Reference: *Biol. Bull.* **205:** 133–143. (October 2003)

Effects of Allogeneic Contact on Life-History Traits of the Colonial Ascidian *Botryllus schlosseri* in Monterey Bay

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Abstract. The formation of chimeric colonies following allogeneic contact between benthic invertebrates may strongly affect colony fitness. Here we show that, in a field population of the colonial ascidian *Botryllus schlosseri* in Monterey Bay, California, more than 20% of all colonies occur in allogeneic contact with conspecifics. We experimentally assessed the effects of allogeneic contact on the following life-history traits under natural field conditions: growth, age and size at first reproduction, and egg production (fecundity). When compared with isolated colonies, and in some cohorts also with colonies that rejected allogeneic neighbors, colonies that fused with neighbors incurred reduced fitness in terms of most life-history traits measured. We propose that one of the benefits of precise allorecognition is that, in fused colonies, it limits the unit of selection to chimeric individuals composed of closely related kin.

Introduction

Tissue fusion and chimera formation between allogeneic individuals occurs in sessile invertebrates such as sponges (Ilan and Loya, 1990), corals (Chadwick-Furman and Rinkevich, 1994; Hidaka *et al.,* 1997; Frank *et al.,* 1997), and protochordate ascidians (Chadwick-Furman and Weissman, 1995a; Rinkevich, 1996). The formation of chimeras between kin may confer benefits on colonial invertebrates due to increased body size, early onset of sexual reproduction, and increased survival following partial predation (Buss, 1982; Grosberg and Quinn, 1986; Sabbadin, 1994). However, fusion of different genotypes also may come at a cost to individuals, in terms of germ and somatic cell parasitism (Stoner and Weissman, 1996; Rinkevich, 1996; Stoner *et al.,* 1999).

Studies of fused allogeneic colonies of the protochordate *Botryllus schlosseri* have shown that the eggs of one partner may be retained and brooded by the other partner over several reproductive cycles (Sabbadin and Zaniolo, 1979). In laboratory studies, fusion between allogeneic colonies of *B. schlosseri* leads to costs rather than benefits in terms of several fitness parameters (Rinkevich and Weissman, 1987, 1992a, b); indeed an inevitable result of such fusion is the death and resorption of all zooids (colonial units) of one colony, and the survival of the zooids of the other colony for up to many weeks after fusion (Rinkevich and Weissman, 1992a, b). In nature, however, resorption is not the inevitable conclusion of fusion prior to the onset of reproductive competence (Chadwick-Furman and Weissman, 1995a). Further, the resorbed partner also may parasitize the germ cell line of the resorbing partner in chimeras under both laboratory and field conditions (Pancer *et al.,* 1995; Stoner and Weissman, 1996; Stoner *et al.,* 1999). Thus, the genetic composition of chimeric colonies in nature may be more complex than previously observed in the laboratory.

Previously we reported on seasonal variation in life history traits of *B. schlosseri* colonies in a field population in Monterey Bay, California (Chadwick-Furman and Wiessman, 1995b). Here we determine natural frequencies of allogeneic contact in the same field population, and assess the resulting impacts on life-history traits in this colonial ascidian. We also describe the morphology and stability of chimeric colonies under natural field conditions.

Received 3 July 2002; accepted 8 July 2003.

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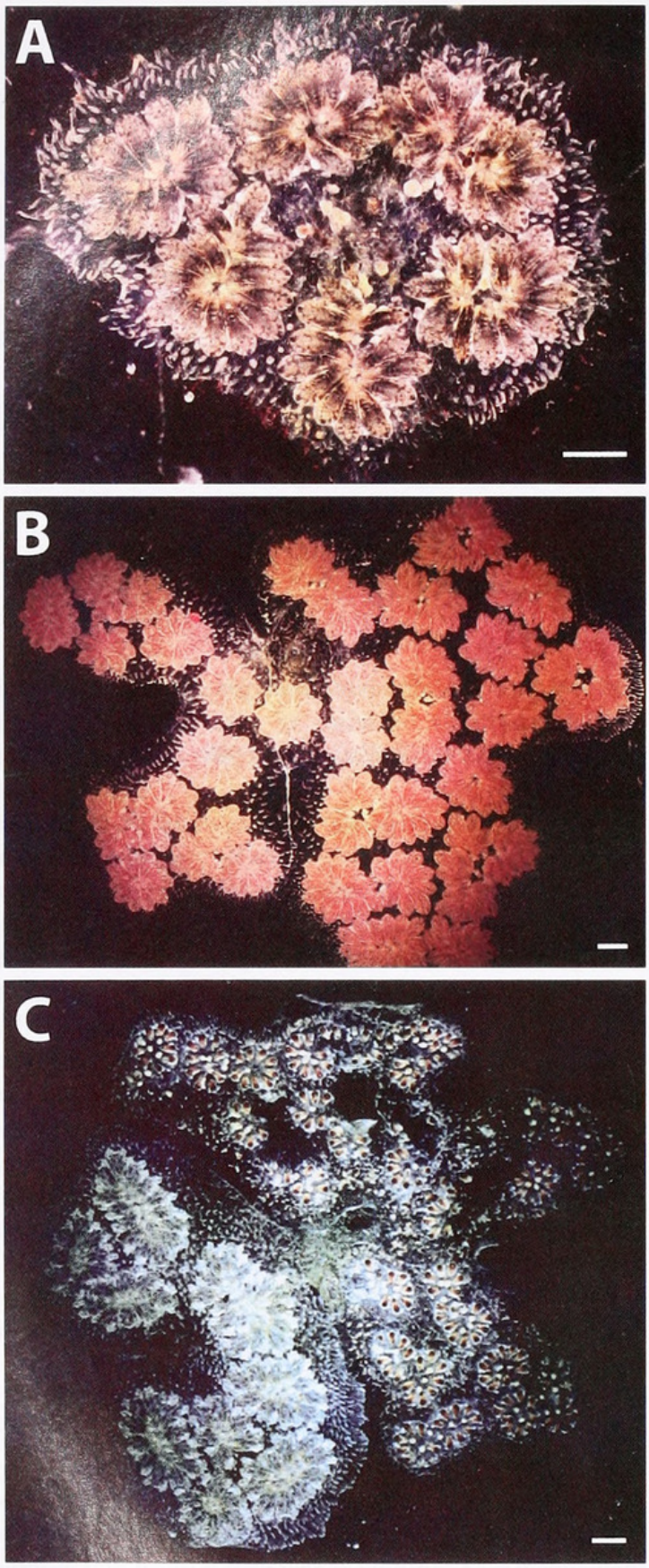

