Int. J. Dev. Biol. 47: 433-438 (2003)

Original Article

Allogeneic interactions in *Hydractinia*#: is the transitory chimera beneficial?

SHARON GILD, URI FRANK[1] and OFER MOKADY*

The Institute for Nature Conservation Research, Tel Aviv University, Tel Aviv, Israel and [1]Institute of Zoology, University of Heidelberg, INF 230, Heidelberg, Germany

ABSTRACT The colonial marine hydroid, *Hydractinia,* exhibits four possible outcomes to allogeneic contacts: passive rejection, aggressive rejection, stable fusion and transitory fusion. In the special case of transitory fusion, *Hydractinia* colonies undergo tissue fusion, followed by tissue death at the original contact area, and colony separation. This type of rejection is different in several aspects from the rejection process that accompanies incompatible encounters. It has been suggested that in transitory fusion, the colonies gain immediate benefits from fusion, mainly due to size increase, without succumbing to costs associated with fusion (germ line parasitism). We report a long-term observation of repeated fusion and separation cycles in clones featuring transitory fusion that revealed a slow-down of specific growth rates following fusion, and recovery in growth rates following separation. Very rapid transfer of stained material between partners in transitory chimeras provides suggestive evidence that protection against germ line parasitism is far from being guaranteed by separation. Our data cast doubt as to whether the benefits considered for transitory fusion are sustainable and support the already made suggestion that fusion with self, rather than fusion with kin, has been the major selective force governing the evolution of allorecognition in colonial invertebrates.

KEY WORDS: *Invertebrate allorecognition, germ line parasitism, fusion, rejection, transitory fusion*

Introduction

Invertebrate allorecognition has been widely investigated and continues to raise intriguing questions as to the origin and evolution of the vertebrate immune system (Grosberg and Quinn, 1988; Shenk, 1991; Feldgarden and Yund, 1992; Crampton and Hurst, 1994; Humphreys and Reinherz, 1994; Mokady, 1996; Rinkevich, 1998, Laird *et al.,* 2000). Many sedentary, colonial invertebrates, from the more ancient groups of sponges and cnidarians to the protochordates, are characterized by genetically controlled allorecognition systems and effector mechanisms for accepting self and kin tissue and rejecting non-related allogeneic grafts (Grosberg, 1988; Buss, 1990).

Hydractinia, a colonial marine hydroid, colonizes gastropod shells inhabited by hermit crabs (Schijfsma, 1935; Hauenschild, 1954; Müller, 1964). Several colonies may sometimes be found located in close vicinity on the same shell (Yund *et al.*, 1987; Hart and Grosberg, 1999). A typical colony of *Hydractinia* is composed of a network of gastrovascular canals, termed stolons, from which polyps arise. The colony grows asexually by lateral extension of the stolons. The progressing stolons may encounter isogeneic as well as allogeneic tissue. These contacts may result in either fusion between the encountering stolons, or rejection.

In a series of tissue transplantation assays with inbred lines Mokady and Buss (1996) determined that fusibility in *H. symbiolongicarpus* is controlled by a one locus system with multiple, codominantly expressed alleles, with one shared allele being sufficient for fusion. Later, Cadavid and Buss (1999) used AFLP markers to define the chromosomal region harboring this locus. Recent studies indicate the existence of more than one recognition gene (*e.g.*, Grosberg, 2000) in a tightly linked complex (allorecognition complex, termed ARC; L. F. Cadavid personal communications). The term 'haplotype' is thus more appropriate

Abbreviations used in this paper: ARC (allorecognition complex); IAR (incompatible allogeneic rejection); IF (isogeneic fusion); SAF (stable allogeneic fusion); TAF (transitory allogeneic fusion).

Note: Allorecognition has been studied intensively in the two closely related species *Hydractinia echinata* and *Hydractinia symbiolongicarpus*, and features very similar phenomena in both. For the sake of simplicity we use the generic term *Hydractinia* throughout this report, referring to both species.

* Address correspondence to: Dr. Ofer Mokady. The Institute for Nature Conservation Research, Tel Aviv University, Tel Aviv 69978 Israel. Fax: +972-3-6407304. e-mail: mokady@post.tau.ac.il

0214-6282/2003/$25.00

Printed in Spain
www.ijdb.ehu.es

than 'allele', and is used hereafter unless a citation of former work is involved.

