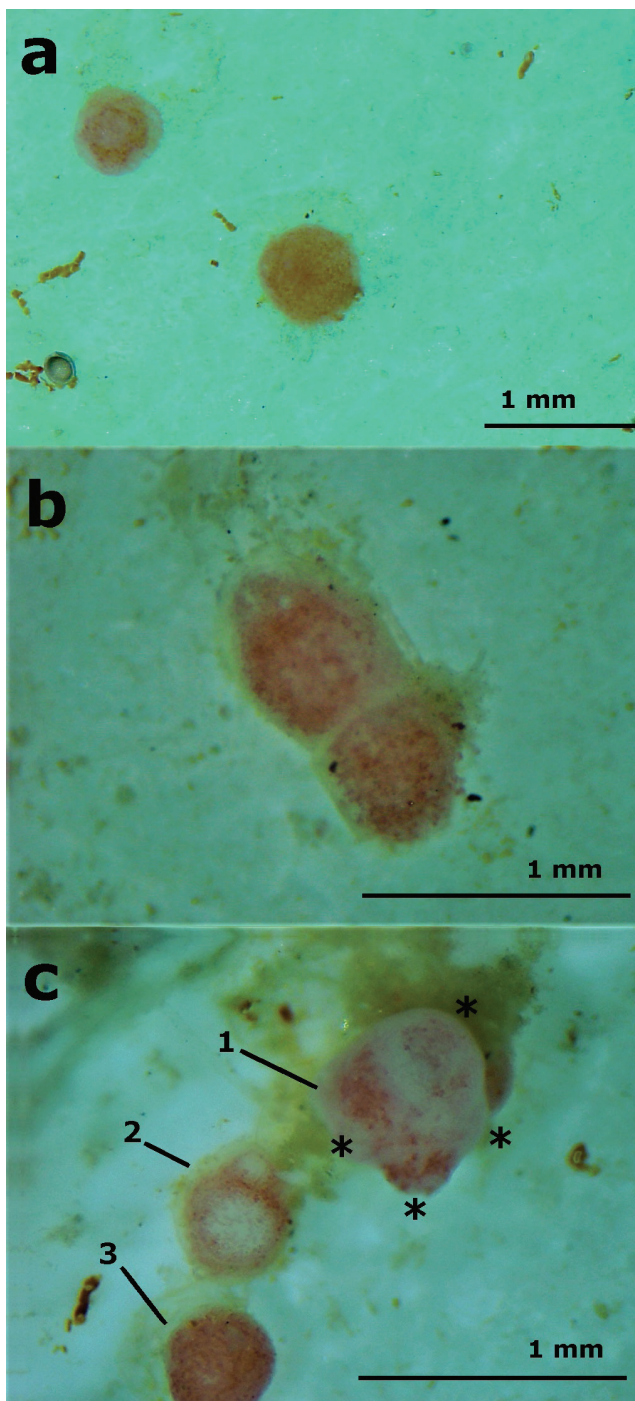


Fig. 1: Chimeric and non-chimeric *C. rubrum* individuals. a: two individuals that are not in close contact (distance > 1 mm). b: two individuals in close contact (distance < 1 mm) that started forming a chimera. c: a chimera composed of 4 different individuals (1) and two close contact individuals that did not end up forming a chimera (2 and 3). Black bars represent 1 mm length. Asterisks indicate the original 4 individuals that merged to form the chimera.