Figure 1. Colonies of the ascidian *Botryllus schlosseri* that were grown under three types of allogeneic contact conditions in Monterey Bay, California. Scale bars = 5 mm. (A) An isolated colony at 49 days old, consisting of 70 clonal units, termed zooids, that are arranged into six circular groups (systems) of 10–14 zooids each. The zooids are embedded in a clear gelatinous tunic and connected by a closed circulatory system. Bulbous ampullae, or sacs of the circulatory system, are visible around the perimeter of the colony. This colony settled in May 1990, began sexually producing eggs in August, and produced a total of 683 eggs in four clutches before it died in September. (B) Fused chimeric colony at 142 days old. The left genotype, according to developmental characters (see text), consists of 151 zooids; the right gonotype, which is slightly darker, consists of 215 zooids. The line of fusion of their tunics is visible at center. Both members of this chimera settled in October 1990, came into contact and fused in January 1991, and died simultaneously in March 1991 at 149 days old, without having produced any eggs. (C) Pair of rejecting colonies at 68 days old. Both colonies settled in May 1990, and came into contact and rejected during June 1990. Along their interacting borders at center, 25 pairs of blood-system ampullae are in allogeneic tissue contact. The right colony, which consists of 129 decaying zooids, is in the process of senescing and dying. The left colony, which consists of 89 zooids, died one week later at 75 days old. Neither colony produced eggs.

Materials and Methods

Under field conditions, individuals of the cosmopolitan ascidian *Botryllus schlosseri* pallas form compact, disc-shaped colonies (Fig. 1a), which occur in protected shallow marine environments, such as bays, harbors, and marinas, in temperate areas of both the northern and southern hemispheres (Chadwick-Furman and Weissman, 1995b, and references therein). Our experimental studies were conducted in the Monterey Municipal Marina, Monterey Country, California (36°37.4′N;121°54′W), where colonies of *B. schlosseri* are a dominant component of the fouling community on hard submerged surfaces (Chadwick-Furman and Weissman, 1995b).

We observed colonies of *B. schlosseri* on submerged columns and docks in the Monterey Marina at depths from the surface to 1 m and determined the frequencies of natural contacts between these colonies and other encrusting macroorganisms. This survey was conducted during November 1990, in the season of low abundance of fouling organisms in the marina (Boyd *et al.*, 1986; Carwile, 1989); thus our estimates represent minimal contact rates. Three hundred and nine colonies of *B. schlosseri* were observed in the marina for determination of their contact status.

To test the effects of allogeneic contact on life-history traits in *B. schlosseri,* we set up three treatments using each of four cohorts of newly settled offspring from field-collected colonies. The four cohorts settled on 19 May 1990, 3 July 1990, 15 October 1990, and 25 January 1991 (after Chadwick-Furman and Weissman, 1995b). In each cohort, newly settled, one-system colonies of 1–7 zooids each (= one circular system of zooids, Fig. 1a), were either (1) isolated on plates, (2) placed in incompatible pairs that rejected each other, or (3) placed in compatible pairs that fused. We distinguished between fusible and incompatible pairs by placing the small, one-system colonies into contact and observing the outcome. We used only colony pairs that established contact during the one-system stage of develop-

ment. In each cohort, offspring from 10 field-collected colonies were assigned randomly to each of the three treatments (n = 8–36 newly settled colonies per treatment). A total of 274 colonies from all cohorts were monitored.

Each experimental colony or pair of colonies was placed on a glass plate measuring 5.0 × 7.5 cm and allowed to attach firmly for 1 week in the laboratory (after Rinkevich and Weissman, 1992a; Chadwick-Furman and Weissman, 1995b). Colonies that did not firmly attach to the plates, or that appeared damaged, were removed from the study at this point. All well-attached colonies were then transferred to the marina field site, in an area where abundant colonies of *B. schlosseri* grow naturally on fouling surfaces. About every 7 days, depending on the time of year, all the zooids in each colony passed through an asexual growth cycle (hereafter termed "cycle"). During each cycle, the zooids produced buds, then shrank and were replaced by their buds; thus a new asexual generation of zooids was formed in each colony. To examine cycle-related life-history traits, every 4–7 days we collected all the experimental colonies, observed them under a dissecting microscope in the laboratory, and returned them to the field within a few hours (after Chadwick-Furman and Weissman, 1995a, b).

We examined the following life-history parameters for each colony: (1) growth rate of somatic tissues, as measured by the number of clonal units (zooids, Fig. 1) produced per cycle; (2) age and size at sexual maturity, defined as the beginning of egg production; and (3) sexual reproductive output, as measured by the number of eggs produced by each zooid during each cycle, the number of cycles in which eggs were produced (# clutches), and the total number of eggs produced by each colony throughout its lifespan (fecundity) (after Sabbadin and Zaniolo, 1979; Sabbadin and Astorri, 1988; Chadwick-Furman and Weissman, 1995b). We assigned zooids in chimeric colonies to genotype on the basis of morphological and developmental characters, such as their relative positions in the chimera, the number of buds produced, and in some cases, color patterns (after Chadwick-Furman and Weissman, 1995a; Yund *et al.,* 1997). Since colonies were observed every 4–7 days, we counted directly the number of buds produced by each zooid at each cycle, and thus accurately assigned each new budded zooid to original colony genotypes in chimeras.