A complex array of effector reactions follows the ARC-mediated recognition. Incompatible allogeneic encounters in *Hydractinia* lead to either passive or aggressive rejection (Buss and Grosberg, 1990), hereafter referred to as incompatible allogeneic rejection: IAR. Passive IAR results in the formation of a barrier between the two counterparts, whereas aggressive IAR results in massive nematocyte accumulation, continuous nematocyst discharge and abnormal growth of hyperplastic stolons (Ivker, 1972; Lange *et al.*, 1989). This may lead to the eventual destruction of at least one of the competitors. Compatible allogeneic encounters usually result in the formation of a stable chimera (hereafter referred to as stable allogeneic fusion: SAF). In some cases, however, the chimera is a temporary state. This special case was first recognized by Hauenschild (1954) and characterized later as a transitory chimera by Shenk and Buss (1991). The transitory interaction (hereafter referred to as transitory allogeneic fusion: TAF) starts with fusion and the formation of a chimera, but eventually a cytotoxic rejection occurs at the original contact area, and the colonies separate. It is not known, however, whether the chimeric state is maintained at the cellular level.

As summarized by Buss and Shenk (1990), all four possible outcomes of allogeneic interactions in *Hydractinia* may carry potential costs and benefits. Rejection occurs to maintain the integrity of self (Grosberg 1988), and to achieve dominance in the competition for the space resource (Buss, 1982, 1990; Grosberg and Quinn, 1988). Costs of IAR for the more aggressive strains may include the energy spent in the assault, possibly causing a delay in the onset of reproductive maturity or reduced fecundity (Ivker, 1972). The less aggressive strains may lose space and associated resources to the competing colony.

Fusion with allogeneic compatible colonies leads to the formation of a genetically heterogeneous entity (*i.e.*, a chimera). Most benefits attributed to chimera formation are related to size increase, which reduces the risks associated with tissue loss (*e.g.*, Harvell and Grosberg, 1988). Earlier onset of reproduction was also invoked as a potential benefit for chimerism due to size increase (Grosberg, 1988; Shenk and Buss, 1991), but the relationship between size and reproduction seems to involve interactions with additional factors (Harvell and Grosberg, 1988). Finally, it has been argued that the genetic diversity within the chimeric soma may offer better survival in an unstable environment as compared to genetically homogeneous entities (Buss, 1982; Grosberg and Quinn, 1986).

The main cost of allogeneic fusion was proposed to be germ line parasitism. Multipotent stem cells may migrate from one genet to the other following fusion, become represented in the germ line, or even take over, completely excluding the host from sexual reproduction. Evidence for germ line parasitism was reported from several chimera-forming organisms (*e.g.* Müller, 1964; Crampton and Hurst, 1994; Pancer *et al.*,1995; Stoner & Weissman, 1996; Stoner *et al.*, 1999). Fusion with close kin only would reduce the costs of germ line parasitism (Buss, 1982; Buss and Green, 1985; Buss and Shenk, 1990; Shenk and Buss, 1991). Transitory chimeras were hypothesized to offer the benefits of fusion, while avoiding, or reducing, its associated costs. At an early age the participating colonies increase their size following fusion, improve their survival, achieve earlier onset of sexual reproduction and may gain advantage from the increased genetic variability of the chimera. Upon reaching reproductive maturity they separate to avoid the risk of germ line parasitism (Buss and Shenk, 1990; Shenk and Buss, 1991). Despite its theoretical appeal, there are practical considerations that may reduce the validity of this hypothesis. Stem cells may migrate from one genet into the other before separation and persist in the host's tissue without being detected by its allorecognition system (allotypic determinants may not be expressed by these cells). Also, the continuous rejection process may consume resources, which would otherwise be allocated to growth and reproduction.

