

NOTE

Do chimeric sponges have improved chances of survival?

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ABSTRACT: It has been suggested that the capacity of fusion with both kin and genetically unrelated conspecifics to form chimeras (i.e. individuals with a mixture of genetically different cells) is evolutionarily retained in several phyla because the resulting organism obtains some selective advantages over non-chimeric conspecifics. Many demosponges are known to have fusible larvae that form young chimeric sponges, but the ecological and evolutionary significance of this phenomenon has seldom been investigated. It is reasoned here that if chimeras have a selective advantage, their formation will be expected to be favored by mechanisms promoting larval encounters at settlement. By using sibling larvae of the demosponge *Tedania ignis* in the laboratory, I tested the hypothesis that larvae show a natural tendency to aggregate and form chimeras. In a 50 d field-transplantation experiment using chimeric sponges obtained from the fusion of 2 sibling larvae, I also tested the hypothesis that size and survival are increased in chimeric individuals compared to non-chimeric ones and investigated the permanent versus transitory character of this chimerism. It was found that larvae did not show any significant tendency to settle spontaneously near siblings, and no fusion between siblings took place. When pairs of larvae were forced to settle in contact, fusion was, however, the outcome in all cases. It was also found that, although chimeric sponges were stable and about twice as large as non-chimeric sponges, they did not show increased survival. These results disprove the common argument that the capacity of fusion with conspecifics is evolutionarily retained in many sponges because the gain in size after fusion improves the chances of survival of the resulting chimeric individual.

KEY WORDS: Chimerism · Larval fusion · Recruitment · Sponge larvae · Demosponges

Tissue fusion between conspecifics is a well-documented event in many invertebrate phyla (Buss 1982). If fusion can occur between genetically different individuals (alogeneics), we might expect chimeras to form. Several benefits have been proposed for chimeras when compared with non-chimeric conspecifics. For example, fusion of either larvae or young individuals leads to an immediate size increase in the

resulting chimera. The increase of size may reduce the impact of mortality by partial damage, enhance the performance in competition for space, and increase reproductive output by lowering the onset of the first reproduction (Buss 1982, 1990, Grosberg & Quinn 1986, Rinkevich & Weissman 1987a). In addition, a chimera has a greater genetic variability than a genetically homogeneous individual. The gain in genetic variability is expected to confer wider ranges of physiological resistance (Rinkevich & Weissman 1987a). On the other hand, chimerism may involve some costs. For example, cell lineages within a chimera may compete for limited resources (Buss 1982, Rinkevich & Weissman 1987a). Besides, allogeneic fusion entails the risk that distinct genotypes in the chimera compete for positions in the germ line and that gametes produced in the chimera may disproportionately favor just one of its components (Buss 1982, Rinkevich & Weissman 1992, Pancer et al. 1995). In some cases, fusion between 2 genetically different individuals was found to be a transitory condition under ontogenetic control, because, after a variable period, most chimeras regressed by either separation or resorption of one of the partners (Rinkevich & Weissman 1987b, 1989, Buss 1990, Ilan & Loya 1990). It has also been suggested that transitory fusion may be highly advantageous, since the organisms acquire the benefits of fusion (i.e. increased size), but evade the costs of somatic cell parasitism (Buss 1990).

The occurrence of chimerism is recorded in at least 9 phyla in several kingdoms (Buss 1982), but most of the recent knowledge is from studies of Cnidaria and Tunicata. In sponges, the formation of chimeras in the field upon contact of adult individuals is still the subject of debate because of technical and interpretational problems in the empirical approach. Some histocompatibility studies have found that tissue fusion occurs exclusively in encounters between genetically identical individuals (isogenic encounters), whereas others

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reported that fusion may occur between genetically distinct (allogeneic) conspecifics (see Wulff 1986, Van de Vyver 1988, Grosberg 1988a and references therein). In contrast, there is irrefutable evidence from laboratory studies that sibling larvae of some sponges may settle together to form chimeric individuals (e.g. Warburton 1958, Fell 1974, Uriz 1982, Ilan & Loya 1990). The argument that fusion of larvae produces a chimeric sponge is based on the assumption that they are sexually produced, each therefore representing a new, unique genotype. Parthenogenetically produced larvae have been detected in a few species of corals (Stoddart 1983) and have been suggested for some sponges, though the latter remains unproved (Fell 1974, Bergquist 1978).

