Int. J. Dev. Biol. 46: 1023-1033 (2002)

Original Article

Autoaggressive, multi-headed and other mutant phenotypes in *Hydractinia echinata* (Cnidaria: Hydrozoa)

WERNER A. MÜLLER*

Institute of Zoology, University of Heidelberg, Germany

ABSTRACT In an inbreeding program conducted with the colonial hydroid *Hydractinia echinata*, each F1 mating produced up to 50% F2 offspring displaying an aberrant, clone-constant phenotype, hence referred to as mutant strain. In autoaggressive strains, in one or several areas of the colony autoreactive stolons direct their aggressive devices (stolon tips filled with cytotoxic stinging cells), normally used to kill allogeneic competitors for living space, towards neighboring stolons or polyps (hydranths) of their own colony. In these areas tumor-like masses of self-aggressive stolons were formed, in severe cases causing the death of the colony. Based on previous genetic studies, the interpretation proposed here attributes autoaggressive behavior to a mosaic-type alternative expression of *arl (allorecognition)* alleles in heterozygous individuals. Developmental mutant strains termed *He-mh* form supernumerary heads during regeneration and normal development as well. Common to all *He-mh* phenotypes is the production of additional heads along the body column of fully-grown polyps. The heads give rise to complete hydranths connected by a tube that derives from the gastric region of the original polyp and eventually transforms into a stolon. In *bastol* strains, polyps convert the basal region of their body column into a periderm-covered stolon from which the residual apical hydranth detaches. Colonies expressing both the *He-mh* and the *bastol (bst)* phenotype frequently lose detaching multi-headed hydranths and the colony disintegrates. The large number of mutant F2 offspring reveals high genetic variability in *Hydractinia*.

KEY WORDS: *Allorecognition, autoimmunity, development, Hydractinia, Cnidaria*

Introduction

Mutants are valuable tools in the analysis of developmental processes. 1) Mutant phenotypes pave the way for the identification and cloning of genes required to perform a developmental program, and they point to organismic or even molecular functions of those genes. 2) Even if irksome genetics do not allow the causative genes to be identified by methods such as positional cloning or AFLP, mutants can still be valuable tools in experiments designed to answer questions arising in classical developmental biology, such as: what are the developmental potencies of stem cells?

For instance, the fresh-water polyp *Hydra* is resistant to classic genetic analysis. Nevertheless, the few mutants available, found and described by Novak and Lenhoff (1981) and Sugiyama and coworkers (Sugiyama and Fujisawa, 1977; Nishimiya, *et al.,* 1986), facilitated the analysis of developmental processes in the experimental laboratory. Thus, multipotency of the migratory interstitial stem cells (i-cells) of hydra was verified by introducing i-cells from wild-type donors into mutant strains of *Hydra magnipapillata* (Marcum and Campbell, 1978; Sugiyama and Fujisawa, 1979; Sugiyama, 1982; Bosch and David, 1987).

At present, the few mutants of *Hydra* are the only known mutants in the entire phylum of the Cnidaria. The members of this phylum display basic features which constitute the “eu”metazoa, - that is, the “true” animals, - as they possess typical animal cells such as sensory cells, nerve cells and muscle cells. On the other hand, the circular or radial arrangement of their organs place the Cnidaria in a position of an outgroup to all bilaterian phyla. A representative of this phylum proposed as a model organism of unmatched versatility is the colonial hydroid *Hydractinia* (Frank *et al.,* 2001).

Our proposal to assign to *Hydractinia* the role of a pioneer model organism is based on features not shared by *Hydra* or reef corals, such as the feasibility of applying classical genetics. Within the phylum Cnidaria the only animals with which genetics beyond F1 or F2 offspring have been performed are the colonial sibling species *Hydractinia echinata* (Hauenschild, 1954, 1956) and *H.*

Abbreviations used in this paper: arl, allorecognition allele; bst, bastol phenotype.

***Address correspondence to:** Dr. Werner A. Müller. Institute of Zoology, INF 230, D 69120 Heidelberg, Germany. Fax: +49-6221-545-639.
e-mail: w.muller@zoo.uni-heidelberg.de