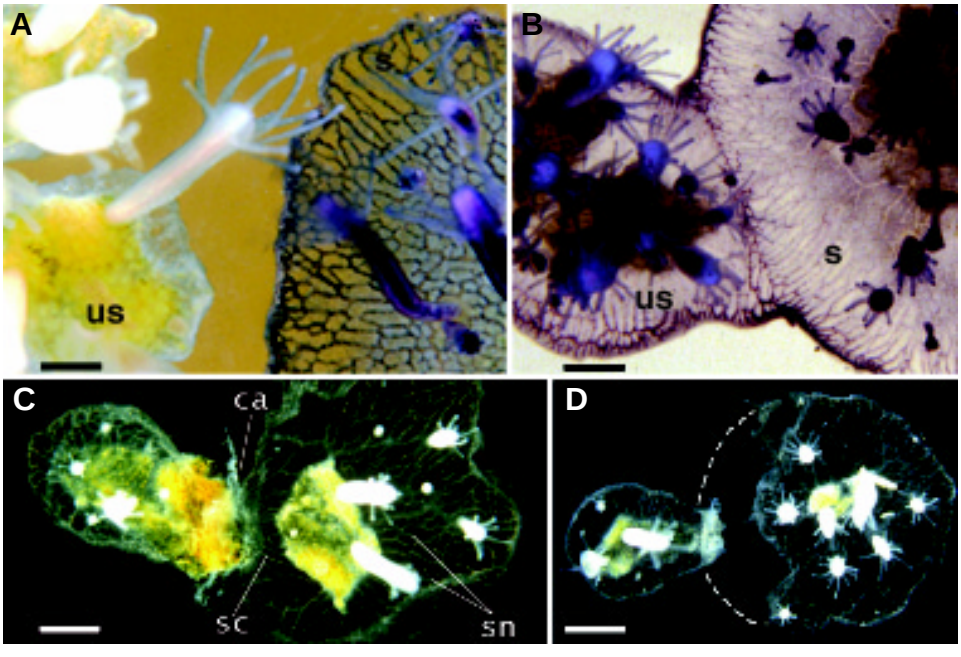

Fig. 1. Transitory chimeras in *Hydractinia*. *(A,B) Transfer of dye within a transitory chimera.* **(A)** *An unstained colony explanted near a stained colony.* **(B)** *A stained transitory chimera which was formed following fusion between an ustained (ff_A) and a stained (fx) colony. (C,D) Tissue dynamics in a transitory chimera.* **(C)** *Stolons in a transitory chimera are denser near the contact area.* **(D)** *A transitory chimera in the process of rejection. The broken line follows the original outline of the right colony prior to contact. ca, contact area; s, stained; sc, stolons at the contact area; sn, 'naive' stolons (away from the contact area; as seen in naive colonies); us, unstained (in B, us indicates the formerly unstained participant in the chimera). Scale bars: ca. 0.5 mm (A); 1 mm (B); 3 mm (C,D).*

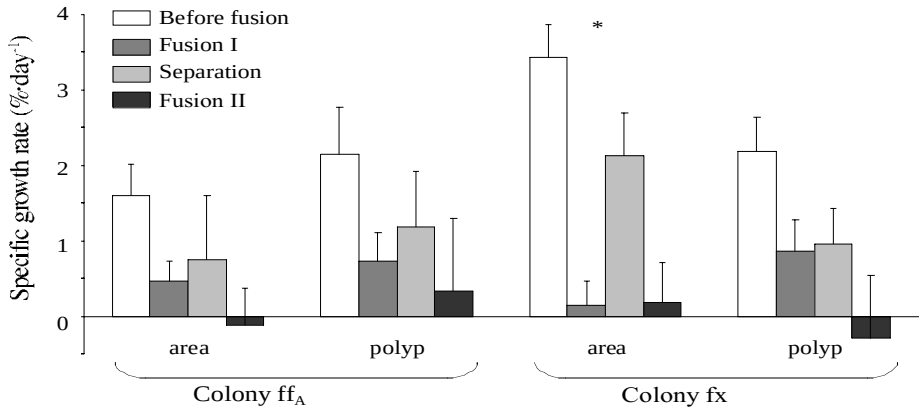

Fig. 2. Specific growth rates of participants in transitory chimeras. *Average rates of area and polyp increase (+SE) are shown for each genet, for each phase along the fusion/separation cycles. For number of colonies, see Table 1. * p<0.05.*

In the current study special attention was given to TAF. This was done by comparing long- and short-term outcomes of transitory fusion with the outcomes of the other types of alloresponses exhibited by *H. symbiolongicarpus*. Additionally, the alleged benefits of TAF were reconsidered by comparing the different phases of transitory fusion (fusion and separation cycles) with regard to changes in growth dynamics.

Results

All but one of the 6 pairwise combinations between 4 colonies of known haplotypes tested in the first experiment resulted in a SAF reaction. Interestingly, contacts involving heterozygous and homozygous colonies (fx/y and ff) resulted in different outcomes - either TAF (fx and ff$_A$) or SAF (fx and ff$_B$; fy and ff$_A$; fy and ff$_B$) were formed.