All statistical analyses were performed using STATA, version 7.0 (Statacorp, 2001). Effects of allogeneic contact treatment on life-history traits were examined only within each cohort, since between-cohort comparison of life-history traits were made previously (Chadwick-Furman and Weissman, 1995b). For life-history traits that were examined on a per-cycle basis (*i.e.,* number of zooids produced per cycle and number of eggs per zooid per cycle, see above), we measured the value for each cycle within a colony, but we present only the mean of these values for each colony. Thus, at one-way model was used in analyzing these traits. Log-transformed values of all life-history traits had approximately equal variances between treatment groups within each cohort, so ANOVA tests were applied to the data.

Results

Frequencies of natural contacts

We observed high frequencies of natural contact between colonies of *Botryllus schlosseri* and other encrusting macroorganisms. About one-third of all colonies (28.2%, n = 309) contacted encrusting bryozoans. Many colonies of *B. schlosseri* contacted the other colonial ascidians *Botryllioides violaceous* (23.6%) or *Diplosoma macdonaldi* (4.8%), or individuals of solitary ascidians (5.5%). In addition, 21.4% of *Botryllus schlosseri* colonies occurred in allogeneic contact with conspecifics. Only one colony was observed to contact macroalgae (0.3%), and some colonies were isolated from contact with other sessile macroorganisms (16.2%).

Morphology and growth

Colony morphology was similar in all cohorts and experimental treatments. All colonies were flat and disc-shaped when small, with closely spaced groups of zooids (Fig. 1a). As they grew, some of the colonies developed irregular outlines, but the zooid systems remained compact and close together (Fig. 1b, c). In fused chimeric colonies, the area of fusion became barely visible over time, and some zooid systems straddled the area of initial fusion (Fig. 1b). The zooids of all genotypes in fused colonies appeared to grow constantly and to coexist in chimeras during their entire lifespan (Fig. 1b). We did not observe any shrinkage or somatic resorption of one genotype by another in chimeras. Until the time of chimeric colony senescence and death, robust blastozooids from all partners appeared to coexist within a single fused colony (Fig. 1b).

Colonies that contacted noncompatible partners underwent rejection reactions that persisted along an extensive border of contacting tissues (Fig. 1c). As colonies grew, the contact area expanded along this border, and the number of points of rejection increased. Up to 15 points of rejection were observed during each sampling period throughout the lifespan of rejecting colonies. All rejecting colonies maintained a long, continuous border throughout their lifespans, until one of them senesced and died (Fig. 1c). Pairs of rejecting colonies were compact, grew actively, and neither retreated nor grew away from each other.

Colonies grew until they reached the edges of the glass culture plate, then grew around the plate edges, and continued to spread over the back sides of the plate. None of the colonies filled all of the space available on both sides of the plate (Fig. 1).

Juvenile colonies grew exponentially, regardless of treatment (Fig. 2). During January and October, exponential growth began after a lag time of 3–5 cycles (= 32 to 64 days, Fig. 2). In all cohorts, colonies in the isolated treatment reached the largest maximum size (Fig. 2). This pattern persisted even in the October cohort, in which some isolated colonies experienced partial predation during cycle 9 that reduced their size to almost zero, after which they recovered and became the largest colonies in the cohort (Fig. 2). Growth rate slowed upon commencement of sexual reproduction in all cohorts (Fig. 2).

In most cohorts, there was a significant effect of treatment on colony growth rate (Tables 1 and 2, Fig. 3a). Isolated colonies grew faster than did both rejected and fused colonies in the cohorts born during January and May (Table 2). In two of the cohorts, rejected colonies also grew faster than did colonies that fused to become chimeras (Table 2). In the October cohort, none of the fused colonies grew past the juvenile stage, and so were not included in statistical analyses of life-history differences among colonies that reached sexual maturity (Fig. 3, Tables 1 and 2).

Sexual reproduction

There was no effect of allogeneic contact treatment on the age at which colonies reached first reproduction, except in the May cohort, where rejected colonies reached sexual maturity at a significantly later age than did both isolated and fused colonies (Tables 1 and 2, Fig. 3b). The age at which colonies began to reproduce sexually appeared to be