0214-6282/2002/$25.00

Printed in Spain
www.ijdb.ehu.es

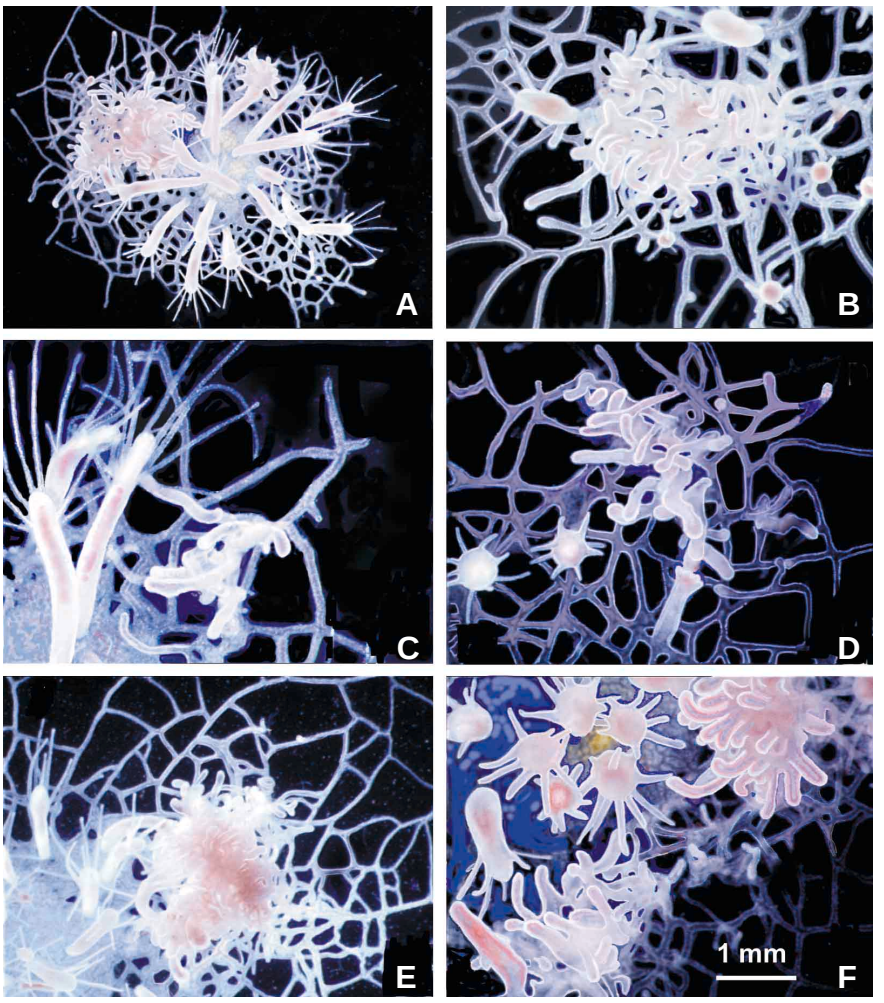


Fig. 1. Examples of autoaggressive mutants. (A) *Survey of a young colony. Tumor-like tangles of (reddish) stolons develop in the upper-left area.* **(B,C,D)** *Developing hyperblastic stolons (white, rub-like protrusions) in different offspring.* **(E,F)** *Tumor-like masses of hyperblastic stolons which would kill their own colony if not surgically removed.*

symbiolongicarpus (Mokady and Buss, 1996; Grosberg and Hart, 2000), which inhabit near-shore waters of the Atlantic Ocean, but can be maintained and bred in inland laboratories. The phenotypic trait studied was histo-incompatibility and rejection versus histo-compatibility and tolerance among allogeneic individuals.

The term individual in the present context refers to a colony of polyps. A colony is composed of a network of vascular canals, called stolons, which cover the substratum and produce, as iterative modules, a varying number of feeding polyps (gastrozooids) and sexual polyps (gonozooids, blastostyles) by budding. Budding is based on mitotic cell proliferation. All polyps of a colony are isogeneic, sharing the same genotype. In its ontogeny, each colony arises from one primary polyp, this primary polyp from one planula larva, and this larva from a fertilized egg. Therefore, each colony is isogeneic in itself but allogeneic to any other sexually generated colony. Artificially, the colonial individuals can be propagated and multiplied through a simple cloning procedure: explants give rise to new isogeneic colonies (see Methods).

Hydractinia has the capacity to discriminate between self and non-self, and was among the first invertebrates shown to display a genetically based system of intolerance against allogeneic tissue (Hauenschild, 1954, 1956; Müller, 1964; Buss *et al.,* 1984; Lange *et al.,* 1989, 1992; Mokady and Buss, 1996; Grosberg and Hart, 2000; Fuchs *et al.,* 2002).

The inherited type of histocompatibility in *Hydractinia* not only determines whether or not allogeneic transplants are tolerated or rejected. The ability to recognize and destroy foreign tissue becomes even more conspicuous in natural situations, when comparing the behaviors of expanding colonies in incompatible and compatible encounters. When a colony comes into contact with competitors for the limited substrate space, it employs aggressive devices to ward off any competitors, the surface markers of which indicate genotypic differences; these include distantly related conspecific individuals. In particular, by accumulating and discharging batteries of specialized, toxin-ejecting stinging cells at all contact sites, each colony tries to kill allogeneic adversaries. By contrast, isogeneic colonies produced by cloning, or close kin sharing the same type of histocompatibility markers, tolerate each other, grow together and form a unified colony. Fusion of close kin results in the formation of a genetic chimera. Confluent chimeric colonies are morphologically and physiologically unified and exchange stem cells (Müller, 1964).

Genetically, the type of self-markers, and hence of histocompatibility, is determined by a highly polymorphic one-locus system (Hauenschild, 1956; Mokady and Buss, 1996), termed the *allorecognition* locus *arl* (Cadavid and Buss, 1999). Sharing one or both alleles at the *arl* confers compatibility and allows fusion regardless of the sex of a clone. Therefore, even male and female colonies can fuse, if compatible, and form sexual chimeras. In such chimeras the migratory precursors of germ cells can invade the area of the former neighbor and eventually contribute to its gametes or even displace the host's own germ cell precursors (Müller, 1964). The evolutionary forces to develop mechanisms of histo-incompatibility are currently being interpreted as deriving from the need to prevent parasitism of a host by immigrated germ-line cells carrying a foreign genotype (Buss, 1982; Buss *et al.,* 1984).

In an inbreeding project aimed at finding combinations of compatible heterosexual pairs of clones, performed as a prologue

TABLE 1A

HISTOCOMPATIBILITY AMONG F1 SIBLINGS

	♀1	♀2	♀3	♀4	♀6
♂1	–	+	+/–	+	+
♂2	+	+	–	+/–	+/–
♂3	–	–	+	+	+
♂6	+/–	–	+	+	+
♂9	–	+/–	+/–	+	+

Compatible (+), incompatible (-) and transiently compatible (+/-); F1 offspring, tested in pair-wise combinations of sisters with brothers.

to studies on germ cell parasitism, I found among the F2 offspring individuals showing self-aggressive behavior. Stolons began to attack each other as if they belonged to foreign colonies. In addition, in several inbred families other aberrant, clone-constant and therefore apparently mutant phenotypes arose, such as colonies producing multi-headed polyps.

The terms "mutant" and "mutant phenotype" in the present context are used in the conventional, classic sense. It refers to aberrant phenotypes which hitherto were not, or only very infrequently, observed in natural populations, have a genetic background as revealed by sexual crosses, and can be assigned by formal genetics to putative, rare alleles having a decisive influence on its occurrence. These traditional criteria apply to the mutant phenotypes described here. Subclones of these phenotypes may facilitate new approaches with which to investigate pattern formation, cell lineage analyses or microchimerism.