Short term responses

In the experiments involving transfer of dye, no difference was detected in the dynamics of staining of an unstained chimera partner, between SAF, TAF and IF (isogeneic fusion) interactions. In all cases staining of the unstained partner occurred within a short time after contact. First, the contact area filled with stained cells and after about 12 hours the dye has reached throughout the formerly unstained partner (Fig. 1A,B). In one case, a stained polyp budded at the contact zone of a transitory chimera. Following separation of transitory chimeras established between a stained and an unstained colony, both colonies remained tinted. As previously shown (Ivker, 1972) no transfer of dye was observed in the rejection response (IAR).

Influence of the chimeric phase on growth dynamics

Twenty three allogeneic encounters of fx vs ff$_A$ resulted in 21 TAF chimeras. Two pairs did not come into contact, and the four individual colonies were left to grow as control (naive) colonies. The chimeras were observed for a period of 6 to 7 months.

All interacting colonies followed the same general pattern of repeated cycles of fusion and gradual rejection (Table 1). In both chimeric phases, after a lag period, both colonies gradually reduced their growth rates until eventually the rate became negative and the colonies decreased in size (Fig. 2). The chimeric phases ended following gradual tissue death at the contact area (without hyperplastic stolon formation), which eventually resulted in the separation of the chimera-partners. Considering both chimeric phases, 91% of the colonies decreased their growth rate after fusing and 98% of the colonies increased growth rate after the chimera separated (Table 1). The variation between replicates in the time period of each phase may be attributed, among other factors, to differences in shape and length of the initial contact zone and the relative sizes of the interacting colonies at the time of contact.

Average specific growth rates decreased in both chimeric phases, and increased in both separation phases (Fig. 2). It should be noted that only the difference in area specific growth-rate between the four phases for colony fx was statistically significant (ANOVA by randomization, $p<0.05$). Colony fx was also the one to display a higher growth rate as a naive colony. Replicates of colony fx being part of a chimera for 180 days reached a size of 13.8±13.92 mm^2 (n=15). By contrast, explants of similar size (ca. 5 mm^2) of colony fx, maintained naively through the same period reached a size of 55.0±8.66 mm^2 (n=3).

TABLE 1

CHANGES IN GROWTH DYNAMICS OF *HYDRACTINIA SYMBIOLONGICARPUS* COLONIES PARTICIPATING IN TRANSITORY CHIMERAS, DURING TWO FUSION/REJECTION CYCLES

Phase within fusion/rejection cycle (n)	Changes in growth dynamics			
	fx		ff$_A$	
	Average time (range)[a] (days)	Colonies[b] (%)	Average time (range)[a] (days)	Colonies[b] (%)
First Chimera (21)	16 (4-42)	100	16.9 (1-44)	100
First Separation (19)	8.1 (0-50)	94	4.1 (1-7)	100
Second Chimera (13)	15.2 (1-66)	76	18.2 (0-34)	92
Second Separation (4)	9.7 (0-34)	100	9 (0-46)	100

Changes consisted of a decrease of growth rate following fusion (chimera formation) and an increase following separation. n - number of chimeras for which at least two data readings are available within the reported growth phase.

[a]The number of days from the beginning of the phase (chimera formation or separation) until the change in growth rate was observed; [b]The proportion of the colonies displaying the change in growth rate at the reported phase

Transitory chimeras displayed a unique morphological phenomenon, never observed in stable chimeras, in which the network of stolons became denser at the contact zone (Fig. 1C). This occurred during the first stages of rejection, before the flow between the two colonies completely ceased. Although local, rejection reactions within chimeras affected large proportions of both colonies. Along the rejection process the colonies narrowed toward the contact area, and the border area became characterized by an incision in the otherwise circular form of the colonies, causing a "bay" formation at the former contact zone (Fig. 1D).

Discussion

Similarities and dissimilarities between transitory fusion and other interactions

The fusion process in transitory chimeras followed the same pattern as that observed in stable, allogeneic chimeras and isogeneic fusions. By contrast, the rejection process in the transitory chimeras was found to be markedly different from that shown in IAR. Transitory rejection had a gradual nature, it was reversible and although in both cases the rejection was restricted to the original contact zone, in transitory chimeras a larger area around this zone was affected (Fig 1D). The fundamental differences between the rejection in TAF and IAR suggest the existence of separate mechanisms underlying the two reactions. We hypothesize that IAR is directly triggered by contact and is therefore probably activated by a cell surface molecule, possibly encoded at the recently defined chromosomal complex (L.F. Cadavid personal communications). Rejection in TAF, on the other hand, may be triggered by gradually accumulating factors, after such factors locally reach threshold concentration levels.

