DYNAMICS OF SOMATIC CELL-LINEAGE COMPETITION IN CHIMERAS OF *Hydractinia symbiolongicarpus* (CNIDARIA: HYDROZOA)

Dinámica de competencia entre líneas celulares somáticas en quimeras de *Hydractinia symbiolongicarpus* (Cnidaria: Hydrozoa)

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ABSTRACT

Sessile colonial invertebrates often fuse with conspecifics to form chimeras. Chimerism represents an unequivocal instance of within-individual selection where genetically different cell-lineages compete for representation in the somatic and gametic pools. We analyzed temporal and spatial variations in somatic cell-lineage composition of laboratory-established chimeras of the hydroid *Hydractinia symbiolongicarpus* (Cnidaria: Hydrozoa). Using three clones with different allotypic specificities (*i.e.*, two rejecting one another but fusing with a third one), we established two classes of two-way chimeras, a single three-way chimera class, and an incompatible interaction as control. Chimeras were sampled at five time intervals for a year. Cell-lineages in samples were identified by polyp fusibility assays against tester colonies of known fusibility. The cell lineages composing the chimeras showed a differential competitive ability, with one of them representing close to 80% by the end of the study. Rare cell-lineages stabilized at low frequencies but preserved their ability to gain somatic representation and to colonize distant parts of the chimera. This behavior characterizes cell parasites. As a consequence of the reproductive plasticity of most colonial invertebrates, cell-lineage variability may be transmitted to the offspring both sexually and asexually. Successful somatic competitors are expected to be preferentially transmitted asexually, whereas cell parasites would be preferentially transmitted sexually.

Key words: Allorecognition, *Hydractinia*, units-of-selection, cell-lineage competition, chimera

RESUMEN

Los invertebrados coloniales y sésiles con frecuencia se fusionan con conespecíficos para formar quimeras. Estas quimeras son un ejemplo de selección natural actuando al interior del individuo en donde células genéticamente distintas compiten por acceso tanto a la línea somática como a la germinal. En este estudio se analizaron las variaciones temporal y espacial de linajes celulares somáticos en quimeras establecidas en el laboratorio del hidroide colonial *Hydractinia symbiolongicarpus* (Cnidaria: Hydrozoa). Usando tres clones con distintas especificidades alotípicas (dos de ellas se rechazaban pero ambas se fusionaban a una tercera), se establecieron dos clases de biquimeras, una triquimera y una interacción incompatible como control. Muestras de tejido de quimeras se obtuvieron en cinco intervalos de tiempo durante 50 semanas. La identidad celular de cada muestra se determinó por ensayos de fusibilidad de pólipos con colonias estándar de fusibilidad conocida. Los distintos linajes celulares de cada quimera mostraron una habilidad competitiva diferencial, con una de ellas representando cerca del 80% de las quimeras hacia el final del estudio. Las líneas celulares con menor representación se estabilizaron a bajas frecuencias pero mantuvieron la capacidad de aumentar en frecuencia y de colonizar partes distantes en la quimera. Este comportamiento caracteriza los parásitos celulares. Como consecuencia de la plasticidad reproductiva de la mayoría de invertebrados coloniales, la variabilidad de los linajes celulares puede ser trasmitida a la descendencia tanto sexualmente como asexualmente. Linajes celulares somáticos con alta capacidad competitiva serían heredados asexualmente, mientras que los linajes celulares parásitos se transmitirían preferencialmente por reproducción sexual.

Palabras clave: alorreconocimiento, *Hydractinia*, unidades de selección, competición, quimeras

INTRODUCTION

Sessile colonial invertebrates frequently encounter members of their own species as they grow on hard surfaces, with the encounter typically culminating in either fusion of kin or rejection of unrelated colonies (Grosberg, 1988). These allorecognition responses have been unambiguously described in sponges, cnidarians, bryozoans, and ascidians (reviewed in Grosberg, 1988) and have excited interest in various disciplines. Specifically, these phenomena have attracted the attention of evolutionary theorists as a paradigm of conflicts between units-of-selection (Buss and Shenk, 1990), have represented a challenge to conventional notions on the origin and maintenance of genetic variability in natural populations (Grosberg *et al.*, 1996; Grosberg and Hart, 2000), and have led to debates amongst comparative immunologists as to whether such phenomena represent the ancestral state of key elements of the vertebrate immune system (Burnet, 1971; Scofield *et al.*, 1982; Buss and Green, 1985).