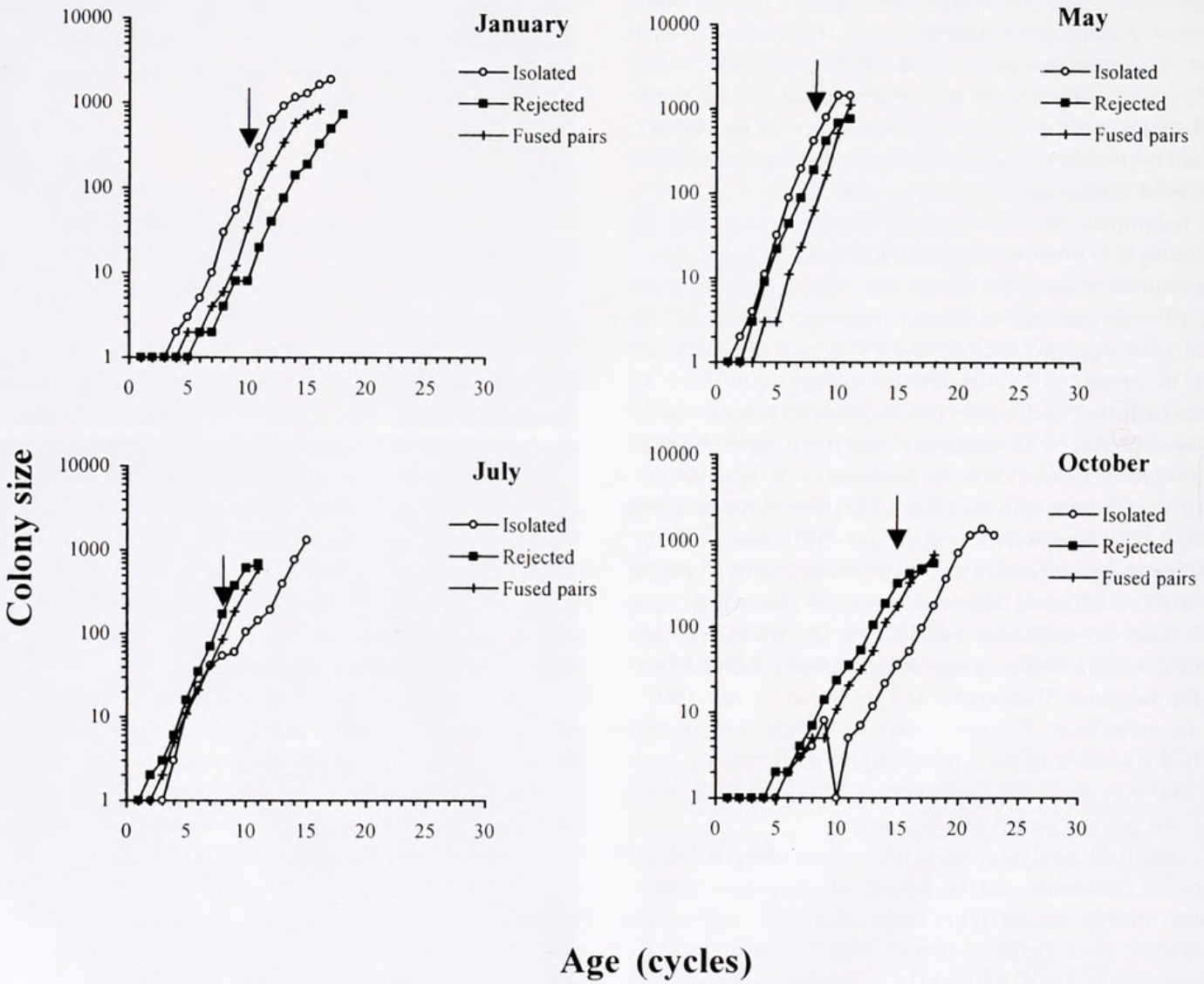

Figure 2. Typical growth curves of colonies of the ascidian *Botryllus schlosseri* for three allogeneic contact treatments and four cohorts in Monterey Bay, California. The shape of growth curves varied widely within each treatment, so mean and error values cannot be shown clearly here. Thus, only the largest colony in each treatment is shown for each cohort (for mean growth rates, see Fig. 3a). Note that colony size is plotted on a logarithmic scale. Arrows mark the commencement of sexual reproduction (egg production) for the first colonies to reach maturity in each cohort. Data on isolated colonies were published previously as Figure 1 in Chadwick-Furman and Weissman (1995b).

Table 1

One-way ANOVAs of life-history traits between allogeneic contact treatments within each of four cohorts of the colonial ascidian Botryllus schlosseri *grown in Monterey Bay, California*

Life-history trait	Cohort	Source of variation	DF	Mean square	*F*	*P*
Growth rate	January	Treatment	2	0.105	10.44	***
		Error	33	0.010		
	May	Treatment	2	0.521	41.20	***
		Error	53	0.013		
	July	Treatment	2	0.200	4.71	*
		Error	47	0.049		
	October	Treatment	1	0.030	1.62	ns
		Error	8	0.019		
Age at first reproduction	January	Treatment	2	0.356	3.02	ns
		Error	33	0.118		
	May	Treatment	2	0.073	9.90	***
		Error	53	0.007		
	July	Treatment	2	0.016	1.03	ns
		Error	47	0.016		
	October	Treatment	1	0.107	4.61	ns
		Error	8	0.023		
Size at first reproduction	January	Treatment	2	1.953	3.72	*
		Error	33	0.525		
	May	Treatment	2	14.400	41.66	***
		Error	53	0.346		
	July	Treatment	2	3.036	3.35	*
		Error	47	0.907		
	October	Treatment	1	2.142	4.56	ns
		Error	8	0.470		
Number of eggs/zooid/cycle	January	Treatment	2	0.641	3.25	ns
		Error	33	0.197		
	May	Treatment	2	1.008	5.00	*
		Error	53	0.202		
	July	Treatment	2	0.343	2.31	ns
		Error	47	0.148		
	October	Treatment	1	0.305	4.10	ns
		Error	8	0.074		
Clutch number	January	Treatment	2	2.696	9.83	***
		Error	33	0.274		
	May	Treatment	2	0.179	1.34	ns
		Error	53	0.133		
	July	Treatment	2	0.795	2.35	ns
		Error	47	0.339		
	October	Treatment	1	1.692	12.04	**
		Error	8	0.140		
Fecundity	January	Treatment	2	12.844	15.79	***
		Error	33	0.813		
	May	Treatment	2	19.587	25.71	***
		Error	53	0.762		
	July	Treatment	2	4.695	3.17	*
		Error	47	1.480		
	October	Treatment	1	16.656	23.43	**
		Error	8	0.711		

* $P < 0.05$; ** $P < 0.01$; $P < 0.001$; ns = not significant.

controlled mainly by environmental factors, such as temperature, that varied with season of birth (see Chadwick-Furman and Weissman, 1995b).