Hydractinia TAF is different from the few cases of transitory chimeras recorded in other organisms (Ilan and Loya, 1990; Shapiro, 1992, 1996; Frank *et al*., 1997). In the other cases (sponges, bryozoans and corals), allogeneic fusion is age dependent, whereas in *Hydractinia*, although age is a factor that influences the process of fusion (Shenk and Buss, 1991), additional genetic elements are probably also involved. Furthermore, *Hydractinia* colonies forming transitory chimeras, as our results show, will fuse each time they meet, even after sexual maturity, whereas participants of transitory chimeras in the other systems will not (*e.g.*, Frank et. al 1997 and references therein).

Is the transitory chimera beneficial?

Our results suggest that the fusion phase of transitory chimeras in *Hydractinia* has no apparent benefit to the involved colonies. We have shown that chimeric phases were associated with a decrease in growth rate while separation was followed by the opposite. The growth dynamics did not change immediately following phase transition. A lag of a varying number of days occurred between change of phase and change in growth dynamics (Table 1). The natural growth of *Hydractinia* colonies is characterized by a continuous increase in size through time (Buss *et al*., 1984; Blackstone and Yund, 1989; Buss and Blackstone, 1991). In our experiments, naive subclones followed this pattern. However, subclones of colony fx being part of a transitory chimera grew ca. 4 times less then non-interacting subclones. It would be safe to conclude that the negative growth rates result from the interaction with ff_A. As can be appreciated from Fig. 1 (C vs. D), the short-lived advantage of size-gain upon fusion is soon outweighed by the disadvantage posed by the negative growth rate.

In addition, our results suggest that the morphological separation of transitory chimeras holds no insurance against germ line parasitism. As opposed to rejecting responses where dye was not transferred between interacting colonies (see also Ivker, 1972), no difference in passage of material from one colony to the other was observed between transitory and stable chimeras. It took less than a day after fusion for dye to move from the stained to the unstained participant in the chimera, and about 24 hours for naive gonozooids to become stained. Precautions taken to avoid free dye in the stained colony (see Materials and Methods) suggest that the transfer of dye to the other colony in the chimera represents cell migration. It must be noted, however, that we cannot rule out the possibility of phagocytosis and transport of dead stained cells. Our conclusions are somewhat contradictory to those of Buss and Shenk (1990), who performed experiments with mixed sex chimeras. They found that in transitory chimeras both colonies expressed only their own sex whereas in permanent chimeras, deviation from the expected sex ratio was recorded (Buss and Shenk 1990). Using sex (Buss and Shenk 1990) or dye (the present study) as markers may have biased the true results. The discrepancy between those outcomes and the present study may have to await the establishment of microsatellite markers, which is in progress in our laboratories.

Buss and Shenk (1990) suggested that genets in transitory chimeras avoid germ line parasitism by separation prior to sexual maturity. Our results show, however, that colonies exhibiting the TAF reaction are destined to meet and fuse time and again during their (theoretically unlimited) life span and "infection" by allogeneic stem cells may occur with each additional fusion. Furthermore, *Hydractinia* colonies are capable of rejection without costly tissue death (passive rejection; Buss and Grosberg, 1990), whereas transitory chimerism is an expensive response with respect to size. This involves both a slowdown of growth rate while the colonies are fused as well as a massive tissue loss during the rejection process.

The evolution of allorecognition

One of the theories as to the selective forces governing the evolution of allorecognition advocates kin selection, by claiming that the need to defend against germ line parasitism on the one hand, and selective advantages of chimerism on the other hand, would favor chimera formation by relatives (Grosberg and Quinn, 1986, 1988; Shenk, 1991). Problems with this theory include the inability of kin selection to maintain high levels of polymorphism observed at the recognition loci (Grosberg, 1988; Feldgarden and Yund, 1992), and the improbability that encounters between relatives, which are expected to be rare, would be the selective force governing allorecognition (Feldgarden and Yund, 1992). Recent research, though, argues that genetically related colonies of *Hydractinia* are often found located in close vicinity on the same shell (Hart and Grosberg, 1999). It is currently unknown if paguroid crabs revisit the same specific mating grounds (W. A. Müller, personal communications). If so, a greater chance exists that a *Hydractinia* individual will meet relative colonies established in former recruitment cycles (although this would mean meeting an older and probably mature colony).