Allorecognition responses in the colonial hydroid of the genus *Hydractinia* (Cnidaria: Hydrozoa) are better understood than in perhaps any other colonial invertebrate (*e.g.*,

Teissier, 1929; Schijfsma, 1939; Crowell, 1950; Hauenschild, 1954; Hauenschild, 1956; Müller, 1964; Müller, 1967; Toth, 1967; Ivker, 1972; Gallien and Govaere, 1974; Buss *et al.*, 1984; Buss *et al.*, 1985; Buss, 1987; Müller *et al.*, 1987; Grosberg, 1988; Lange *et al.*, 1989; Buss and Grosberg, 1990; Shenk and Buss, 1991; Lange *et al.*, 1992; Grosberg *et al.*, 1996; Mokady and Buss, 1996; Hart and Grosberg, 1999). *Hydractinia* is a colonial athecate hydroid found in near-shore oceanic waters growing as a surface incrustation on gastropod shells inhabited by pagurid hermit crabs (Buss and Yund, 1989). Colonies are diploblastic and composed of three morphological modules: polyps, stolons and the stolonal mat. Polyps are feeding structures and gamete carriers, and are embedded in the stolonal mat, a two-dimensional basal plate that consists of two ectodermal layers sandwiching a network of endodermal canals. These endodermal canals provide vascular continuity between the polyps' gastric cavities and may extend beyond the stolonal mat, in which case they are called stolons. Colonies release their gametes to the ocean where fertilization occurs. Fertilized eggs develop into crawling planula larvae which settle on hermit crab-occupied shells, and subsequently metamorphose into primary polyps. As stolons extend, bifurcate, and anastomose, new polyps bud from the stolons yielding thus a mature colony (Ballard, 1942; Berking, 1991).

Contacts between *Hydractinia* colonies results in one of three outcomes: (i) Fusion: After contact, compatible colonies dissolve their periderm coat and, within an hour, they adhere to one another by their epithelial cells. Two to four hours post-contact, colonies establish a common gastrovascular system forming a permanent chimera. (ii) Rejection: Upon contact, incompatible colonies fail to adhere, and within the first 12 hours, interacting tissues begin to swell due to massive migration of "stinging cells" or nematocytes, phylum-defining cells containing specialized organelles called nematocysts. Nematocysts discharge a harpoon-like thread which delivers toxins causing extensive tissue destruction in the opponent (Müller, 1964; Buss *et al.*, 1984). Subsequent rejection takes two forms depending upon the colony morphology (Buss and Grosberg, 1990). Encounters between incompatible stoloniferous colonies (i.e., those with predominance of free stolons over stolonal mat) result in aggressive rejections and are characterized by the induction of a specialized organ of defense, the hyperplastic stolon (Ivker, 1972). In these reactions, differentiation and recruitment of nematocytes continue until one colony has eliminated the other (Buss *et al.*, 1984; Lange *et al.*, 1989). Confrontations between stolonless colonies (*i.e.*, those with predominance of stolonal mat over free stolons), produce passive rejections. These responses are characterized by the secretion of a fibrous matrix by both colonies, accompanied by cessation of growth along the contact margin (Buss and Grosberg, 1990). (iii) Transitory fusion: In this reaction, colonies initially fuse only to separate days or weeks later (Hauenschild, 1954; Shenk and Buss, 1991; Grosberg *et al.*, 1996; Gild *et al.*, 2003; Cadavid *et al.*, 2004). When initial fusion is established through the stolons, the reaction is characterized by initial occlusion of vascular spaces, followed by local necrosis and separation of interacting stolons (Shenk and Buss, 1991). When initial fusion is established through the stolonal mats, a necrotic band appears at the point where colonies initially contacted. This band subsequently spreads to form a line spanning the original contact zone. The emergence of the necrotic line is accompanied by occlusion of the once fused endo-

dermal canals. Within days or weeks after the first appearance of the necrotic line, colonies separate from one another. From this point on, the response is indistinguishable from a passive rejection, except at the growing edges of the contact zone which, upon contact, display the same time course and phenomenology described above (Cadavid *et al.*, 2004). Transitory fusion may in fact represent a composite of phenotypes varying in time course, progression and developmental regulation. In *H. symbiolongicarpus*, these allorecognition responses are mediated by a dose-dependent interaction of two linked allorecognition loci, with fusion occurring when colonies share at least one allele at each locus, rejection resulting when they share no alleles at either locus, and transitory fusion occurring when colonies share a single allele at only one of the two loci (Cadavid, 2004; Cadavid *et al.*, 2004).