Results

Survival Rates and Aberrant Phenotypes observed in the F1 and F2 Generations

Common to all investigations aimed at identifying mutant phenotypes was the inbreeding scheme. A single pair of one reproductive male and one female wild-type individual was selected to start the inbreeding project. Pairs of F1 full-siblings were mated to generate F2 offspring. This inbreeding scheme apparently revealed many genetic defects hidden in wild-type, heterozygous individuals. Mortality in F2 offspring was high. Even in cases in which the fertilization rate was high and development of the zygotes proceeded through embryogenesis, the larval stage and metamorphosis, many of the primary polyps that emerged from metamorphosis with apparently normal morphology eventually died from starvation. They were unable to catch food due to the lack of nematocysts and/or failed to ingest food due to deficits in the nervous system (as verified by microscopic examinations of selected immuno-stained individuals; see Methods). Both these failures point to defects in the founder cells that give rise to nematocytes and/or nerve cells. Mortality among the F2 primary polyps was, as a rule, between 10 and 50%, in the cross ♂7 x ♀3 it was 92% (n = 76).

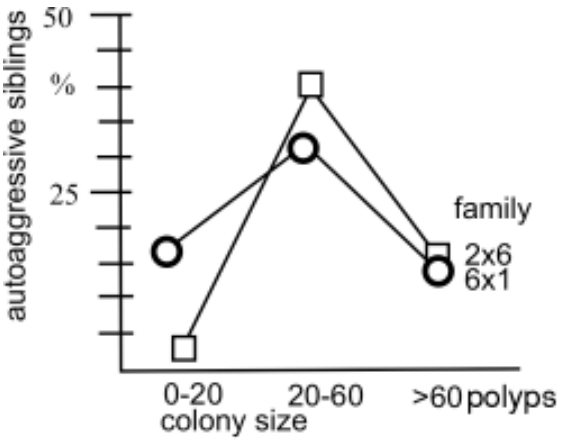


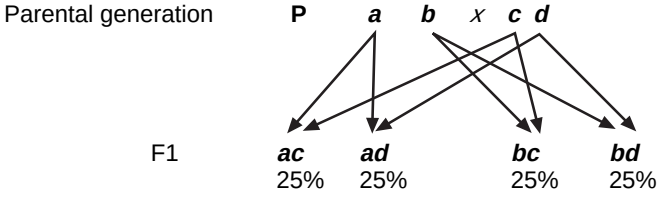
Fig. 2. Autoaggressive episodes in two F2 families. *Within the two evaluated families, up to 40% of siblings developed tangles of autoaggressive stolons when the colony size reached and surpassed 20 feeding polyps. The incidence decreased when the growth of the colonies ceased and the number of actively moving stolon tips declined.*

Unless protocols are developed to nourish primary polyps with liquid media, primary polyps that are unable to catch or ingest solid food cannot be raised to maturity nor can they be subcloned. Therefore, only clones displaying aberrant phenotypes in post-metamorphic development but able to consume solid food were maintained and investigated.

In the notation used in this paper F1 colonies are indicated by simple Arabic numbers, in crosses with the male in the first and the female in the second place. For instance 6x4 means: male number 6 of the F1 offspring mated with his sister number 4. The notation 6x4-7 designates an F2 and means offspring number 7 of the couple 6x4.

TABLE 1B

HISTOCOMPATIBILITY GENOTYPES IN F1



Compatible, incompatible and transiently compatible F1 pairs arranged according to putative genotypes, with *a, b, c, d* being different alleles at the allorecognition-locus. ♀4 and ♀6 had the same genotype with respect to the *ar*/locus.

Histocompatibility between the F1 siblings

Explants with the genetic constitution → / in contact with ↓	♀ ***ac*** ♀ 1	♀ ***ad*** ♀ 2	♀ ***bc*** ♀ 3	♀ ***bd*** ♀ 4, ♀ 6
♂ *ac* not present	(++)	(+/-)	(+/-)	(- -)
♂ ***ad*** ♂ 2	+/-	++	- -	+/-
♂ ***bc*** ♂ 3, ♂ 6	+/-	- -	++	+/-
♂ ***bd*** ♂ 1, ♂ 9	- -	+/-	+/-	++

Fully compatible ++ 4/16 = 25%
fairly compatible +/- 8/16 = 50% (or only transiently compatible)
compatible total.....up to.....75%
incompatible - - 4/16 = 25% (up to 75%, if all +/- are only transitorily compatible).

Compatibility / Incompatibility Ratios among the F1 Offspring and the Development of Aggressive Stolons in Colonies encountering Allogeneic, Incompatible Conspecifics

In the labortory as well as in nature histo-incompatibility among colonies of *Hydractinia* becomes manifest by development of aggressive stolons along the boundaries of adjacent colonies. The following description applies not only to wild-type colonies encountering incompatible conspecifis but with modifications also to the behavior of autoaggressive stolons within colonies displaying the mutant phenotype described below.

The manner in which the stolonal network in *Hydractinia* grows and colonizes available space paral-

lels angiogenesis in vertebrates. Capillary-like pioneering stolons, hence called free stolons or runners, sprout from the base of a primary polyp and advance over the substratum by means of a terminal, locomotory pathfinding organ, the pulsatile stolon tip (e.g. Donaldson, 1974, in *Proboscydactyla*; in *Hydractinia*: Müller and Plickert, 1982; Müller *et al.,* 1987; Videorecording: Müller, 1996c). Elongating stolons ramify by forming new lateral stolon tips at more or less regular intervals and distances. If an advancing tip comes into contact with the flank of another, transversely running stolon, it induces a lateral tip in the touched stolon (Müller *et al.,* 1987; Lange and Müller, 1991). In isogeneic encounters, both tips, the inducing and the induced tip, fuse and make way for a traversing canal: an anastomosis is formed through which fluid flows. In forming an anastomosis both tips abandon their existence as locomotory tip-organs.