Our results cast further doubts with respect to the advantages attributed to chimerism with allogeneic tissue, as also suggested

for colonial ascidians (Rinkevich and Weissman, 1992; Chadwick-Furman and Weissman, 1995). We thus support the suggestion of Feldgarden and Yund (1992), that fusion with self rather than fusion with kin has been the major force governing the evolution of allorecognition. A mechanism for self-recognition based on partial matching of alleles (or haplotypes) at the allorecognition locus, could have been sufficient for self recognition and thus selected for in spite of incidences of non-advantageous kin fusions. These incidences could have been neutral enough so as not to be selected against, in light of the selective benefits of efficient self-recognition.

Materials and methods

Research organism

Colonies of *Hydractinia symbiolongicarpus* were obtained from the laboratory of L.W. Buss at Yale University. Two colonies, homozygous for the ARC haplotype *f* are hereafter termed ff_A (male) and ff_B (female), and two heterozygous colonies termed fx (male) and fy (female). Fx and fy are siblings from a mating between a wild type female and an F6 male of a line inbred for fusibility, considered homozygous (see Fig. 1 in Mokady and Buss, 1996). Thus, *x* and *y* may actually be identical (Mokady and Buss, 1996; alleles *r* and *q*). Ff_A and ff_B are siblings from a mating between fx and fy, determined homozygous by fusibility analysis (Mokady and Buss, 1996). Colonies were grown in aerated aquaria containing artificial seawater at 16-17ºc and fed 2-3 times a week with 3-5 day old *Artemia salina* nauplii. Colonies were grown and propagated on glass slides from which subclones (ramets) could conveniently be made.

Allogeneic interactions

Explants from chosen colonies were positioned on the same glass slide, 2-5 mm apart, and allowed to grow toward each other until tissue contact was established within a few days. Tissue contacts were made for three types of experiments. In the first experiment 3-6 replicates of all 6 possible combinations between the four colonies (ff_A, ff_B, fx and fy) were made. Only one combination (fx with ff_A) proved to exhibit TAF as the sole outcome. In the second experiment 23 replicates of this combination were made, and changes in size, polyp number and contact zone length were followed through repeated cycles of fusion and rejection. In this experiment data were taken from five phases: naive growth (before contact), first chimeric phase, post separation (inter-chimeric) phase, second chimeric phase and following second separation.

Specific growth rates were calculated for the time interval between every two readings (3-10 days), as the increment in colony surface area or polyp number per time per average area or polyp number, respectively, for that time interval (Blackstone and Yund, 1989). Growth rates were compared between the different growth phases using ANOVA by randomization (RT package; Manly, 1997).

The third experiment followed the migration of material between fused colonies. Twelve subclones of colony fx were immersed for 15 minutes in neutral red (0.1% in filtered artificial sea water), after which they were rinsed in sea water and allowed to recover for a period of 1 hour. The colonies were then immersed in a solution of toluidine blue (0.01% in filtered artificial seawater) for 2 hours, and then rinsed again. Three days later, explants of unstained colonies were placed 2-4 mm away from the stained colonies to establish tissue contacts. This procedure is very similar to the one employed by Lange *et al.* (1992), to track the origin of cells in chimeric embryos, except that they waited only 12 hours prior to grafting the embryo halves onto each other. Four types of interactions were thus compared: isogeneic fusion, hereafter termed IF (fx with fx), stable allogeneic fusion (SAF; fx with fy), transitory allogeneic fusion (TAF; fx with ff_A) and allogeneic rejection (IAR; fx with a wild type colony). Three replicates were made of each type of interaction. Before setting the interactions, precautions were taken to ensure that there was no free dye in any of the stained colonies. This was done by checking under a microscope to ensure that stained cells could be distinguished (*i.e.*, no free intercellular dye), and by checking that no dye flowed out of the stolons when the colony edge was intentionally bruised (*i.e.*, no free dye in the stolons).

Acknowledgements

This research was supported by a generous grant from the Deutche Forschungemeinschaft (DFG; grant No. FR 1346/2-1). We wish to thank M. Wolberg and J. Delaria for preparation of specimens for microscopic analysis, A. Shoob for photography, and V. Wexler for graphic assistance.

References

BLACKSTONE, N.W. and YUND, P.O. (1989). Morphological variation in a colonial marine hydroid: a comparison of size-based heterochrony. *Paleobiology* 15: 1-10.