Variation in the size of colonies at first reproduction followed the same pattern as did colony growth rate (Table 2), but the differences between groups were magnified (compare Figs. 3a and c). In both the January and May cohorts, isolated colonies, which grew relatively rapidly as juveniles (Fig. 3a), were significantly larger at maturity than were fused and rejected colonies (Fig. 3c, Table 2). Where

Table 2

Tukey-Kramer multiple comparisons tests for differences in life-history traits between allogeneic contact treatments within each of 4 cohorts of the colonial ascidian Botryllus schlosseri *grown in Monterey Bay, California*

Life-history trait	Cohort	Treatment*
Growth rate (# buds/zooid/cycle)	January	I > R F
	May	I > R > F
	July	I R > F
	October	I R
Age at first reproduction (# cycles)	January	I R F
	May	I F > R
	July	I R F
	October	I R
Size at first reproduction (# zooids)	January	I > R F
	May	I > R > F
	July	I R > F
	October	I R
Number of eggs/zooid/cycle	January	I R F
	May	I > R F
	July	I R F
	October	I R
Clutch number	January	I > R F
	May	I R F
	July	I R F
	October	I > R
Fecundity (total # eggs/colony)	January	I > R F
	May	I > R F
	July	I R F
	October	I > R

* Symbols for treatments: I = isolated, R = rejected, F = fused. Treatments that did not differ significantly ($P > 0.05$) are conjointly underlined. > signs indicate which treatments had significantly larger values of each life-history trait within each cohort. In the October cohort, none of the fused colonies survived to reproduce, and so they were not included in analysis of variation in life-history traits among colonies that survived to maturity.

there were significant differences, isolated colonies were, on average, 1.5–2.5 times larger at sexual maturity than rejected colonies, and 2–3 times larger than colonies that fused to form chimeras (Fig. 3c).

The number of eggs produced per zooid per cycle (= reproductive effort) varied widely between colonies within each treatment, and did not vary between treatments, except in the May cohort (Table 1, Fig. 3d). For colonies born during May, isolated individuals produced significantly more eggs per zooid per cycle than did colonies in either of the allogeneic contact treatments (Table 2).

The total number of egg clutches produced by each colony was affected by treatment in only the two cohorts that overwintered, those born during October and January (Table 1, Fig. 3d). In both cases, colonies that were isolated from contact produced significantly more egg clutches than did those in either of the two allogeneic contact treatments (on average, 2–3 times more clutches, Fig. 3e, Table 2).

The lifetime fecundity of colonies varied significantly with treatment in all cohorts (Table 1). The combined effects of relatively rapid somatic growth, large size at maturity, and a large number of egg clutches in the isolated colony treatment (Fig. 3a–e) resulted in much higher lifetime fecundity in isolated than in either the fused or rejected treatments (Fig. 3f, Table 2). The mean fecundity of isolated colonies ranged from 1.8 to 2.5 times that of fused or rejected colonies in summer cohorts (May and July). In the winter cohorts (January and October), the mean fecundity of isolated colonies was more than 5–10 times that of fused or rejected colonies. Fused colonies that were born in October did not produce eggs at all (Fig. 3f).

Colony longevity and survivorship

Colonies in all treatments and cohorts had short, subannual lifespans (Fig. 4). Within each cohort, colonies in all treatments reached sexual maturity at about the same age (Fig. 3b), reproduced sexually for a few cycles, and then all died within a few cycles of each other (Fig. 4). The percentage of colonies that survived to reproduce was high and did not vary significantly among treatments in the January and May cohorts (chi-square tests, $\chi^2_{0.05(2)} = 5.99$, $G = 0.68$ and 3.78 respectively, Fig. 5). In the July cohort, survivorship also was high, but did vary with treatment; rejected colonies had the lowest survivorship to maturity ($G = 13.16$). Colonies born in October had low survivorship that did not vary significantly with treatment, even though all colonies in the fused treatment died as juveniles ($G = 5.04$, Fig. 5).

Colony longevity in all treatments and cohorts was controlled mainly by the timing of colony senescence (Fig. 1c). Senescence occurred in four distinct stages that began 1–2 weeks before death (details in Chadwick-Furman and Weissman, 1995b). The stages of senescence did not vary with treatment or cohort.

When senescence began in the zooids of one genotype, it spread to the zooids of fused, but not rejected, partners (Fig. 1b, c). After one of the partners in a rejecting pair died, the other colony continued to live for a few cycles (Fig. 1c).

Discussion

We document here that allogeneic contacts, whether they lead to fusion or rejection, result in significantly reduced fitness in field-grown colonies of the colonial ascidian *Botryllus schlosseri*. This is the first demonstration in a protochordate that, under natural field conditions, allogeneic contacts leading to both fusion and rejection come at a cost to life-history processes such as growth and reproduction.

We also show that colonies of *Botryllus schlosseri* in the wild frequently contact those of conspecifics and of other species of sessile invertebrates, so associated fitness costs may be a ubiquitous and important phenomenon in nature. Contact rates with the colonial ascidian *Botrylloides viola-*