The capacity of fusion between sponge larvae has been known for a long time (e.g. Wilson 1907), but its ecological consequences and evolutionary significance have received little attention, with only 1 empirical study (Ilan & Loya 1990). In present study, I investigated chimerism in the demosponge *Tedania ignis*, the larvae of which fuse with each other when they are maintained at high density in the laboratory (Maldonado & Young 1996). It was reasoned that if chimeras resulting from fusion of sibling larvae have some selective advantage, their formation would be favored by mechanisms promoting larval encounters or aggregated settlement. Therefore, I examined the tendency of larvae to aggregate and fuse at settlement to form chimeras in laboratory conditions. In a 50 d field-transplantation experiment, I also tested the hypothesis that size and survival are increased in chimeric individuals when compared with non-chimeric sponges and investigated the permanent versus transitory character of this chimerism. The results are discussed in the light of potential benefits and costs of chimerism.

Material and methods. The sponge *Tedania ignis* is common in marine and brackish habitats in the Caribbean (Zea 1987). Ripe individuals were collected from a sponge-dominated community established between 0.5 and 2 m depth in the Indian River Lagoon, near the Fort Pierce Inlet, Florida, USA, in June 1995. Larvae were spontaneously released by ripe sponges after 1 to 4 d in aquaria. Additional data on adult abundance, recruitment and larval behavior of this species can be found in Jaekle (1995) and Maldonado & Young (1996).

The tendency of larvae to fuse at settlement was examined in a set of 30 polystyrene petri dishes (5 cm in diameter) filled with 30 ml of 0.45 μm filtered seawater and containing 2 sibling larvae each. Larvae were obtained from 3 ripe sponges. Dishes were maintained at room temperature (20 to 24°C) in a dark room until larvae settled. This prevented aggregation induced by light cues. Densities greater than 2 larvae per

30 ml of seawater were not considered, because previous field observations indicated that such densities are unlikely in the wild. Fusion occurs when 2 larvae settle in contact or close enough to establish contact during metamorphosis or immediately after. To calculate the likelihood of this event occurring in the dishes, larvae and early settlers were considered to be quadrats of 1 mm². This figure is slightly larger than the actual area projected by a crawling larva (750 \times 300 μm on average) and similar to that of an early (12 h old) settler. Hence estimates of contact probability should be regarded as conservative. As the bottom of a dish contains 1963 squares of 1 mm², the probability of a random settlement in a dish is $p_1 = 1/1963$. According to the method followed by Ilan & Loya (1990) the second larva will contact the first one only if it settles on top of the former or in one of the 4 neighboring quadrats. The probability of the second larva contacting the first one by chance is $p_2 = 5 \times p_1$. A total of 30 pairs of larvae were used in the experiment; random contact between the larvae is expected in $30 \times p_2 \times 100 = 7.64\%$ of the dishes. Any observed value significantly greater than this prediction indicates an aggregated settlement that may promote formation of chimeras.

I compared survival and body size between a set of young chimeric sponges (N = 28) and a set of non-chimeric sponges (N = 30) by rearing sponges in polystyrene petri dishes and transplanting them to the field 1 d after larval settlement. To obtain a chimera, I forced 2 sibling larvae to settle in contact, promoting larval encounters during the crawling phase immediately prior to settlement by producing a gentle water flux from a Pasteur pipette. Larvae were obtained from 3 ripe individuals. Dishes, containing a single either chimeric or non-chimeric sponge each, were attached to racks by rubber bands and placed facing downward under the docks at Link Port (Indian River Lagoon). This study site is a shaded habitat which a variety of sponges and other sessile filter-feeders also inhabit. Dishes containing sponges settled at corners were discarded. This facilitated subsequent sponge measurements, but also protected the analysis from undesirable, non-controlled effects (i.e. corners may potentially function as dish microhabitats where sponge survival is increased). Data on sponge survival, growth and sponge shape were recorded at 2, 5, 10, 20, 30 and 50 d after transplantation by taking the dishes to the laboratory. Sponge size was measured as the area projected by the sponge on the bottom of the container. Sponge shape (patch contour) was assessed by using the circularity index (C) of Turon & Becerro (1992), where C is the result of dividing the projected area of the sponge by the area of a circle with a perimeter equivalent to that of the sponge. These variables were measured by using a Microcomp image analysis sys-

