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Author(s): Nanette E. Chadwick-Furman and Irving L. Weissman
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Life history plasticity in chimaeras of the colonial ascidian *Botryllus schlosseri*

NANETTE E. CHADWICK-FURMAN1 AND IRVING L. WEISSMAN2

1 Interuniversity Institute for Marine Science, P. O. Box 409, Eilat, Israel
2 Departments of Pathology, Developmental Biology, and Biology, Stanford University, Stanford, California 94305, U.S.A.

SUMMARY

Colonies of the ascidian *Botryllus schlosseri* may fuse with kin to form chimaeras which vary their life histories depending on environmental conditions. We placed chimaeric colonies of this species in Monterey Bay, California, U.S.A., where they received planktonic food continuously. In the field, chimaeras grew rapidly, attained large sizes, and produced many eggs. They formed compact disc-shaped colonies in which genotypic composition remained stable throughout their lifespan. In most cases, genotypic partners in field chimaeras senesced and died synchronously. We also cultured genetically identical replicates of the same chimaeras under laboratory conditions, where they were fed once daily. In the laboratory environment, chimaeras grew slowly, shrank, and fragmented. Most genotypes in chimaeric colonies produced significantly fewer zooids and eggs in the laboratory than they did in the field. Somatic cell parasitism, in the form of resorption of tissues of one genotype by the other, occurred mainly in the laboratory environment, and not in the field. The phenomenon of resorption may thus be a dispensable strategy of fused genotypes depending on environmental conditions. Genotypes in field chimaeras may grow and reproduce rapidly because of the non-limiting food resources available. These data demonstrate that chimaeras of *B. schlosseri* have extremely plastic life histories, and employ different strategies depending on the environment.

1. INTRODUCTION

Chimaeric colonies consisting of two or more distinct genotypes in one soma are known to form in several types of sessile marine invertebrates (Buss 1982). In some cases, one of the genotypes may parasitize the somatic tissue of the other, possibly to produce more of its own offspring (Buss 1982; Rinkevich & Weissman 1992). Barriers to tissue fusion between distinct genotypes are thought to have evolved partly in response to the danger of somatic cell parasitism in chimaeras (Buss 1982; Rinkevich & Weissman 1987b, 1992; Grosberg & Quinn 1989). The origins of the Major Histocompatibility Complex (MHC), which controls vertebrate allograft immunity, may be linked to this ancestral requirement for non-fusion with distantly related genotypes (Scofield et al. 1982; Weissman et al. 1990; Rinkevich & Weissman 1992).

The life history patterns of chimaeric colonies of ascidians (ancestral chordates) have been described from chimaeras cultured only under laboratory conditions. These studies have documented fragmentation of chimaeric colonies, retreat growth, and resorption of the tissue of one genotype by the other (Rinkevich & Weissman 1989; Sabbadin & Astorri 1988). Colonies of the common ascidian *Botryllus schlosseri* may, however, exhibit quite different life histories under different environmental conditions (Grosberg 1988; Chadwick-Furman & Weissman 1995). It is important to understand the degree of plasticity of life history traits in *B. schlosseri* chimaeras, to make accurate predictions concerning the evolution of somatic cell parasitism, alloimmune responses, and other important traits in these ancestral chordates.

We demonstrate here that genetically identical replicates of chimaeras of *B. schlosseri* are extremely plastic in their patterns of growth, reproduction and morphology. Field-cultured chimaeras grow to large size and are genetically stable. Phenomena observed in laboratory replicates, such as resorption, do not appear to occur in most field chimaeras. These results are discussed in terms of implications for the evolution of life history patterns in chimaeric organisms.