Even in incompatible encounters an approaching stolon tip induces a lateral tip in the flank of the foreign stolon, but the two opposing tips do not fuse; they retain their tip properties and continue to advance and mutually induce further tips at contact sites. Thus in combat zones between incompatible neighbors more and more stolon tips are formed by both adjacent colonies. The terminal section of the stolon bearing the pulsatile tip lifts from the substratum and seeks contact with other, upright stolons in its proximity. With time a dense barrier of stolon tangles marks the boundary between incompatible opponents (compare Fig. 1).

Within hours or days, the stolons near contact sites swell and acquire a hyperblastic appearance as they incorporate into their ectodermal layer increasing numbers of immigrating nematocytes (microbasic mastigophores, Lange *et al.,* 1989). These accumulate at contact sites to form a palisade-like arrangement with their cnidocil directed toward the opponent. Suddenly and synchronously they discharge their toxin into the tissue of the opponent (Buss *et al.,* 1982; Lange, *et al.,* 1989, Müller, 1996c). Beseiged stolon tissue becomes paralysed and necrotic. After several attacks, one colony will die, and the winner proceeds to occupy the living space unhindered by conspecific competition.

TABLE 2A

FREQUENCY OF AUTOAGGRESSIVE OFFSPRING

Parents (F1) ♂x♀	Number of survived offspring	No. autoaggressive offspring	% autoaggressive offspring	statistical significance
2 x 1	23	6	26	
6 x 1	24	12	50	
1 x 3	**39**	**19**	**49**	$\chi 2 = 10.53$
2 x 3	**22**	**2**	**5**	**0.001>P>0.01**
6 x 3	18	1	6	
7 x 3	too few surviving (< 10%)			
1 x 4	12	3	25	
1 x 6	13	4	31	
2 x 6	**35**	**18**	**51**	$\chi 2 = 6.75$
3 x 6	**22**	**3**	**14**	0.001<P<0.01
6 x 6	20	3	15	
7 x 6	6	1		
Unrelated wild♂ x 6	27	8	30	
1 x 7	12	4	33	
7 x 7	21	7	33	
Unrelated wild♂ x 7	44	10	23	

Frequency of autoaggressive offspring in the F2 families, arranged according to common mothers. Crosses selected to show the influence of the mother (in bold: selected for significance test).

The histocompatibility responses of the F1 offspring were assessed in pair-wise combinations of explants (subclones) from 10 full-siblings. When expanding colonies touch each other, one of three reactions takes place: (1) They fuse forming a permanent chimera. (2) They do not fuse but reject and one is defeated. (3) In some instances, especially in combinations of full- and half-siblings, initially the two adjacent explants fuse at several, or even all, contact sites. But within days or weeks, the allogeneic tissues separate, and with time the aggressive devices for destroying foreign tissue are employed. This type of reaction has been called transitory fusion (Buss *et al.,* 1984).

On the basis of the one-locus *arl* system, and assuming transitory compatibility as late expression of partial mismatches of the self-markers (heterozygous *arl*-alleles), the frequencies of compatibility versus incompatibility in pairs of F1 siblings can be predicted from Table 1b. The two field-collected colonies that have been used to start the inbreeding program are considered not closely related and to share neither allele at the *arl* locus. Many unsuccessful attempts to find compatible field-collected colonies (Hauenschild 1954; Buss *et al.,* 1984, and hundreds of my own tests) justify this assumption. Moreover, incompatibility of the selected couple was verified in control tests using several explants from both parental colonies.

When compatibility is tested in the F1 offspring in pair-wise combinations of all potential genotypes, 25% of the pairings are expected to share both allorecognition alleles in identical edition and should therefore be fully compatible. In another 25% of the combinations the partners share neither *arl*-allele and hence are expected to be incompatible. In 50% of the combinations the two genotypes share one *arl*-allele but differ in the second (being semi-allogeneic). These are expected to fuse, though sometimes only transiently, depending on the degree of self-marker mismatch (see Discussion). Thus, compatibility could amount up to 75%, but, conversely, also incompatibility could reach this percentage.

The actual observations are compiled in Table 1a. They fit the expectation very well if it is assumed that one of four possible genotypes was not among the five F1 brothers chosen for the compatibility assay. In Table 1b the genotype of the F1 siblings was deduced from the compatibility data.

Autoaggressive colonies were first observed among the F1 offspring. Later also three F1 clones were identified (♀4,†♀6, ♂7,) showing a weak autoaggressive behavior as described in the following section.

Autoaggressive Strains

In the F2 families up to 50% of the offspring developed stolons displaying aggressive behavior in one or several areas of their own colony, although the individuals had never been in contact with any allogeneic conspecific tissue (Table 2a, 2b). Stolon tips were raised away from the substratum, acquired a hyperblastic appearance, bent to neighboring stolons or polyps, and discharged their toxin into them. Frequently feeding polyps adjacent to autoreactive areas tried to engulf the injured tissue and were subsequently injured themselves and became necrotic. The tangles of aggressive stolons formed dense masses resembling tumors (Fig. 1).

Thus, autoreactive areas often were surrounded by polyps undergoing necrosis. In severely autoaggressive colonies these tumor-like masses had to be removed from time to time to prevent the suicide of the whole colony. Upon their removal, often residual parts of the stolons resumed growth and aggressive behavior. Unless removal of "tumors" was done at regular intervals, several strongly autoaggressive colonies were lost.

Cloned replicates of those individuals showed the same symptoms, but they did not do so in all instances or during the entire time of observation. In most colonies the autoaggressive episode began when the colony surpassed the size of about 30 polyps. Only in the family 1x7 did autoaggressive behavior begin in colonies comprising as few as two to three polyps. The number of autoreactive siblings within each family was counted at weekly intervals until the size of a colony, quantified in terms of polyp numbers, surpassed 80-120 polyps, at which size expansion of the stolonal network and also the autoaggressive episode often ceased (Fig. 2). As a rule, the area available on coverslips did not allow unhindered growth beyond this size because the peripheral pioneering stolons had reached the edge of the substrate. Now the number of free, advancing stolon tips declined, as normally behaving tips fused with the established stolons they encountered, and lost their tip properties. Decreased number of functional tips was correlated with declining autoaggressivity. The autoreactive episode could also brought to an end by a change in the pattern of growth.