tem linked to a Zeiss dissecting microscope. Measurements of projected area were assumed to be good descriptors of growth dynamics at early life-history stages, because young sponges are flat patches that show negligible growth in height for the first 2 mo. Sponge circularity was used to assess differences in body form between chimeric and non-chimeric sponges, and to describe the dynamics of the separation process, if any, between partners of a chimera.

Differences between the proportion of chimeric and non-chimeric individuals that survived after 50 d were tested by using the Z-test (Zar 1984). Differences in survival time (days) between chimeric and non-chimeric sponges were tested using the Cox *F*-test (Cox 1964). This method is the most powerful out of a variety of tests to compare survival time between 2 samples of small size (i.e. $N < 50$; Gehan & Thomas 1969, Lee 1980).

Differences in sponge size (area) and circularity as a function of sponge type (chimeric versus non-chimeric) and time were first analyzed by 2-way analyses of variance with repeated measures on factor time (ANOVAR). However, the assumption of circularity required for the ANOVAR was violated (Mauchly's sphericity test). The multivariate equivalent of this analysis (MANOVAR) was not applicable in this case either, because data did not meet the assumption of homogeneity of the variance-covariance matrix (Box *M*-test). Therefore, the repeated-measures size data and circularity data were finally analyzed using a randomization method that consisted of a 2-stage permutation process (Manly 1991). First, individuals were randomly reassigned to the levels of the sponge factor (chimeric versus non-chimeric). Then, readings for each individual were randomly rearranged among time levels. According to Manly (1991), the total between- and within-subject sums of squares (SS), as well as the overall total SS, remain unchanged after this randomization. The SS associated with each factor and their interaction is, therefore, a suitable statistic to test the significance of each effect. The randomization distribution of the SS for each factor and the interaction term (null hypothesis) was approximated by using 9999 random permutations of the data set plus the observed one. Then, an effect was considered to be statistically significant when the observed sum of squares value (SS_{obs}) was exceeded by less than 5% of the randomization sum of squares values (SS_{ran}). Data randomization was performed using a computer routine written in Turbo Pascal v6.0 by Turon et al. (1998). An interesting advantage of this non-parametric statistical approach compared to ANOVAR or MANOVAR methods is that sponges that were not present at all time levels (as a result of mortality) are included in the analysis. In these cases, permutation is done only

within those time levels comprised within the survival period of each particular individual (Turon et al. 1998).

The relationship between body size (projected area) and survival time was assessed by the Spearman rank correlation coefficient (Zar 1984).

Results. No chimeric sponge was obtained by spontaneous fusion of cosettled larvae in dishes containing 2 sibling larvae. Indeed, larvae settled relatively far from each other in all 30 dishes. However, when 30 pairs of sibling larvae were forced to settle in contact, they fused in all cases.