Allorecognition responses in colonial invertebrates mediate competitive interactions at two different levels of biological organization: the colony and the cell-lineage (Buss, 1990; Buss and Shenk, 1990). Rejecting colonies compete for habitable space by interfering with their competitor's growth, where the outcome of such competition is a function of colony size and morphology (Buss and Grosberg, 1990). Fusing colonies, on the other hand, form discrete physiological entities composed by genetically different cell-lineages which compete for representation in the chimera's somatic and germ cell lines (Stoner *et al.*, 1999). Chimerism represents an example of within-individual selection and potentially plays a prominent role in the adaptive evolution of colonial invertebrates (Buss, 1987). Natural selection acts on differences in cell growth and survival between cell lineages within the chimera. Yet, for cell-lineage selection to have an evolutionary significance, this variation ought to be inherited to the offspring. In contrast to solitary organisms for which evolutionary important variation is restricted to the germ line, colonial invertebrates may transmit both somatic and germ line variation to the progeny. These organisms often lack discrete germ lines but possess multi-potent stem cells that are able to differentiate into somatic and germ cells at any time during ontogeny. Thus, variation in fitness-related traits between cell-lineages composing a chimera can be sexually inherited after their eventual differentiation into gametes, or asexually transmitted through fragmentation, fission or budding. Chimerism, therefore, is an important mechanism to introduce heritable cell-lineage variation in colonial invertebrates. The evolutionary significance of cell-lineage selection due to somatic fusion largely depends on both the frequency of chimerism in natural populations and the competitive behavior of cell-lineages. While a number of studies have shown that chimerism is of relatively common occurrence among colonial invertebrates (Stoner and Weissman, 1996; Hart and Grosberg, 1999; Stoner *et al.*, 1999; Sommerfeldt *et al.*, 2003), our understanding of the nature and dynamics of cell-lineage competition and selection in chimeras of colonial invertebrates is still fragmentary. In this study, we analyzed differences in fitness-related traits, such as replication and survival rates, between somatic cell-lineages in laboratory-established chimeras of *H. symbiolongicarpus*. Using unique tissue compatibility specificities as markers for individual cell-lineages, we measured the cell-lineage composition of different classes of chimeras and determined how such composition varies in time and space.

MATERIALS AND METHODS

Animals and Chimera Generation

Three *Hydractinia symbiolongicarpus* clones were used in this study (833-8, 4117-2, and 431-63). They were derived from the inbreeding program designed to genetically characterize the *Hydractinia* allorecognition complex (Mokady and Buss, 1996; Cadavid *et al.*, 2004). Colony 833-8 was the product of eight generations of brother-sister matings from two wild-type colonies. Clone 4117-2 resulted from seven generations of inbreeding from a wild-type animal and a six-generation clone from the 833-8's inbred line. Clone 431-63 was derived from three generations of backcrossing from the 833-8's line into the 4117-2's line. These clones differed in their allorecognition specificities such that 833-8 and 4117-2 reject one another and both fuse to 431-63. Animals were grown on microscope glass slides and maintained in 35 liter aquaria in re-circulating artificial seawater (Reef Crystals) at 16 ± 2 °C with three changes per week of one-quarter of the water volume. Colonies were fed three times a week to repletion with 3-4 day old nauplii of *Artemia salina*. Colonies 833-8 and 4117-2 also served as testers to identify individual cell-lineages in the chimeras by fusibility assays (see below). Two classes of two-way chimeras, 833-8/431-63 and 4117-2/431-63, and one three-way chimera, 833-8/431-63/4117-2, were established. Two-way chimeras were generated by placing a five-polyp fragment of stolonal mat from each partner at 0.5 cm from one another on a glass slide, and held in position with a thread. Three-way chimeras were established in a linear disposition with colony 431-63 placed in the center between 833-8 and 4117-2 colonies. An incompatible interaction (833-8/4117-2) was also established as control. Explants attached to the glass slide within 36-48 hours and the thread was then removed. Clones grew into contact within 3-6 days, and were subsequently maintained as described above.