Genetically different colonies of *Hydractinia* vary considerably in their growth patterns (McFadden *et. al.* 1984; Van Winkle and Blackstone, 2002). (1) Some clones form a coherent sheet-like mat almost from the outset and cover the substratum slowly by the expanding margin of the mat. (2) Other clones initially form wide networks of tubular stolons, thus quickly seizing the entire substratum. Only when the available space is colonized do patches of mats surround the bases of the feeding polyps and fuse to form larger areas with time. (3) Most clones display an intermediate growth pattern: a central, slowly expanding mat is surrounded by a network of quickly extending free stolons, which play a pioneering role in the colonization of the substratum. When the substrate is seized and covered by a vascular network, the pathfinding organs of stolon tips decline in number. In correlation to these growth patterns, autoaggressive behavior was observed in growth type (2) and (3), but only very seldom (estimated below 5%) in colonies displaying the mat-type growth pattern. In these rare cases one or several upright stolons sprouted from the mat at or near its margin and began to attack adjacent polyps. In most cases transition of the growth pattern from net-type to mat-type was associated with recovery from the autoreactive disease.

However, several individuals as well as their subclones displayed autoaggressive behavior over the entire period of observation, - that is, up to six months.

TABLE 2B

FREQUENCY OF AUTOAGGRESSIVE OFFSPRING

Parents (F1) ♂x♀	Number of survived offspring	No. autoaggressive offspring	% auto aggressive offspring	statistical significance
1 x 3	39	19	49	
1 x 4	12	3	25	
1 x 6	13	4	31	
1 x 7	12	4	33	
2 x 1	23	12	50	
2 x 3	**22**	**1**	**5**	$\chi 2 = 9.06$
2 x 6	**35**	**16**	**51**	**0.001>P>0.01**
3 x 6	22	3	14	
6 x 1	**24**	**12**	**50**	$\chi 2 = 7.54$
6 x 3	**18**	**1**	**6**	**0.001>P>0.01**
6 x 6	20	3	15	
7 x 3	too few surviving (< 10%)			
7 x 6	6	1		
7 x 7	21	7	33	

Frequency of autoaggressive offspring in the F2 families, arranged according to common fathers. Crosses selected to show the influence of the father (in bold: selected for significance test).

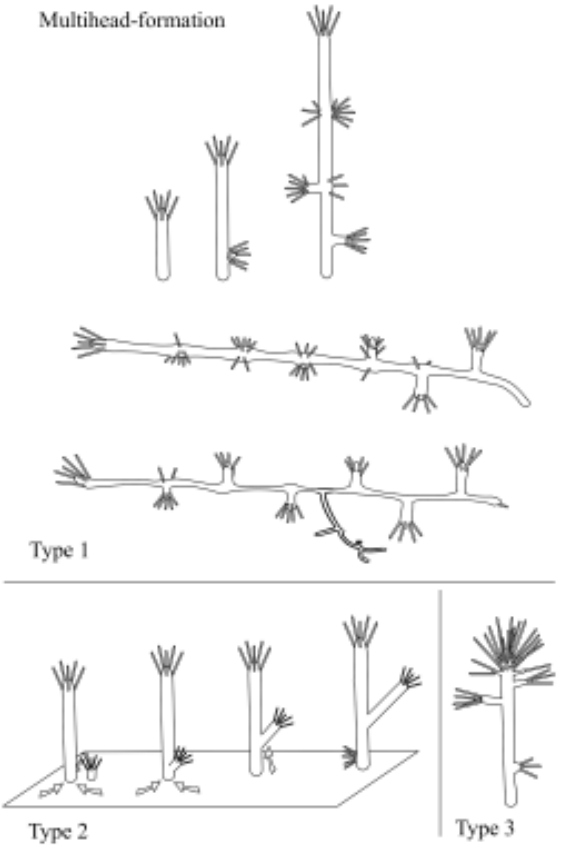


Fig. 3. Development of multi-headed polyps. *Overview of the three different modes in which multi-headed forms can arise.* **(Type 1)** *Feeding polyps elongate and form supernumerary lateral heads at regular intervals and distances.* **(Type 2)** *In the clone 3x6-20, buds form in close vicinity to established, full-grown polyps. While developing into a new polyp, the bud is shifted to the older polyp and incorporated into it.* **(Type 3)** *Supernumerary head structures as they often occur in a particular clone (7x7-21).*

Genetics of the Autoaggressive Disease

In all F2 families at least one of the offspring displayed symptoms of self-intolerance. The frequencies of the disease varied in the F2 families, and reached about 50% in the families ♂1x♀3, and ♂2x♀6; the strongest phenotypes, however, were offspring of the parents ♂1x♀7.

Of the F1 parents having self-intolerant offspring, only ♀6 was among those that showed signs of self-intolerance themselves. If the frequencies among siblings and their half-siblings sharing the