The percentages of chimeric and non-chimeric sponges surviving for more than 50 d in the field were 16.7 and 30%, respectively (Fig. 1a). According to a Z-test, these proportions are not significantly different ($Z = 0.892$, $p = 0.141$). Differences in survival time between sponge groups were also non-significant, according to a Cox *F*-test ($T_{chimeric} = 19.3$, $T_{non-chimeric} = 24.6$, $F_{56,60} = 1.274$, $p = 0.178$). Mortality patterns over time were parallel in both sponge groups. Mortality began after only 10 d in the field, peaked dramatically

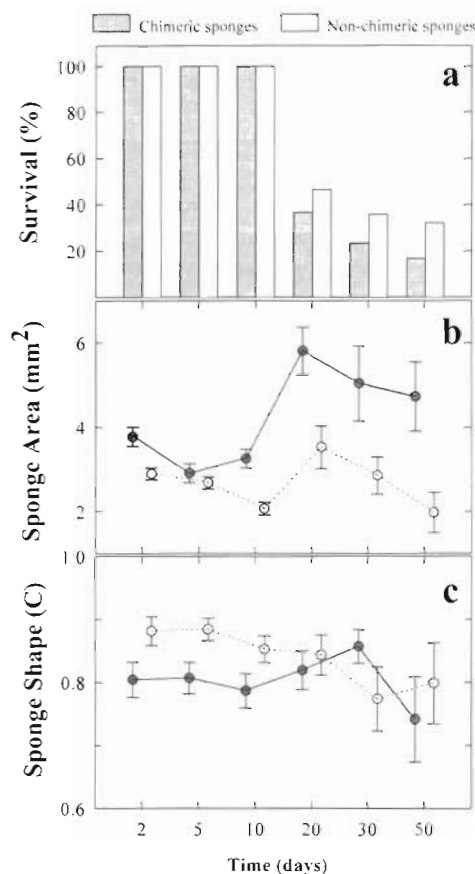


Fig. 1. *Tedania ignis*. (a) Survival (cumulative percentage), (b) values of area (mean \pm SE), and (c) values of the circularity index (*C*; mean \pm SE) for chimeric and non-chimeric sponges over time

Table 1. Results of the randomization analysis for the repeated-measures size data and circularity data. In both analyses, factors are sponge type (chimeric vs non-chimeric) and time (2, 5, 10, 20, 30, 50 d). The $SS_{ran} > SS_{obs}$ columns are the percentage of randomization sum of squares (SS_{ran}) that exceed observed sum of squares (SS_{obs}) for each factor and their interaction. Statistically significant effects at the 95 and 99% confidence levels are indicated by * and **, respectively

Source of variation	Size $SS_{ran} > SS_{obs}$	Circularity $SS_{ran} > SS_{obs}$
Sponge type	0.06**	1.42*
Time	<0.01**	36.04
Sponge type × Time	4.74*	99.88

in the subsequent 10 d period, then decreased gradually with time (Fig. 1a).

Randomization analyses for the repeated-measures size data indicated that chimeric sponges were significantly larger than non-chimeric sponges, and that size was also dependent on time (Fig. 1b, Table 1). A statistically significant interaction term revealed that differences in size between chimeric and non-chimeric sponges depended on level (Table 1). A significant increase in mean size took place in both sponge groups between 10 and 20 d, coincidental with the onset of mortality. Maximal size was reached in both sponge groups 20 d after transplantation (Figs. 1b & 2). Then, most sponges shrank for the following 30 d. Mean size and time were not linearly related (Fig. 2). According to the Spearman rank correlation coefficient, size and survival time were clearly unrelated both in chimeric and non-chimeric individuals. The periods of negative growth reflected internal reorganization, as tissues became denser and secretion of spicules increased. It is noteworthy, however, that many individuals shrank progressively before death (Fig. 2).

Chimeras appeared to be stable organisms, as no sponge split into 2 distinct individuals during the 50 d of study. Both types of sponge maintained a nearly circular shape throughout the study (Fig. 1c). Nevertheless, chimeric sponges had slightly lower mean values of circularity index than those of non-chimeric sponges, irrespective of time (Table 1).