Tissue Sampling and polyp fusibility Assays

Cell lineage composition in each chimera was determined by polyp fusibility assays on defined sampling regions and at various time intervals, as follows. Microscope glass slides serving as substrata for chimera growth were divided into fourteen regions of 1 x 1.25 cm each, distributed in two rows and seven columns (Fig. 1A). From each sampling region containing tissue, two to four polyps were surgically removed with a scalpel and used for polyp fusibility assays (Lange *et al.*, 1992) against tester clones 833-8 or 4117-2. Briefly, an excised polyp from a sampling region was held with its cut aboral end in contact with an excised polyp from either 833-8 or 4117-2 tester clones. Interacting polyps were maintained in position by passing a human hair through their gastric cavities. Compatible polyps developed continuous endodermal and ectodermal cell layers forming a common gastric cavity within 12-24 hours whereas incompatible polyps remained separated (Fig. 1B). Accordingly with the different allorecognition specificities, if a polyp from the 4117-2/431-63 chimera failed to fuse with the 833-8 tester it was considered to be derived from the 4117-2 cell-lineage. If, however, the assay resulted in fusion, the polyp was considered to be derived from the 431-63 cell-lineage. Likewise, if a polyp sample from the 833-8/431-63 chimera failed to fuse with the 4117-2 tester, it was assigned to the 833-8 cell-lineage, but if the interaction resulted in fusion,

the polyp was considered to be derived from the 431-63 cell-lineage. In three-way chimeras four to six polyps were excised from each sampling region and divided into two groups of equal size. One was assayed against the 4117-2 tester and other against 833-8 tester. Assignment of polyps to a given cell-lineage in the three-way chimera as described may underestimate the actual contribution of 833-8 and 4117-2 cell lineages. That is, if a polyp from the three-way chimera fails to fuse with the 4117-2 tester, it is considered to be derived from the 833-8 cell-lineage. But if the interaction results in fusion, it could be derived from either 4117-2 or 431-63 cell-lineages. We, therefore, registered the minimum number of samples derived form 833-8 and 4117-2 cell lineages in the three-way chimera. For the incompatible control (833-8/4117-2), two to four polyps from each sampling region were tested with polyps from the 4117-2 tester. Assays resulting in fusion indicated that the polyp was derived from cell lineage 4117-2, whereas failure to fuse indicated that it was derived from cell lineage 833-8. As sampling destroys tissue and might bias cell lineage composition in time, temporal changes in cell lineage composition was evaluated on chimera replicas. Five replicas of each chimera and incompatible control were established. Each replica was sampled only once at either 2, 8, 37.5, 44.5, or 50 weeks post-contact. No data was collected at week 50 post-contact for the three-way chimera. To evaluate reproducibility, three experimental trials of identical design were performed simultaneously. Only one trial was performed for the incompatible interaction (833-8/4117-2).

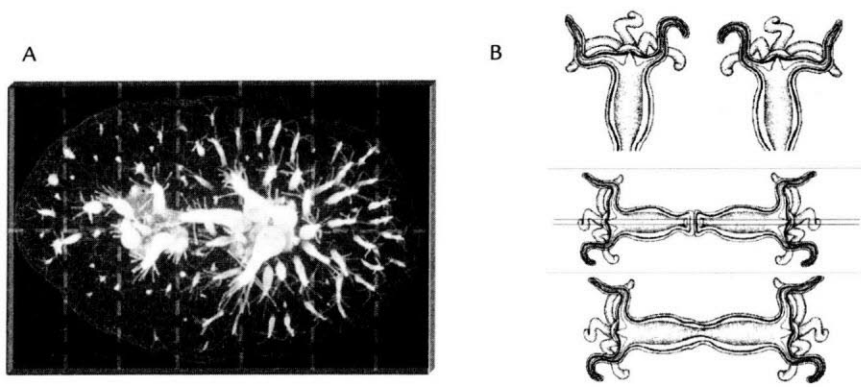

Figure 1. Sampling and typing strategy. A, five copies of each chimera class were established and sampled at five different time intervals at fixed locations in a grid. B, polyp fusibility assay for identifying cell-lineages in chimeras. This assay is based on allorecognition differences between the cell-lineages composing the chimera (see text).