2. MATERIALS AND METHODS

This study was conducted during summer 1991 at the Hopkins Marine Station of Stanford University, Pacific Grove, California, U.S.A. Colonies of the ascidian *Botryllus schlosseri* from a large stock of genetically defined laboratory cultures were tested for their fusibility (for review, see Rinkevich & Weissman 1992). Two genotypes which were known to fuse and which were of sufficiently large colony size, were sub-cloned into eight fragments each, using standard laboratory procedures (Rinkevich & Weissman 1987a; Sabbadin & Astorri 1988). Each fragment contained 9-17 zooids. The eight replicates of the first genotype, termed here genotype A, were allowed to attach to glass plates in the laboratory. A fragment of a fusible partner, genotype B, was
positioned within 1–2 mm of contact from each of the eight fragments of genotype A, and allowed to attach to the plate. A second set of eight replicate pairs was set up with other compatible colonies, genotypes C and D. Thus, in total we set up eight replicates of each of two compatible pairs, obtained from four distinct, single-genotype colonies (A, B, C, and D).

Within one week, all fragments had fused with their partners and fully attached to the culture plates. Then, four replicates of each fused chimaera (AB and CD) were transplanted to the field site, whereas the other four were maintained in laboratory culture. The field site was at the Monterey Marina, at approximately one metre depth in hanging racks as described by Boyd et al. (1986) and Chadwick-Furman & Weissman (1995). B. schlosseri grows abundantly in this habitat, and may dominate submerged surfaces in the marina during certain times of year (Chadwick-Furman & Weissman 1995). The laboratory replicates were cultured at Hopkins Marine Station using standard procedures. In the laboratory, the colonies were fed once per day with commercial invertebrate food (Liquify) and cultured algae (Dunaliehia tertiolecta, Thalassiosira pseudonana, and T. cloe), and maintained in aerated standing seawater, unchanged once per week, at ambient sea temperatures (after Boyd et al. 1986; Rinkevich & Weissman 1987a, 1989).

All 16 chimaeras were examined under a dissecting microscope every 4–7 days for the duration of their lifespans (maximum five months). The field replicates were removed from the field site for less than three hours during each observation, and were kept constantly submerged in seawater. During each observation period, we recorded for each genotype the number of zooids, systems (= groups of zooids), buds per zooid (budding process described in Mukai & Watanabe 1976), and eggs, and also the shape and condition of the entire chimaera (figure 1). The zooids belonging to each genotype were distinguished by: their relative positions in the chimaera, the number of buds they produced, and in some cases, their colour patterns (see figure 1).

To analyse shape differences among colonies, we traced the outline of each chimaera at maximum size, then measured its total surface area and length of perimeter. We then compared the ratio of surface area to perimeter length, which is an indicator of compactness, for chimaeras cultured in the laboratory versus the field environment.

3. RESULTS
(a) Field environment

All replicates of chimaeras AB and CD grew rapidly under field conditions (see figures 2, 3). In all cases, genotypes A and C grew at faster rates than did their respective partners, genotypes B and D. Consequently, genotypes A and C attained significantly larger sizes in the field than they did in the laboratory (Mann–Whitney U-tests, $U = 16, p < 0.05$ for both) but genotypes B and D did not ($U = 9$ and $14$ respectively, $p > 0.05$) (see figure 4a). In one field replicate, genotype D shrunk and died three weeks before its partner did (see figure 3).

Under field conditions, the chimaeras formed compact, rounded disks with closely-spaced zooids (figures 1, 5). There was no morphological separation between the partners in each chimaera, and they did not disconnect at any time after fusion. The ratio of surface area to perimeter, an indicator of compactness of shape, was significantly higher in field versus laboratory-raised chimaeras (Mann–Whitney U-test, $U = 57, p < 0.01$). Most field chimaeras were stable, in that both partners grew continuously and died simultaneously (figures 2, 3).

Most field-raised genotypes produced large numbers of eggs (see figure 4b). Sexual reproduction started at 2–3 weeks after fusion, and then each genotype produced one clutch of eggs per week until the end of
the lifespan (at least four weeks). Each replicate still contained a clutch of eggs at death. Genotypes A, C, and D produced significantly more eggs in the field than they did in the laboratory environment (Mann–Whitney U-test, U = 16, p < 0.05 for all), but genotype B did not (U = 9, p > 0.05). Field chimaeras had short lifespans of 6–9 weeks, which in most cases ended in the synchronized senescence and death of both partners (figures 2, 3).