Discussion. It was predicted that, if chimeric sponges had selective advantages, larval behavior would have evolved to favor aggregated settlement and subsequent fusion of individuals. I found, however, that sibling larvae of *Tedania ignis* did not show any significant tendency to aggregate spontaneously at settlement and form chimeric sponges. Moreover, the study of chimeras obtained under experimental conditions revealed that, although chimeric individuals were stable and about twice as large as non-chimeric sponges, they did not show increased survival in the field. The causes

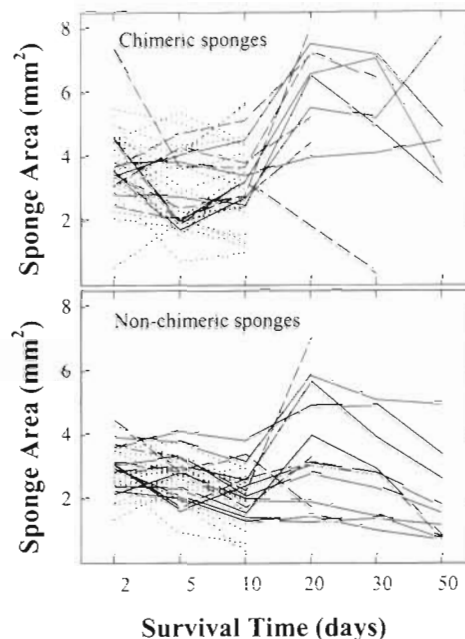


Fig. 2. *Tedania ignis*. Evolution of body size (projected area in mm^2) over time for each sponge individual. Solid, dashed and dotted lines indicate individuals that survived throughout the 50 d study, individuals that died between Days 20 and 50, and individuals that survived less than 20 d, respectively

of mortality remained unclear. Nevertheless, the finding that many sponges shrank progressively before death (Fig. 2) suggests that physiological failure may have been the major cause of mortality in both chimeric and non-chimeric sponges. According to my observations, predation and overgrowth by spatial competitors were apparently minor mortality factors, at least at the study site and during the period studied. The present results suggest that, if the chance of survival among young sponges is size-dependent (e.g. Ayling 1980), the initial size required to confer any advantage to the chimeric individual must be larger than that resulting from the fusion of 2 larvae. However, it is unlikely that more than 2 sibling larvae of *T. ignis* settle together and fuse in the field. The fact that a ripe individual of *T. ignis* needs multiple release events to expel the entire brood (Maldonado & Young 1996) suggests that the mechanism of larval release has not evolved to favor encounters between sibling larvae. In addition, larval release in this species is known to be asynchronous at the population level and to last at least 2 mo. The finding that *T. ignis* larvae did not tend to aggregate at settlement and form chimeras is therefore consistent with the observation that survival appears not to be increased among young chimeric sponges. These results are also consistent with a recent laboratory study on the compound ascidian *Botryllus schlosseri*, in which chimeric colonies did not show higher survival than

non-chimeric colonies (Rinkevich & Weissman 1992). Similarly, settlement of *B. schlosseri* larvae on the parental colony resulted in increased offspring mortality (Rinkevich & Weissman 1987c).

It is usually reasoned that since the capacity to fuse with siblings must be maintained by some selective advantage, the benefits of chimerism should outweigh the potential costs of somatic cell parasitism (Buss 1982, Grosberg 1988a, Rinkevich & Weissman 1992). Most of the proposed benefits (except for physiological resistance) are derived from the immediate increase in size. However, my results do not support the hypothesis that chimerism is mainly maintained in sponges by size-derived advantages. There is only 1 other empirical study of chimerism in sponges (Ilan & Loya 1990). These authors reported that larvae of an unidentified species of sponge (*Chalinula* sp.) showed a significant tendency to aggregate and form chimeras with both sibling and genetically unrelated larvae in laboratory conditions. Unfortunately, they did not test for differences in body size or survival between these chimeric and non-chimeric sponges.