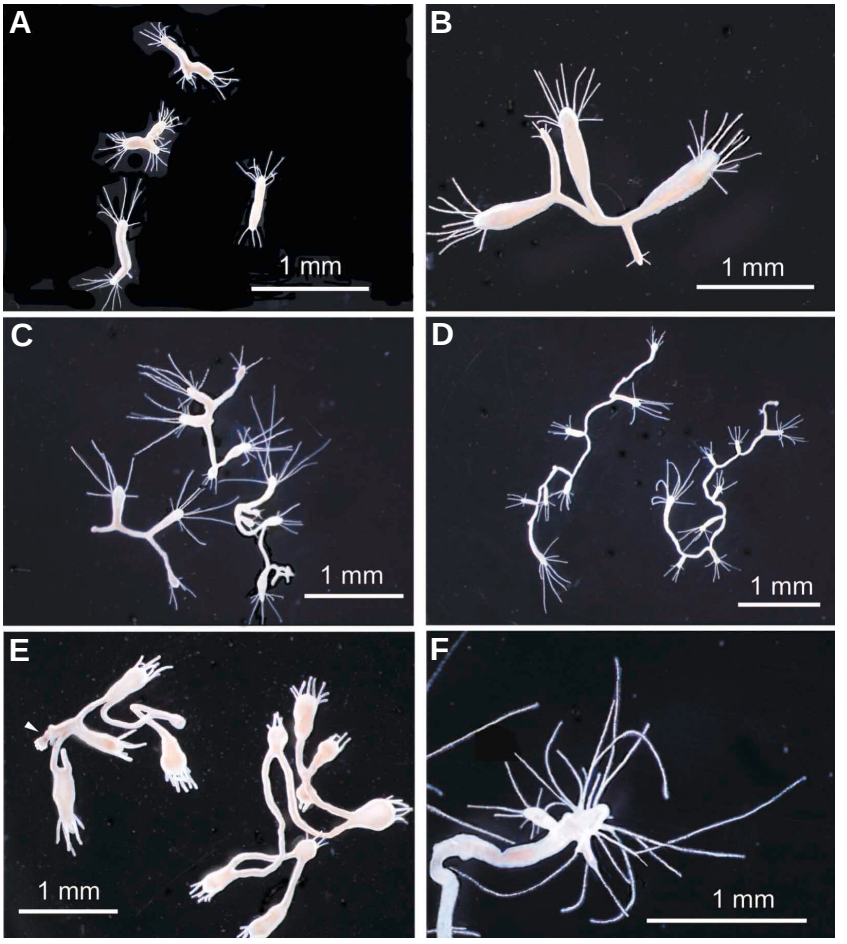


Fig. 4. Development of multi-headed polyps. **(A)** *Polyps that became detached from a colony displaying both the* mh *and the* bst *phenotypes. The isolated polyps formed a head at their lower (aboral) end and, simultaneously or subsequently, also in the middle of their body. With time, multi-headed chains arose like those in shown in B,C,D and E.* **(B,C,D,E)** *The additional heads organized the development of a complete polyp (hydranth) each. The connecting tube derived from the gastric region of the original polyp.* **(E)** *One of the 6 polyps (arrowhead) is a gonozooid which lacks long tentacles.* **(F)** *A head with supernumerary tentacles and additional hydranth-like structures as they were regularly formed by clone 7x7-21.*

same mother or the same father are compared, no consistent bias of the maternal or paternal genotype is apparent (Table 2a, 2b). To further examine a possible maternal predominance (as reported by Buss *et al.,* 1985, for *H. symbiolongicarpus*), the mothers ♀6 and ♀7 of strongly affected families were crossed with an unrelated wild-type male. Both couples generated an unexpected high ratio of sick offspring (Table 2a). This might indicate a maternal predominance but can also be explained in terms of conventional Mendelian inheritance. All scored ratios can be attributed to a pair of infrequent alleles if the strength of their expression varies between recessive and dominant, depending on the individual genetic background in the offspring.

Which alleles are of significance? When the putative genotype at the *arl*-locus in the autoaggressive F2 children is deduced from Table 1b, a non-random correlation between a particular *arl*genotype and the occurrence of self-intolerance becomes evident. In those crosses (1x3; 2x6; 6x1) that yielded the highest percentages of sick offspring, one of the F1 parents carried the *arl* allele *b.* In the crosses 3x6 and 6x6 not only was the female (genotype *bd*) identical, but also the males possessed an identical *arl* genotype (*bc*). Both these couples generated the same percentage of sick children. Common denominator in all these crosses was the possible transmission of a *b* allele from one or both parents to their offspring. In the offspring, the inherited *arl* genotype determines whether or not an individual is prone to express the disease.

On the other hand, the mother of the 2x3 family that consisted of healthy siblings (96%) also carried the *b* allele. Moreover, the *b* allele was introduced into the inbreeding families by a healthy P1 male or female (healthy at least during sexual maturity). According to the known *arl*genetics, each *arl*allele is codominantly expressed and contributes to the molecular composition of the self-marker molecules on the surface of the individual (Mokady and Buss, 1996). Thus, expression of this allele as such is not the direct cause of hereditary diseases occurring in F2 offspring. Therefore, the mutant phenotype must be assigned to a gene, or to genes, outside the *arl*-locus. A frequency of up to 50% offspring with mutant characteristics is compatible with Mendelian genetics if the decisive alleles display hypomorphic features (s. Discussion).

Multi-Headed Strains

In the Hydrozoa, the head of polyps has properties of an organizing center comparable to the Spemann-organizer in vertebrates. Upon transplantation head tissue can induce the development of secondary body axes (review: Müller, 1996a). In *Hydra*, and most probably in all polyp-type Cnidarians, in the center of arising heads the WNT-signalling system is installed (Hobmayer *et al.,* 2000). This system governs axis formation in many eumetazoan animals.

Among the Cnidarian species and strains maintained in laboratories only two putative mutants are known to exhibit a striking aberrant morphological phenotype: a multi-headed “non-budding” strain of *Hydra viridis* (Novak and Lenhoff, 1981) and the multi-headed mutant *mh-1* of *Hydra magnipapillata* (Sugiyama, 1982). Phenocopies of such mutants can be produced by periodic treatment of wild-type hydras (*H. magnipapillata*, wt 105) with activators of protein kinase C such as tumor-promoting phorbol esters or diacylglycerol. The polyps elongate and successively form additional, lateral organizing centers in more or less regular intervals and distances; these develop heads structures and these give rise to heads-bearing lateral branches (Müller, 1989, 1990).

In the present study multihead-formation was observed in several F2 colonies and their subclones. Fully-grown polyps were detected bearing supernumerary, ectopic heads along their body column. Observations of their ontogeny revealed three modes of origin of multiple heads.