BUSS, L.W. (1982). Somatic cell parasitism and the evolution of somatic tissue compatibility. *Proc. Natl. Acad. Sci. USA.* 79: 5337-5341.

BUSS, L.W. (1990). Competition within and between encrusting clonal invertebrates. *Trends Ecol. Evol.* 5: 352-356.

BUSS, L.W. and BLACKSTONE, N.W. (1991). An experimental exploration of Waddington's epigenetic landscape. *Phil. Trans. R . Soc. Lond. B.* 332: 49-58.

BUSS, L.W. and GREEN, D.R. (1985). Histoincompatibility in vertebrates: the relict hypothesis. *Dev. Comp. Immunol.* 9: 191-201.

BUSS, L.W. and GROSBERG, R.K., (1990). Morphogenic basis for phenotypic differences in hydroid competitive behavior. *Nature* 343: 63-66.

BUSS, L.W., MCFADDEN, C.S. and KEENE, D.R. (1984). Biology of hydractiniid hydroids. 2. Histocompatibility effector system/competitive mechanism mediated by nematocyst discharge. *Biol. Bull.* 167: 139-158.

BUSS, L.W. and SHENK, A.M. (1990). Hydroid allorecognition regulates competition at both the level of the colony and at the level of the cell lineage. In: Marchalonis, J.J. and Reinish, C. (Ed.). *Defense Molecules*. A.R. Liss Press, New York, pp. 85-106.

CADAVID, L.F. and BUSS, L.W. (1999). Genetic mapping of the allorecognition locus in *Hydractinia. Proc. 8th Int. Workshop on Hydroid Development.* Tutzing: p. 115.

CHADWICK-FURMAN, N. and WEISSMAN, I.L. (1995). Life history plasticity in chimeras of the colonial ascidian *Botryllus schlosseri*. *Proc. R. Soc. Lond. B.* 262: 157-162.

CRAMPTON, W.G.R. and HURST, L.D. (1994). True kin recognition in the form of somatic incompatibility has multiple independent origins. *Anim. Behav.* 47: 230-234.

FELDGARDEN, M. and YUND, P.O. (1992). Allorecognition in colonial marine invertebrates: does selection favor fusion with kin or fusion with self? *Biol. Bull.* 182: 155-158.

FRANK, U., OREN, U., LOYA, Y. and RINKEVICH, B. (1997). Alloimmune maturation in the coral *Stylophora pistillata* is achieved through three distinctive stages, 4 months post metamorphosis. *Proc. R. Soc. Lond. B.* 264: 99-104.

GROSBERG, R.K. (1988). The evolution of allorecognition specificity. *Quart. Rev. Biol.* 63: 377-412.

GROSBERG, R.K. (2000). Mate selection and the evolution of highly polymorphic self/nonself recognition genes. *Science* 289: 2111-2114.

GROSBERG, R.K. and QUINN, J.F. (1988). The evolution of allrecognition specificity. In: Grosberg, R.K., Hedgecock, D. and Nelson, K. (Ed.). *Invertabrate Historecognition*. Plenum Press, New York, pp. 157-167.

GROSBERG, R.K. and QUINN, J.F. (1986). The genetic control and consequences of kin recognition by the larvae of a colonial marine invertebrate. *Nature* 322: 456-459.

GROSBERG, R.K. and STRATHMANN, R.R. (1998). One cell, two cell, red cell, blue cell: the persistence of a unicellular stage in multicelluar life histories. *Trends Ecol. Evol.* 13: 112-116.

HART, M.W. and GROSBERG, R.K. (1999). Kin interactions in a colonial hydrozoan (*Hydractinia symbiolongicarpus*): population structure on a mobile landscape. *Evolution* 53: 793-805.

HARVELL, C.D. and GROSBERG, R.K. (1988). The timing of sexual maturation in clonal animals. *Ecology* 69: 1885-1864.

HAUENSCHILD, V.C. (1954). Genetische und entwicklungsphysiologische Untersuchungen über Intersexualität und Gewebeverträglichkeit bei *Hydractionia echinata* Flem. (Hydroz. Bougainvill.) *Roux Arch. Entwicklugsmech.* 147: 1-41.

HUMPHREYS, T. and REINHERZ, E.L (1994). Invertebrate immune recognition, natural immunity and the evolution of positive selection. *Immunol. Today* 15: 316-320.

ILAN, M. and LOYA, Y. (1990). Ontogenetic variation in sponge histocompatibility responses. *Biol. Bull.* 179: 279-286.