Statistical Analysis

As repeated sampling destroys tissue and can bias cell lineage composition and distribution in time, replicas of each chimera class were established to be sampled only once at either 2, 8, 37.5, 44.5, or 50 weeks post-contact. Two or three replicas were established for each sampling time. The General Lineal Model on an analysis of variance (ANOVA) design was used to test the null hypothesis that the mean number of polyps contributed by each cell lineage in a chimera was the same at the different time intervals. Multiple comparisons of means was performed with the Tukey-Kramer

method to obtain confidence intervals and P values for all pairwise differences between level means. Statistical analysis was performed with the Minitab package.

RESULTS

Table 1 shows the mean number of polyps derived from the different cell lineages in each chimera at the different time intervals. The 833-8/431-63 chimera grew linearly in time and showed a significant difference in the mean number of polyps across time intervals (F=18.89, DF=4, P=0.0). Examining pairwise comparisons of mean number of polyps at different weeks post-contact revealed that the overall difference was explained by the difference in mean number of polyps between 2 and 44.5 weeks (T=6.1, P=0.0), 2 and 50 weeks (T=7.4, P=0.0), 8 and 44.5 weeks (T=4.6, P=0.0), and 8 and 50 weeks (T=5.7, P=0.0). In this chimera, the mean number of polyps contributed by cell lineage 431-63 was significantly grater than that contributed by cell lineage 833-8 across time intervals (F=105.1, DF=1, P=0.0). While there was no significant difference between the mean number of polyps contributed by 833-8 and 431-63 cell lineages at 2, 8, and 37.5 weeks post-contact, the number of polyps from 431-63 was significantly higher than those of 833-8 at 44.5 weeks (T=8.9, P=0.0) and 50 weeks (T=9.7, P=0.0) post-contact. At this two time intervals, cell lineage 431-63 represented the 93% and 87%, respectively, of the chimera. The 4117-2/431-63 chimera also showed a linear growth with significant differences in mean number of polyps across time (F=23.8, DF=4, P=0.0). In pairwise comparisons of mean number of polyps for different time intervals, all differences were significant at the 5% level, except those for 2 and 8 weeks, 8 and 37.5 weeks, 37.5 and 50 weeks and 44.5 and 50 weeks post contact. The 4117-2/431-63 chimera also was primarily composed by cell lineage 431-63 (F=48.0, DF=1, P=0.0). The mean number of polyps derived from cell lineage 431-63 was significantly higher than that derived from cell lineage 4117-2 at 44.5 (T=9.1, P=0.0) and 50 (T=5.0, P=0.001) weeks post contact. Cell lineage 431-63 represented the 88% and 71% of the chimera at 44.5 and 50 weeks post contact, respectively. Thus, in both two-way chimeras, cell lineage 431-63 grew at a higher rate than its partner to become the predominant composite of the chimera. Cell lineages 833-8 and 4117-2 remained at low frequency and were relatively stable through time. Additionally, they preserved the ability to increase in frequency after a critical low at 44.5 weeks post-contact. Figure 2A compares the fraction of 833-8 and 4117-2 cell lineages in their respective chimeras.

The three-way chimera 833-8/431-63/4117-2 was set with two rejecting clones bridged with a mutually fusible clone. Surprisingly, this chimera did not show signs of rejection at any time during the study and grew linearly across time intervals (F=9.43 DF=3 P=0.0). This overall difference in growing was explained by differences in the mean number of polyps between 2 and 8 weeks (T=3.1, P=0.02), 2 and 37.5 weeks (T=4.5, P=0), and 2 and 44.5 weeks (T=4.7, P=0) post-contact. A significant difference between the relative contributions of cell lineages to the chimera was also observed across time intervals (F=6.38, DF=3, P=0.002).