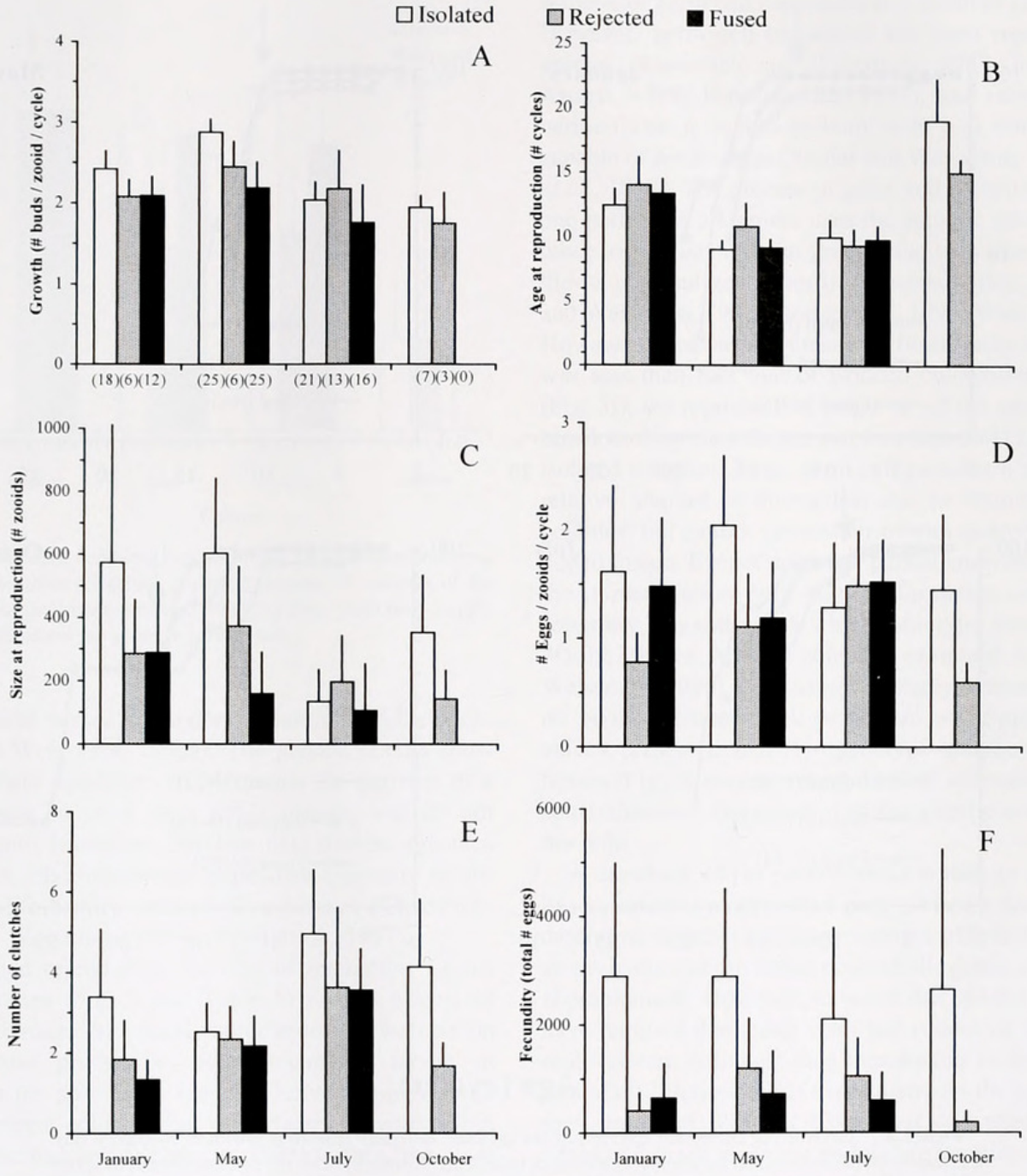

Figure 3. Variation in life-history traits among three allogeneic contact treatments and four cohorts in the colonial ascidian *Botryllus schlosseri,* grown in Monterey Bay, California. Note that for fused colonies, traits are presented for each genotype within the colony. Means plus one standard deviation are shown. Sample sizes for all life-history traits are given in parentheses in graph A. Sample sizes are low in some groups due to mortality of some colonies before reaching sexual maturity (compare sample sizes with those in Fig. 5). Data on isolated colonies were published previously as Figure 2 in Chadwick-Furman and Weissman (1995b).

ceous were especially high at our site (see Results). Yet, because our survey of contact frequencies was based on a one-time observation, which inherently underestimates contacts throughout the life of a colony, lifetime contact rates between colonies of *Botryllus schlosseri* and other sessile organisms at Monterey are even higher than those presented here (see Results). Our limited manipulation of colonies in one of the cohorts of *Botryllus schlosseri* (born on 15 October 1990) indicates that xenogeneic interaction with *Botrylloides violaceous* results in a level of fecundity inter-