Type 1: (Fig. 3, Fig. 4 B-E) This is the classic mode described above: The polyps (hydranths) elongate and develop heads one after the other along the elongating body column. With time, each

new head gives rise to a side branch of the body axis. Each side-branch adopts the quality of a separate hydranth. If such multi-headed forms were isolated from the colony and feeding was carried on, they continued to form heads, and freely floating, long chains of hydranths resulted. The tube-like structure connecting the hydranths is the derivative of the gastric region of the original polyp. It retained a polyp-type organization for several days or weeks, as testified by the lack of a peridermal cover and by its longitudinal contractility. When segments were cut out, they regenerated heads at both ends, as did segments excised from polyps of the same colony still exhibiting a normal morphology (Fig. 4A). When fed, bi-headed polyps developed into chains of multiple hydranths as well.

With time, the tube-like structure connecting the hydranths became thinner and thinner, and eventually parts of these tubes transformed into true, periderm-covered stolons, forming lateral stolon tips, and adhering to the substratum by these tips. Once attached to a substratum, the stolons elongated, branched and gave rise to new polyps by budding. When full-grown, these polyps become multiheaded.

Type 2: (Fig. 3) Developed ramified multi-headed polyps in a different, hitherto unknown mode. In the ontogeny of *Hydractinia* colonies, whether normal or mutant, a growing polyp incorporates cells from the stolonal compartment. Cells of the stolonal tissue flow as a coherent sheet toward and into the polyp, just as an emerging bud of a hydra incorporates cells flowing from the parental body column into the bud.

In the present study, among the full-siblings of the family 3x6 whose members had a high potential to develop or regenerate ectopic heads, one individual (3x6-20) was found to develop ectopic heads differently from its siblings. Within close proximity to growing feeding polyps, a second polyp emerged from stolonal tissue. Subsequently it became shifted and incorporated into its adjacent older companion, resulting in a ramified polyp. In a few cases even a third, adjacent polyp was incorporated. Later in the development additional heads were also formed in elongating polyps according to the Type 1 mode.

Type 3: (Fig. 3; Fig. 4F) This was found in one member of the 7x7 family. The head of the feeding polyps sprouts many tentacles in irregular patterns, and directly below this zone of irregularly arranged tentacles, several small secondary body axes bearing one single, enlarged tentacle or small hypostomes emerged (Fig. 3; Fig. 4F). Segments excised from young, not yet ramified polyps regenerated heads at both ends and gave rise to multi-headed forms. In older colonies, the cellular interior of the stolons retracted in the direction of the head and eventually disappeared, leaving behind an empty peridermal tube. The fate of the disappeared stolon cells is unclear at present.

TABLE 3

***MULTI-HEADED (mh)* AND *BASTOL (bst)* PHENOTYPES IN THE F2 FAMILIES**

Parents	No. of offspr.	*mh*	*bst*	Coincid. *mh+ bst*	Ratio *mh/No. offspr.*	Ratio *bst/No. offspr.*
1x3	39	2	3	1	0.05	0.08
2x3	22	0	2	0	0	0.09
2x6	35	2	7	2	0.06	0.20
3x1	22	4	4	1	0.18	0.18
6x1	29	4	16	3	0.14	0.55
6x3	18	3	4	2	0.17	0.22
6x6	20	3	4	2	0.15	0.20
7x7	21	5	5	2	0.24	0.24

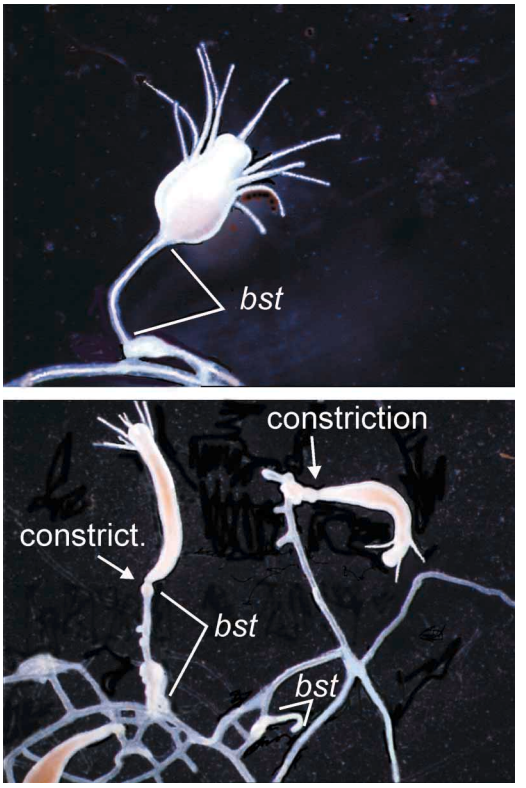


Fig. 5. The *bastol (bst)* phenotype. *The basal (aboral) part of the polyp converts into a stolon. Eventually, the remaining apical (oral) hydranth becomes detached at the point of constriction.*

Formation of Stolons by Multi-Headed Polyps

Fully-grown feeding polyps lose, as a rule, the capacity to regenerate stolons, obeying the developmental "rule of distal transformation" (Müller *et al.,* 1986). Multi-headed polyps formed stolons, though often only weeks after their isolation from the mother colony. Irrespective of their particular mode of origin, the chains of interconnected heads underwent the same characteristic transformation. With time the heads and their adjacent tissue acquired the appearance of normal looking hydranths projecting laterally out of a long interconnecting stolon-like tube (Fig. 3; Fig. 4). This tube derived from the original body column which transformed into the (flexible) stem of a tree bearing heads like blossoms. The tube became thinner with time but retained a polyp-type organization for several days or weeks, as testified by the lack of a peridermal cover and by their longitudinal contractility. But eventually, parts of this tube transformed into true stolons which adhered to the substratum, formed periderm-covered branches, and gave rise to new polyps by budding.