(b) Laboratory environment

Under laboratory conditions, chimaeras grew slowly and then shrank gradually (see figures 2, 3). Genotypes A and B reversed their relative rates of growth and maximum sizes from those observed in the field (figure 4a). In the laboratory, genotype A grew more slowly than did B, and in most cases died before B (figure 2). In all laboratory-cultured chimaeras of CD, genotype D shrank and died long before its partner genotype C (figure 3). The maximum sizes attained by genotypes in the laboratory were relatively small (figure 4a).

In the laboratory environment, chimaeras formed spread-out colonies which produced finger-like extensions along the substratum (see figure 5). Two replicates of chimaera AB fragmented at 9–10 weeks after fusion, leading to complete disconnection between the genotypic partners (two arrows in figure 2). Laboratory chimaeras were significantly less compact in shape than were those cultured in the field environment (see statistics above).

Chimaeras did not reproduce continuously in the laboratory. Genotypic partners began to produce eggs in a non-synchronized, staggered fashion at 1–3 weeks after fusion, continued intermittently with lapsed periods of up to six weeks, and completely ceased reproduction at least three weeks before death. Most genotypes produced significantly fewer eggs in the laboratory than they did in the field (statistics above). Genotypes A and B reversed the relative number of eggs they in the laboratory versus the field (figure 4b).

Laboratory-raised chimaeras had relatively long lifespans of 18–19 weeks. In most cases they died following several weeks of gradual shrinkage (figures 2, 3). One member of the chimaera often died before the other, and was resorbed (see figures 2, 3, 5). Thus, partners did not senesce synchronously and or immediately following rapid growth and reproduction, as observed in field chimaeras. The overall condition of laboratory colonies deteriorated for several weeks before death.
4. DISCUSSION

We present here the first description of chimaeric tunicates grown under field conditions. Our observations here (and in additional studies on more than 40 chimaeras grown in Monterey Bay from newly-settled oozoids, Chadwick-Furman & Weissman in preparation) indicate that in most cases the colonial ascidian Botryllus schlosseri produces stable chimaeras in the field. In other organisms, chimaeras that form between compatible individuals also are usually stable (Buss 1982). Chimaeras cultured here in the field environment show significant differences from genetically identical replicates maintained in the laboratory. Some genotypes even reverse their relative growth and reproductive rates depending on the environment. Chimaeras of B. schlosseri thus appear to be extremely plastic in their expression of life history traits.

The main environmental factors that differ between laboratory and field environments include levels of water motion and the presence of natural predators, parasites and other organisms. In addition, the frequency of food delivery and the diversity of food types are lower in the laboratory than in the field (Brunetti & Copello 1978; Boyd et al. 1986; Chadwick-Furman & Weissman 1995). One of the main causes of differences in growth, reproduction and stability between laboratory and field chimaeras may be food limitation in the laboratory. Previous laboratory studies on chimaeric growth in B. schlosseri have all used similar once-daily feeding regimes (Sabbadin & Zaniolo 1979; Rinkevich & Weissman 1987a, 1989; Sabbadin & Astorri 1988; Rinkevich et al. 1994).

Figure 4. Comparison of two chimaeras (AB and CD) of the colonial ascidian Botryllus schlosseri, grown under field versus laboratory conditions. (a) Maximal size attained by each genotype, in number of zooids. (b) Egg production by each genotype. Bars represent means plus positive standard deviations. Four replicates of each chimaera were cultured in each environment. Note that the relative size and egg production of genotypes A and B reversed in the two environments.