Indeed, chimerism is just one of the possible alternatives that may lead to the advantages attributable to an immediate increase in body size. Most recent discussions on the costs and benefits of chimerism have overlooked the possibility that similar size-derived advantages may also be obtained by non-chimeric individuals or colonies resulting from fusion of either asexual propagules, parthenogenetic larvae or isogenic body fragments (Van Duyl et al. 1981, Feldgarden & Yund 1992). In addition, an asexual mechanism prevents the threat of germ cell parasitism. Therefore, the capacity to fuse with siblings may have been maintained by some selective advantages unrelated to size-derived benefits. It would be paradoxical if 2 opposed biological conditions such as chimerism and clonemating were maintained by the same selective advantage. Moreover, the chimeric individuals may even not be larger than the non-chimeric conspecifics. For example, some long-term observations have demonstrated that chimeras of the compound ascidian *Botryllus schlosseri* were not larger than non-chimeric colonies at the end of the study, since partial resorption of 1 of the 2 colonies occurred after several months (Rinkevich & Weissman 1987b, 1989, 1992, Pancer et al. 1995).

In the present study, I did not test the hypothesis that the lag period needed for the onset of first reproduction may be shortened in chimeras as a result of the increase in size. This may be a possible explanation for the evolutionary retention of chimerism in some cases, but it is not a plausible hypothesis for *Tedania ignis*. Under such a hypothesis, fusion of 2 individuals is expected to have a synergistic effect on the growth rate of the resulting organism. So, chimeras should be

far larger than non-chimeric sponges after 50 d. The observed difference in mean size between chimeric (4.19 mm²) and non-chimeric (2.62 mm²) sponges after 50 d did not appear to be large enough to ensure the hastening of the reproductive onset. Moreover, size patterns over time were virtually identical in chimeras and non-chimeric sponges (Fig. 1b). The suggestion that chimerism may lower the onset of reproduction has been tested twice. Both studies were based on the same organism, *Botryllus schlosseri*, and both reported conflicting results. In a 2 yr study, Grosberg (1988b) found that age at first reproduction and survival were correlated with colony size. In contrast, a 6 mo laboratory study by Rinkevich & Weissman (1992) concluded that the formation of chimeras (between siblings and non-related genotypes) does not improve survival, growth rate or onset of reproduction when compared with isolated colonies. Therefore, it appears that this theoretical advantage proposed for chimeric organisms needs further testing.

An alternative hypothesis to explain the evolutionary retention of chimerism in sponges is that the capacity of fusion is a consequence of the absence of immune response in larvae and early juveniles (Ilan & Loya 1990). Juvenile immune incompetence is known in vertebrates (Cooper 1976) and has also been suggested in cnidarians (Hidaka 1985, Shenk & Buss 1991, and references therein). The transitory absence of either allorecognition capacity or immunocompetence allows initial fusion between allogeneics. However, the outcome of most of these chimeras appears to be death, separation between partners or resorption (Rinkevich & Weissman 1989, Shenk & Buss 1991, Pancer et al. 1995). In the present study, I did not detect any sign of separation or resorption in the chimeras. Therefore, if juvenile lack of allorecognition capacity is the case in *Tedania ignis*, the lag period required for the activation of the allorecognition system must be at least 2 mo.

Finally, chimerism may be just a case of intraspecific parasitism by exogenous germ cell lines. The coexistence of 2 germ cell lineages has been demonstrated in chimeric tunicates and suggested in some other groups, such as cellular slime molds, myxomycetes, fungi, sponges and hydroids (Pancer et al. 1995 and references therein). Chimerism would be maintained by the same selective forces acting in interspecific parasitisms, but now operating on lines of totipotent cells (e.g. sponge archeocytes or tunicate stem cells), rather than at the individual or colony level (Tuomi & Vuorisalo 1989, Pancer et al. 1995).

The results of this 50 d study and the literature available do not support one particular hypothesis out of the pool of potential explanations for the fact that larvae of *Tedania ignis* and many other sponges retain the capacity of fusing with siblings. The most common

argument used to explain the evolutionary persistence of chimerism in invertebrates is that chimeras obtain fitness benefits as a result of the increase in both body size and genetic variability inherent to chimerism. However, the present study indicates that this is not the outcome in *T. ignis*. This result is consistent with a small, but growing, body of literature leading to the notion that the outcome of a chimera and the mechanisms controlling its formation are distinct in different organisms. Therefore, chimerism may have evolved separately and be retained for different selective advantages, depending upon particular cases.

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