		Mean number of polyps (StDev) derived from[1]					
	Weeks					833-8 OR	4117-2 OR
Interactions	post-contact	N[2]	833-8	4117-2	431-63	431-63	431-63
833-8/	2	15	2.3 (1.5)	–	2.7 (1.1)	–	–
431-63	8	34	1.7 (2.1)	–	9.7 (1.5)	–	–
	37.5	38	4.5 (2.1)	–	14.5 (4.9)	–	–
	44.5	92	2.0 (1.0)	–	28.7 (2.5)	–	–
	50	136	4.5 (4.2)	–	29.5 (7.0)	–	–
4117-2/	2	12	–	1.7 (0.6)	2.3 (0.6)	–	–
431-63	8	40	–	4.3 (1.5)	9.0 (3.6)	–	–
	37.5	52	–	7.5 (0.7)	11.5 (2.1)	–	–
	44.5	106	–	4.3 (1.1)	31.0 (2.6)	–	–
	50	119	–	8.5 (3.7)	21.2 (6.1)	–	–
833-8/	2	31	2.0 (0.0)	1.7 (0.6)	–	3.0 (1.0)	3.7 (0.6)
431-63/	8	88	3.3 (1.5)	8.3 (3.1)	–	6.7 (1.5)	11.0 (1.0)
4117-2	37.5	114	1.3 (0.6)	5.7 (3.8)	–	12.7 (9.3)	18.3 (9.0)
	44.5	129	8.0 (2.6)	13.3 (1.5)	–	9.7 (3.0)	8.0 (2.6)
833-8/	2	11	5	6	–	–	–
4117-2	8	15	8	7	–	–	–
37.5		30	1	29	–	–	–
44.5		21	0	21	–	–	–
50		12	0	12	–	–	–

Table 1. Time variation of cell-lineage composition in *H. symbiolongicarpus* chimeras [1]The mean and StDev were calculated over three experimental trials except at 37.5 weeks post-contact for which it was calculated over two experimental trials. [2]Total number of polyps sampled.

This overall difference was accounted only by the differences in number of polyps at 37.5 weeks post contact between cell lineages 833-8 and [833-8 OR 431-63] (T=3.7, P=0.05), 833-8 and [4117-2 OR 431-63] (T=5.5, P=0.0004), and 4117-2 and [4117-2 OR 431-63] (T=4.1, P=0.02). Thus, contrary to what was observed in the two-way chimeras, all cell-lineages contributed nearly equally to the three-way chimera. Figure 2B shows the fraction of positively identified 833-8 and 4117-2 cell lineages that contributed to the three-way chimera. While 4117-2 showed higher representation during the last three time intervals than its 833-8 counterpart, the difference was not significant. As it was the case for the two-way chimeras, no total replacement of cell-lineages was observed in the three-way chimera at the end of the study. The incompatible control 833-8/411-7 elicited a strong rejection response with clone 4117-2 displaying the most aggressive behavior. This clone developed a dense thread of hyperplastic stolons which progressed slowly to eliminate clone 833-8. Table 1 shows the number of polyps derived from both competitors. At the onset, polyps from both colonies were detected at equal numbers, but as the rejection progressed, most of the polyps derived from clone 4117-2. After week 37.5 post-contact, clone 4117-2 completely eradicated clone 833-8. Figure 2C depicts the fraction of each competitor detected at the different time intervals.

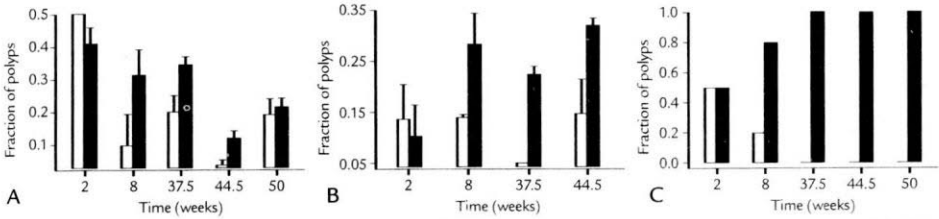

Figure 2. Fraction of cell-lineages composing the chimeras. A, fraction of cell lineages 833-8 (white bars) and 4117-2 (black bars) in their respective two-way chimeras 833-8/431-63 and 4117-2/431-63 in the five time intervals. B, fraction of cell-lineages 833-8 and 4117-2 in the three-way chimera 4117-2/431-63/833-8. C, fraction of cell-lineages in the incompatible encounter 833-8/4117. Bars represent the average fraction of cell-lineages over three (or two) trials, and lines over the bars depict the upper limit confidence interval.