Figure 5. Drawings traced from photographs of chimaeras of the colonial ascidian Botryllus schlosseri. Upper: Chimaera grown in the field in Monterey Bay. Lower: Chimaera grown in the laboratory, modified after Rinkevich & Weissman (1987b). One of the genotypic partners in each chimaera is shaded. Note that in the laboratory chimaera, the unshaded zooids are growing in finger-like extensions, and the shaded zooids are undergoing shrinkage and resorption. Scale bars = 5 mm.
Sabbadin (1969) noted that field colonies of this species usually die soon after transfer to the laboratory. In contrast, when laboratory colonies are moved to the field environment, they often improve in condition and increase their growth rate (N. E. Chadwick-Furman, K. Ishizuka & K. Palmeri, personal observation). Brunetti & Copello (1978) noted similar differences between Mediterranean B. schlosseri colonies grown in the laboratory versus the field, and also implicated food quality as the main cause. Controlled experiments by Grosberg (1988) have shown that feeding level may significantly affect the expression of life history traits in B. schlosseri.

The previously described phenomena of resorption, disconnection and retreat growth in chimaerans of B. schlosseri (Sabbadin & Astorri 1988; Rinkevich & Weissman 1989; Buss 1990) appeared in the present study to be limited mainly to the laboratory environment. Given variation in their expression with environment, these phenomena may represent strategies to increase reproductive output under conditions of resource limitation. Cell lineages within chimaerans may compete strongly with each other when resources are limited (Buss 1982), as evidenced by the resorption of one genotype by another. Certainly, resorption of fusible B. schlosseri colonies occurs with a high frequency in the laboratory (Rinkevich & Weissman 1987a; Sabbadin & Astorri 1988), and could conceivably result in the germ cells of one partner parasitizing the somatic tissues of the other partner. In this speculative circumstance, resorption could result in a means by which the germ cell donor increases low reproductive output. When food resources are not limiting for colony growth, as in isolated field chimaerans supplied with continuous planktonic food, it is possible that genotypes within chimaerans compete less or not at all. This would require an active process wherein the cellular and humoral elements involved in allorecognition and/or response would be inhibited, as in self-tolerance.

There are several limitations to interpretation of the data presented here. First, levels of egg production reported here for genotypes in chimaerans may not be accurate. In B. schlosseri, eggs and ooocytes are known to migrate through the blood stream between zooids (Sabbadin 1969; Sabbadin & Zaniolo 1979; Rinkevich & Weissman 1987b), and so the eggs observed within a zooid may in fact have migrated from elsewhere in the colony. To clearly establish the relative reproductive rates of partners in chimaerans of this species, genetic marker studies of the offspring must be made, as done by Sabbadin & Zaniolo (1979). Second, the sample sizes presented here are small; only eight replicates of each of four genotypes were tested, and so it is difficult to extrapolate. Our results, however, are consistent with those from other studies on chimaerans cultured under laboratory versus field conditions. As noted above, data from more than 40 other chimaerans of B. schlosseri that formed from fusion between newly settled oozoids, show the same patterns of rapid growth, large size and chimaeric stability in the field (Chadwick-Furman & Weissman, unpublished results). In the latter study, no cases of resorption were observed in chimaerans grown during all four seasons of the year in the field. The life histories of the field chimaerans here are similar also to those of single-genotype colonies grown in Monterey Bay (Carwile 1989; Chadwick-Furman & Weissman 1995). In contrast, studies on hundreds of B. schlosseri chimaerans in the laboratory have documented consistent patterns of slow growth, shrinkage, disconnection and resorption (reviewed by Rinkevich & Weissman 1992). The few cases in which both partners in a laboratory chimaera have been observed to senesce and die simultaneously, represent a small minority (less than 10%) of post-fusion outcomes under laboratory conditions (Rinkevich & Weissman 1989; Rinkevich et al. 1992).

In summary, the data presented here, together with those from other studies, indicate that chimaeric colonies of the ascidian Botryllus schlosseri may vary their life history traits widely depending on environmental conditions. Such variation may be adaptive, in that the resulting strategies may allow genotypes to maximize their fitness in different environments. When resources are limited, some fused genotypes may parasitize the tissues of their chimaeric partners, whereas others disconnect to avoid being parasitized. When food resources are not limited, as may occur at the Monterey Bay field site, both partners in a chimaera may grow and reproduce as quickly as their respective genomes allow. These contrasting strategies may form the basis for the different growth patterns of B. schlosseri chimaerans observed in laboratory versus in field environments (see figures 2, 3, 5).

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