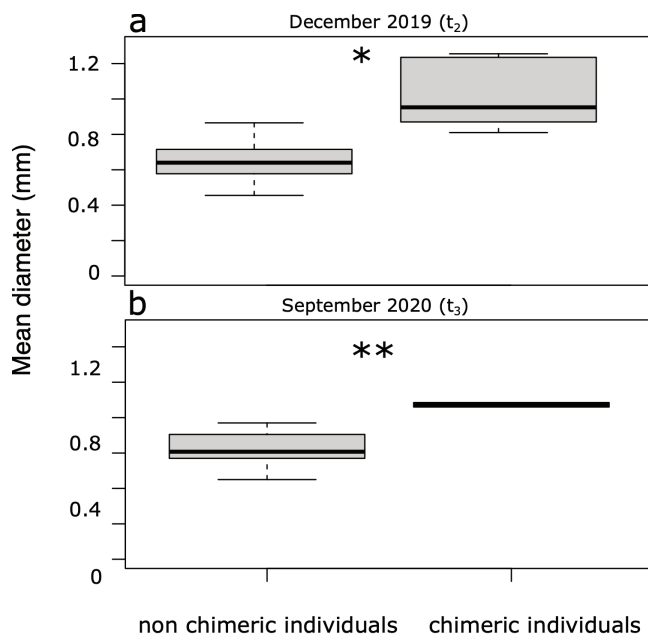


Fig. 2: Mean size of chimeric and non-chimeric individuals. a: mean size 3 months after settlement (December 2019; t_2). b: mean size 1 year after settlement (September 2020; t_3). Asterisks indicate statistically significant differences.

NND of 3.4 mm. Within the 342 settled individuals, 44 had an NND ≤ 1.0 mm (the threshold distance for defining the settled polyps as in “close contact”; see mat and met section), resulting in a 13% aggregation frequency. Three months after settlement, 32% ($n = 14$) of the “close contact” polyps formed 6 different chimeras composed of by 2 or more polyps (Fig. 1b, c). One year after settlement (t_3), only 33% of the chimeras survived ($n = 2$). The mean diameter of solitary polyps and chimeras at 3 months (t_2) was 0.73 ± 0.15 and 1.03 ± 0.32 mm, respectively, and 1 year after settlement (t_3), it was 0.61 ± 10 and 0.87 ± 22 mm, respectively. Results of the T-test showed that chimeric individuals were significantly bigger than non-chimeric ones at t_2 ($t = -4.5$, $df = 5.59$, $p < 0.005$; Fig. 2a) and that the difference was still significant at t_3 ($t = 10.7$, $df = 9.4$, $p < 0.001$; Fig. 2b).

Discussion

We reported the first observation of chimerism in Mediterranean red coral (*C. rubrum*). Our results highlighted that 13% of larvae settle at a very close distance, almost touching one another, suggesting a tendency towards an aggregated spatial distribution. Almost 1/3 (32%) of the close contact settlers (4% of all settled polyps) merged their tissues to form a chimera, suggesting that chimerism in *C. rubrum* is not a rare event. Moreover, 33% of the chimeras were still alive and in good health after 1 year from the chimeras’ formation.

The occurrence of chimerism is a well-known phenomenon that has been observed in numerous phyla (Buss, 1982). Several aspects of chimerism have been studied in cnidarians, including life history traits (e.g., growth rate

and survival, see Shefy *et al.*, 2020), genotypic diversity (Puill-Stephan *et al.*, 2012), and allogenic responses (Hidaka *et al.*, 1985). The majority of studies focus on the cellular mechanisms and genetic implications and are based on chimeras which formation is induced under experimental laboratory conditions (e.g., Rinkevich, 2004; Puill-Stephan *et al.*, 2012). Quantification of the natural frequency of this phenomenon, at least in cnidarians, is still scarce in adult colonies (Puill-Stephan *et al.*, 2009; Oury *et al.*, 2019) and, to our knowledge, there are no data for juveniles.

If the formation of a chimera represents a cost or a benefit is still debated. Some findings suggest that chimerism can provide competitive advantages, such as an increase in body size (Buss, 1982; Amar *et al.*, 2008), reduction of the Allee effect (Rinkevich *et al.*, 2016), increased resistance (Rinkevich, 2019), and reduced intra-specific competition (Hennige *et al.*, 2014). Conversely, several studies highlighted the potential costs of chimerism, such as somatic and germ cell competition (Rinkevich & Yankelevich, 2004; Amar *et al.*, 2008), slower growth (Barki *et al.*, 2002), and morphological re-absorption and necroses (Rinkevich & Weissman, 1987). Our results showed that chimeric individuals are almost 40% larger than non-chimeric ones, both 3 months and 1 year after settlement. In several coral species, the mortality of juveniles decreases with increasing size (Babcock 1985; Fitzhardinge 1988; Smith 1992). Therefore, a boost in growth could result in significant advantages linked to the reduction of the period of vulnerability due to a small size during early life stages. This observation is especially important for slow-growing species, such as *C. rubrum*, for which restoration plans based on sexual reproduction and increased survival should be preferred to direct adult colony transplantation.

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