Spatial distribution of cell-lineages within chimeras was evaluated by counting the number of polyps derived from a given cell lineage for each of the 14 sampling regions and over all experimental trials. Figure 3 shows the location of each cell lineage at each time interval. In this figure, squares represent polyps derived from a given cell-lineage and areas of 4x3 squares correspond to sampling regions. Within a sampling region with more than one cell-lineage, distribution of polyps is arbitrary. The overrepresentation of cell-lineage 431-63 in the two-way chimeras is evident from week 8 post-contact. Although cell lineages 833-8 and 4117-2 were identified at low frequencies, they were highly mobile. Indeed, polyps derived from these two cell lineages were found throughout the chimeras. A similar situation was observed in the three-way chimeras, where polyps derived from 833-8 and 4117-2 cell-lineages were randomly distributed in the clone. While in some cases polyps derived from incompatible cell-lineages occupied the same region, no signs of incompatibility were observed.

DISSCUSION

Most levels of biological organization function as units of natural selection in the evolutionary process, provided that they display heritable variation in fitness (Lewontin, 1970). The heritability nature of cell-lineage variation correlates with certain life-history traits and differ between solitary and colonial organisms (Orive, 2001). Most solitary organisms that reproduce gametically separate the germ and somatic lines early in ontogeny. Thus, in these organisms only variation introduced in the germ line, typically via mutation, is represented in the gametic pool. Heritability of cell-lineage variation in colonial organisms differs from that of solitary animals in at least three fundamental ways. First, cell-lineage variation in colonial organisms is introduced not only by mutation, but also by somatic fusion between conspecifics. In chimeras of colonial organisms, therefore, two or more cell lineages may compete for access to somatic and germ pools. Second, colonial organisms which reproduce gametically do not sequester the germ line. Instead, they possess multipotent stem cells which are competent to produce gametes and the various somatic cell types throughout ontogeny. As a consequence, cell-lineage variants arising either by mitotic mutation or somatic fusion might be represented in the gametic pool. Third, colonial inverte-

brates may reproduce asexually by fission, budding or fragmentation. Propagules produced by these means might differ to their parental clone in cell-lineage composition.

Chimerism is of common occurrence amongst colonial invertebrates. The frequency of chimeras in natural populations of the ascidian Botryllus schlosseri ranges from 6-8% (Karakashian and Milkman, 1967; Ben-Shlomo *et al.*, 2001), whereas in another ascidian species, *Diplosoma listerianum*, it ranges from 3-61% (Sommerfeldt *et al.*, 2003). In natural populations of *H. symbiolongicarpus*, the observed frequency of chimerism is 6-7% (Hart and Grosberg, 1999). Cell-lineage competition is known to occur in chimeras of colonial ascidians. Using microsatellites to trace cell-lineages, Stoner *et al.* (1999) showed that cell-lineages from *Botryllus schlosseri* chimeras compete for access to the gonads and somatic tissues, and that the competitive ability of each lineage is hierarchical and heritable. In this study, we have documented variation in cell-lineage composition through time and space in laboratory-established chimeras of the hydroid *H. symbiolongicarpus*. This variation is largely explained by differences in cell growth rates between cell-lineages composing the chimeras. In all classes of chimeras, cell-lineages from the original partners were detected after nearly a year of establishment, indicating that they are stable through time and that their survival rates are equivalent. They, however, had a different replication rate which resulted in a differential competitive ability. In both two-way chimeras, cell-lineage 431-63 was significantly overrepresented, constituting the majority of the colony early in the study. Thus, cell lineage 431-63 had a higher replication rate than both 833-8 and 4117-2. While two-way chimeras grew linearly, the number of polyps derived from 833-8 and 4117-2 were relatively constant during the study period. A comparison between cell-lineages 833-8 and 4117-2 showed that the latter displayed a higher rate of replication than the former, although no significant difference was detected. In the three-way chimera, differences in competitive abilities of cell lineages were not as evident as in two-way chimeras. As suggested by Stoner *et al.* (1999), competitive interactions between cell-lineages might be modified as a function of the number of competing genotypes. Constancy across replicates suggests that the competitive ability of cell lineages is genetically controlled, and therefore, heritable.