IVKER, F.B. (1972). A hierarchy of histo-incompatibility in *Hydractinia echinata*. *Biol. Bull.* 143: 162-174.

LAIRD, D.J., DE TOMASO, A.W., COOPER, M.D. and WEISSMAN, I.L. (2000). 50 million years of chordate evolution: seeking the origins of adaptive immunity. *Proc. Natl. Acad. Sci. USA* 97: 6924-6926.

LANGE, R.G., DICK, M.H. and MÜLLER, W.A. (1992). Specificity and early ontogeny of historecognition in the hydroid *Hydractinia*. *J. Exp. Zool.* 262: 307-316.

LANGE, R.G., PLICKERT, G. and MÜLLER, W.A. (1989). Histoincompatibility in a low invertebrate, *Hydractinia echinata*: Analysis of the mechanism of rejection *J. Exp. Zool.* 249: 284-292.

MANLY, B.F.J. (1997). RT - A program for randomization testing. Version 2.1. CASM, pp. 1-15. University of Otago, New Zeland.

MOKADY, O. and BUSS, L.W. (1996). Transmission genetics of allorecognition in *Hydractinia symiolongicarpus* (Cnidaria: Hydrozoa). *Genetics* 143: 823-828.

MOKADY, O. (1996). Occam's razor, invertabrate allorecognition and Ig superfamily evolution. *Res. Immunol.* 147: 241-246.

MÜLLER, W.A. (1964). Experimetelle Untersuchungen über Stockentwicklung, Polypendifferenzierung und Sexualchimären bei *Hydractinia echinata. Wilhelm Roux Arch.* 155: 181-268.

PANCER, Z., GERSHON, H. and RINKEVICH, B. (1995). Coexistence and possible parasitism of somatic and germ lines in chimeras of the colonial urochordate *Botryllus schlosseri*. *Biol. Bull.* 109: 106-112.

RINKEVICH, B. (1998). Immonology of human implantation: from the invertebrates' point of view. *Hum. Reprod.* 13: 455-459.

RINKEVICH, B. and WEISSMAN, I.L. (1987). A long term study on fused subclones in the ascidian *Botryllus schlosseri:* the resorption phenomenon (Protochordata: Tunicata). *J. Zool. Lond.* 213: 717-733.

RINKEVICH, B. and WEISSMAN, I.L. (1992). Chimeras vs genetically homogeneous individuals: potential fitness costs and benefits. *Oikos*. 63: 119-124.

SCHIJFSMA, K. (1935). Observations on *Hydractinia echinata* (Flem.) and *Eupagurus bernhardus* (L.). *Arch. Neérl. Zool.* 1: 261–314.

SHAPIRO, D.F. (1992). Intercolony coordination of zooid behavior and a new class of pore plates in a marine bryozoan. *Biol. Bull.* 182: 221-230.

SHAPIRO, D.F. (1996). Size dependent neural integration between genetically different colonies of a marine bryozoan. *J. Exp. Biol.* 199: 1229-1239.

SHENK, A.M. and BUSS, L.W. (1991). Ontogenic changes in fusibility in the colonial hydroid *Hydractinia symbiolongicarpus. J. Exp. Zool.* 257: 80-86.

SHENK, A.M. (1991). Allorecognition in the colonial marine hydroid *Hydractinia* (Cnidaria/Hydrozoa). *Amer. Zool.* 31: 549-557.

STONER, D.S., RINKEVICH, B. and WEISSMAN, I.L. (1999). Heritable germ and somatic cell lineage competition in chimeric colonial protochordates. *Proc. Natl. Acad. Sci. USA.* 96: 9148-9153.

STONER, D.S. and WEISSMAN, I.L. (1996). Somatic and germ cell parasitism in a colonial ascidian: Possible role for a polymorphic allorecognition system. *Proc. Natl. Acad. Sci. USA.* 93: 15254-15259.

VAN DE VYVER, G., HOLVOET, S. and DEWINT, P. (1990). Variability of the immune response in freshwater sponges. *J. Exp. Zool.* 254: 215-227.

YUND, P.O., CUNNINGHAM, C.W. and BUSS, L.W. (1987). Recruitment and postrecruitment interactions in a colonial hydroid. *Ecology*, 68: 971-982.

Received Online: July 2003
Reviewed by Referees: August 2003
Modified by Authors and Accepted for Publication: September 2003