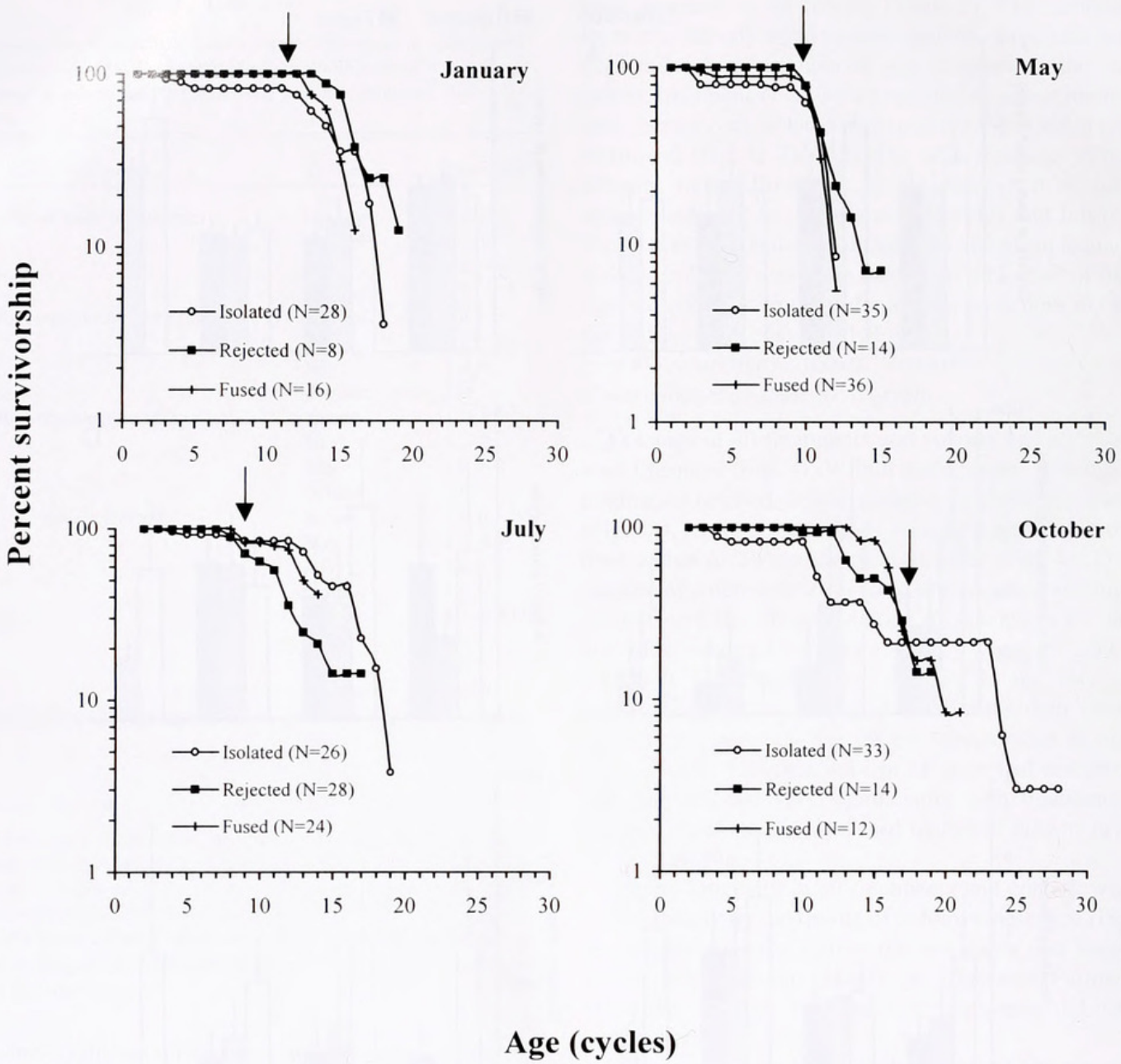

Figure 4. Survivorship curves for colonies of the ascidian *Botryllus schlosseri* grown in Monterey Bay, California, in four cohorts and three allogeneic contact treatments. Arrows indicate the commencement of sexual reproduction in each cohort. The last point in each line represents the last surviving colony of each group. Note that survivorship is plotted on a logarithmic scale. Data on isolated colonies were published previously as Figure 3 in Chadwick-Furman and Weissman (1995b).

mediate between those of isolated and allocontacted colonies [total number of eggs produced = 1383 + 769 ($\bar{x}$ + SD), n = 9 xenocontacted colonies of *Botryllus schlosseri* that survived to maturity, N. E. Chadwick-Furman, pers. obs.; compare with October cohort in Fig. 3f]. Thus, xenogeneic contact appears to affect colony fecundity, but not as severely as allogeneic contact.

The reduced fitness of colonies following fusion or rejection may result from energetic or physiological costs associated with recognizing and reacting to non-self tissue. The process of interaction along the borders of rejecting colonies involves extensive tissue damage and resource demand on both colonies (Scofield and Nagashima, 1983; reviewed in Rinkevich, 1992). In addition, competition between somatic and germ cell lines within fused chimeras also may draw heavily on the physiological resources of the partners involved (Buss, 1982). Colonies that are isolated from allogeneic contact do not face these costs.

The lack of resorption observed here in field-raised chimeras of *Botryllus schlosseri* is in striking contrast to previous results from laboratory studies (Rinkevich and Weissman, 1987, 1992a, b; Pancer *et al.*, 1995). The reduced chimeric stability of laboratory colonies has been demonstrated by growing genetically identical replicates of chime-

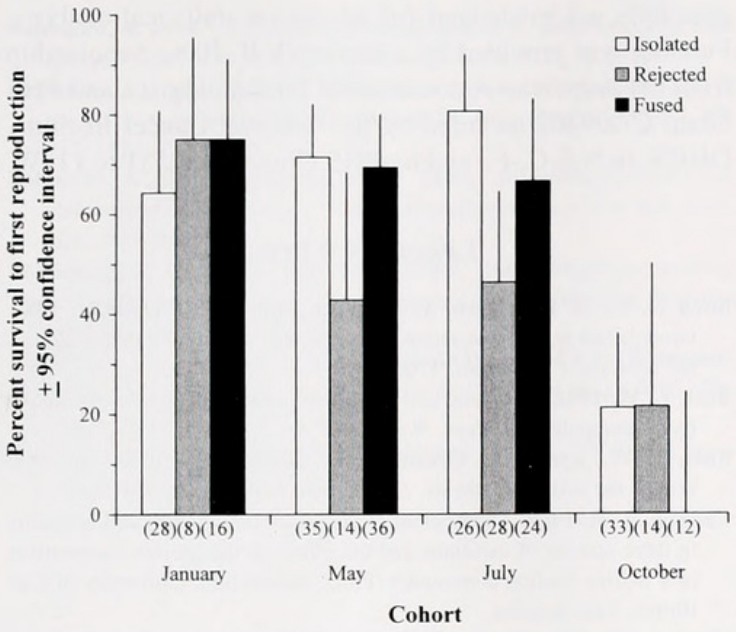

Figure 5. Variation in percent survivorship to first reproduction among four cohorts and three allogeneic contact treatments of colonies of the ascidian *Botryllus schlosseri* grown in Monterey Bay, California. Sample sizes for each treatment are given in parentheses.

ras under field *versus* laboratory conditions (Chadwick-Furman and Weissman, 1995a). The present results show that, under field conditions in Monterey, the partners of a chimera appear to grow in a stable manner and do not undergo somatic resorption. Previous field studies indicate a high level of environmentally dependent plasticity in fitness-related life-history traits of *B. schlosseri* (Chadwick-Furman and Weissman, 1995a; Yund *et al.,* 1997).

The reduced reproductive success of interacting *versus* isolated colonies (Fig. 3 and Table 1) reveals effects of allogeneic contacts on sexual reproduction as well as on somatic tissue production. Recruitment of larvae at Monterey in the springtime may be derived from a small number of parent colonies that overwinter (Carwile, 1989; Chadwick-Furman and Weissman, 1995b). Thus, the costs of interactions in this experiment would have resulted in reduced representation of the offspring of winter allo-contacting colonies in the summer bloom.

Allogeneic interactions do not alter the survivorship of colonies in most cohorts (Fig. 5). The longer lifespans (Figs. 3b and 4) and lower survivorship (Fig. 5) of colonies during winter, as compared to summer, appear to be due to a slowing of colony growth and development during low temperatures in the winter in Monterey Bay (Boyd *et al.,* 1986; Chadwick-Furman and Weissman, 1995b). As found in past studies, whole-colony senescence causes the death of most colonies (Chadwick-Furman and Weissman, 1995a, b) and is genetically controlled (Rinkevich *et al.,* 1992).