Cell-lineages composing a chimera might differ in their somatic versus gametic investments (Buss, 1982). Indeed, a cell-lineage may be disproportionately represented in the gametes at the expense of the chimera's soma. This "cell parasitism" has been observed in various colonial organisms (Buss, 1982; Stoner and Weissman, 1996; Rinkevich, 2002), and might provide an adaptive explanation for the origin and evolution of allorecognition systems in colonial invertebrates (Buss, 1982). As none of the chimeras used in this study reach sexual maturity, it was not possible to evaluate differential somatic versus gametic investment schemes between cell-lineages. Yet, there was indirect evidence that cell-lineages underrepresented in frequency (*i.e.*, 833-8 and 4117-2) behaved as cell parasites. On one hand, they were stable at low frequencies for nearly a year and thoroughly distributed in the chimera. On the other hand, during chimera regression when they were at the lowest frequency (week 44.5 post-contact), they displayed the ability to increase its representation. Thus, 833-8 and 4117-2 cell

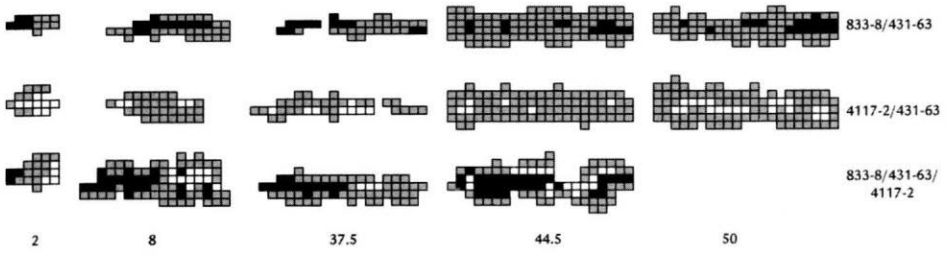

Figure 3. Spatial distribution of cell-lineages in two-way chimeras (first two panels) and the three-way chimera (third panel) at the different time intervals. Chimeras are represented by a grid where each cell is a sampled polyp. Areas of 3 x 4 cells represent a sampling region in the chimera. Black cells indicate polyps derived from cell-lineage 4117-2, gray cells are polyps derived from 431-63, and white cells are polyps derived from 833-8 cell-lineages. In three-way chimeras, grey cells represent polyps derived from 4117-2 OR 431-63 and 833-8 OR 431-63 (see Materials and Methods for an explanation). Numbers in the horizontal axis represent the five time intervals in weeks.

lineages showed a frequency-dependent proliferation ability, which characterizes cell parasites (Buss, 1982). Despite the fact that rare cell-lineages might access the gametic pool in high proportions when they behave as parasites, asexual reproduction confers an advantage to the more frequent cell-lineage. Fragmentation processes by water movements is common in sessile colonial invertebrates (Fautin, 2002), and, in colonies of *Hydractinia*, have been shown to induce a rapid and abundant production of propagules (Bavestrello *et al.*, 2000). Fragments of a chimera are likely to be composed by the most represented cell-lineage of the parental chimera.

Selection acting upon cell-lineages within individuals has long been recognized as a critical phenomenon in development and evolution. For example, cell-lineage selection is thought to act as a sieve eliminating deleterious and spreading beneficial mutations in populations (Otto and Hastings, 1998), and has been invoked as a key factor in the evolutionary transitions leading to multicellularity (Buss, 1987). Furthermore, it largely explains why mutation rates in humans have a paternal-age effect and appear to be higher in male than female gametes (Extavour and García Bellindo, 2001; Goriely *et al.*, 2003). In colonial invertebrates cell-lineage selection is likely to play an important role. First, the frequency of chimerism in natural populations of various colonial organisms is appreciable. Second, somatic cell-lineages composing a chimera display differential competitive abilities that results in a biased chimeric composition. More predominant cell-lineages have a better chance of being represented in progeny produced by asexual reproduction. If, on the other hand, rare cell-lineages behave as parasites, their representation might be higher when reproduction is sexual. Chimerism may confer competitive advantages to the colony by virtue of size increase. Mortality in sessile colonial invertebrates is size-dependent with smaller colonies displaying the highest mortality (Buss, 1990). Chimerism, as shown here, may provide an advantage to small colonies in early stages post-fusion, when cell-lineages contribute equally to the colony. However, beyond a critical size, chimerism did not greatly contribute to increase colony size because competition resulted in a predominance of one cell-lineage over the other.

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