At the time of our experiments, we did not have markers to identify the genotypes of blood cells, bud cells, or gametes in the fused colonies, and so we did not test for somatic or germ cell parasitism as a result of colony fusion. However, germ cell parasitism has been reported in this species (Rinkevich and Weissman, 1987; Sabbadin and Astorri, 1988; Pancer *et al.,* 1995), and recent work has verified that it occurs in both male and female gametes capable of fertilization (Stoner and Weissman, 1996; Stoner *et al.,* 1999). The process of germ cell parasitism, in which one partner in a chimera uses the somatic resources of the other to produce its own germ cells, may alter the relative fitness of fused genotypes in chimeras (Buss, 1982; Stoner and Weissman, 1996; Stoner *et al.,* 1999; Weissman, 2000). However, because the fitness of fused pairs of genotypes was less than half that of isolated colonies in all cohorts (Fig. 3f), the reproductive output of all the genotypes combined in chimeric colonies was less than that of genotypes in isolated colonies. Thus, germ cell parasitism may alter the relative amount of fitness lost due to fusion in chimeric colonies, but cannot prevent an overall reduction in fitness due to fusion. Even if germ cell parasitism were extensive in the chimeras tested here, chimera formation causes reduced fecundity, regardless of which genotype dominates (Fig. 3f). In 30% of the field chimeras examined by Stoner and Weissman (1996) at the same Monterey marina site, little or no germ or somatic cell parasitism was found. Thus, the values presented here for genotype-specific measures of fitness (Fig. 3) may represent realistic estimates for at least some chimeras that retain a stable genetic composition in the wild.

A drawback of the present study is that we could not set up, as controls, undissected pairs of isogeneic colonies, to determine whether isogeneic contact affects fitness. Thus, an evaluation of the actual costs of allogeneic contact *per se* is problematic. However, set-up of this control group would have required dissecting apart and re-uniting systems from multi-system colonies, thus introducing further manipulation of all colonies in this experiment. As the colonies grew, they produced lobes of tissue that contacted along their edges and fused along the undulating margins of the colony in all treatments (Fig. 1b, c). Thus, if isogeneic contact affected fitness, it did so equally in all treatments here.

We show here that egg production in fused colonies is greatly reduced (Fig. 3f), possibly due to competition between the genetically different individuals that fused to make up that colony. Thus, one benefit of precise allorecognition in this species may be that it limits the unit of selection to chimeras composed of closely related kin (Grosberg and Quinn, 1986; Rinkevich and Weissman, 1987; Stoner and Weissman, 1996; Stoner *et al.,* 1999). Because of the high polymorphism of the *Fu/HC* gene locus (that permits fusion rather than rejection to occur; Scofield *et al.,* 1982), fused individuals in the wild most likely represent kin rather than a random assortment of genotypes (Grosberg and Quinn, 1986). In *Botryllus schlosseri,* the proportion of fusions occurring between siblings is higher

than between nonsiblings (Scofield *et al.*, 1982; Magor *et al.*, 1999). Thus, the unit of genetic inheritance for chimeric colonies of fused siblings would be the outcome of germline competition between the mother colony and the diverse sperm that fertilized her. In addition to kin fusion, regulated by *Fu/HC* matching, kin cosettlement is encouraged by the limited dispersal of tadpole larvae from the maternal colony and nonrandom cosettlement according to shared *Fu/HC* genotype (Grosberg and Quinn, 1986). A common selected trait in these chimeras is allele-sharing at the *Fu/HC* locus (Weissman *et al.*, 1990). Kin selection would act also on shared genes other than the selected *Fu/HC* types that are common to these siblings. Reproductive outcomes in these chimeras could be as simple as the direct gametic representation of the diverse blastozooid units in the chimera; or could be as complex as the outcomes of selective resorption or germ cell parasitism that generate skewing from that simple representation (Pancer *et al.*, 1995; Stoner and Weissman, 1996; Stoner *et al.*, 1999; Weissman, 2000).

No matter whether allogeneic colony contact results in fusion or rejection, if it leads to reduced fitness, as measured by growth and fecundity, with no increase in survivorship, why have these organisms developed and maintained an elaborate system of allorecognition? Perhaps, in this species, genetically based allorecognition is nonadaptive. It may be linked to other processes that are adaptive, and thus have evolved as a by-product of processes such as disease recognition (Buss and Green, 1985; Magor *et al.*, 1999) or gametic compatibility (Scofield *et al.*, 1982). However, the ability to recognize and reject nonrelated colonies, and to fuse only with closely related kin that share alleles at the *Fu/HC* locus, may be directly beneficial in that it reduces the costs of germ cell parasitism in colonies (Stoner *et al.*, 1999).

The phenomena of cosettlement, fusion, and development of reproductive competence in chimeras are not limited to protochordates, and may be important selective factors in other sessile organisms as diverse as fungi, sponges, and cnidarians (reviewed in Buss, 1982; Rinkevich and Weissman, 1987; Pancer *et al.*, 1995). Our findings that allogeneic contact, and especially chimera formation, reduce individual fitness under natural field conditions may have broad implications for the evolution of allorecognition systems.

Acknowledgments

We thank Buki Rinkevich for discussions and collaborations that led to and resulted from these studies. We also thank Kathi Ishizuki and Karla Palmeri for assistance in data collection, Simona Bartl and Robert Lauzon for discussions, and Uri Frank and Buki Rinkevich for comments that improved the manuscript. Karen Tarnaruder assisted with the graphics. Prof. Maureen Lahiff of the School of Public Health at the University of California at Berkeley is gratefully acknowledged for advice on statistical analysis. Funding was provided by a Frederick B. Bang Scholarship from the American Association of Immunologists, and PHS Grant CA09302 awarded by the National Cancer Institute, DHHS, to N.E.C.-F., and by PHS Grant CA42551 to I.L.W.

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