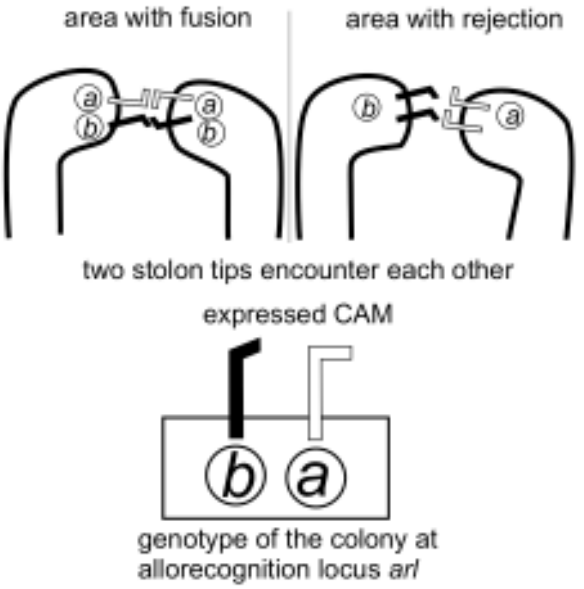


Fig. 6. A self-intolerance hypothesis.

The "Bastol" Phenotype

In several F2 colonies older polyps converted the basal region of their body column, topographically corresponding (homologous?) to the region just above the budding zone in *Hydra,* into a periderm-covered, upright stolon (Fig. 5). The remaining apical hydranths on top of these upright stolons detached upon a constriction of this site. In some colonies, this constriction was seen even before the basal part of the polyp lost contractility and secreted a periderm. The phenotype is called *bastol* (from *bas*al and *sto*lonization, abbreviated *bst*).

Detachment of polyps from a parental producer is common in *Hydra* but not known from *Hydractinia*. The fate of the detached polyp was different: Some few detached hydranths formed a stolon and gave rise to a subclone, some remained unchanged until observation was finished several weeks after their detachment, and some formed a head at their lower end. Such bipolar head-bearing forms derived mainly from colonies displaying besides the *bst* phenotype also an *mh* phenotype. When fed, they gave rise to long chains of hydranths as described above.

The *bastol* phenotype was frequently, but not always, correlated with *mh* phenotypes (Table 3). In some *bst* and *mh* mutants, chains of hydranths detached from the colony. Loss of polyps could lead to the disintegration and the eventual death of the respective colony.

Stability and Reproducibility of the Mutant Phenotypes

As outlined above, autoaggressive behavior was an episodic phenomenon in most clones. On the other hand, if new subclones were established, they again developed autoaggressive stolons as long as the colonies expanded and free stolons with pulsatile, advancing stolon tips were present. Clones with persisting strong autoreactivity were lost as they eventually detoriated.

The expression of the multiheaded phenotype was strongly dependent on the feeding scheme. Multi-head formation could be avoided in most clones if the colonies were fed on poor fare (*Artemia* larvae 5-7 days old, 2 x per week) which allowed no or only weak growth of the colony. With conventional feeding schemes used in our laboratory since years (*Artemia* 3-4 days old, 5 times per week), with time the *mh*-phenotypes and their subclones regularly developed multiheaded polyps whereas other clones and field collected wild-type colonies did not respond in this way.

Discussion

Self-Intolerance and Autoaggression

In vertebrates, with their adaptive immune system, discrimination between self and non-self is based on a learning process. In the thymus, potential autoreactive T-lymphocytes are eliminated. Autoimmune diseases may result from incomplete elimination of such potential autoreactive lymphocytes or by other mistakes of the adaptive immune system.

Hydractinia echinata, like other invertebrates, does not have such an elaborate immune system at its disposal. Its ability to distinguish self from non-self is based on innate mechanisms. Errors in genetically based historecognition systems call for explanations different from those adduced to explain self-intolerance in vertebrates, although mutant phenotypes in vertebrates and invertebrates may display common traits, for instance develop tumors. However, the tumor-like masses of autoaggressive stolons shown here (Fig. 1) cannot be equated with true tumors because their development is a normal response in the contact zone between incompatible colonies.

Autoaggressive behavior similar to that described here has previously been observed and described in the sibling species *Hydractinia symbiolongicarpus* (Buss *et al.,* 1985). However, though the phenotypic expression of the genetic disease was similar in both species, the investigation reported by Buss *et al.* (1985) and the analysis of the phenomenon described here arrived at different conclusions in two important aspects. (1) In *H. symbiolongicarpus* the onset of the autoreactive episode occurred at times and colony sizes at which germ cells matured in normal siblings. Autoreactive behavior was interpreted as a response to self-markers expressed only with the onset of germ-cell maturation. In *H. echinata* self-intolerance occurred much earlier in post-metamorphic life, shortly after self-markers are expressed and incompatibility responses are observed in natural encounters as well as in transplantation (Fuchs *et. al.,* 2002). (2) Autoreactivity in *H. symbiolongicarpus* was interpreted as a maternally inherited defect. In animals, maternal effects are common in the early embryonic phase (mainly based on mRNA transcribed and stored during oogenesis) but very rare in the postembryonic phase of life (mainly based on maternally imprinted or mitochondrial genes). Although a maternal bias cannot be definitively excluded in the phenotypes analysed here, a paternal contribution is clearly demonstrated. Even within inbred families the father was of significance. One and the same female mated consecutively with different brothers produced sick offspring at different rates depending on which brother was allowed to become the father (Table 2a).

The following proposal to explain the phenomenon is based on my interpretation of the molecular nature of the self / nonself markers. I assume that the polymorphic *arl*-alleles code for various isoforms of a homophilic cell adhesion molecule X-CAM. Since both *arl* alleles are codominantly expressed, each heterozygous individual presents two different CAM's on the surface of its ectodermal cells (Fig. 6). In allogeneic encounters mismatch of both CAM's evokes mechanisms of defence. Fusion can occur if

the two counterparts share at least one *arl* allele and, therefore, expose at least one matching CAM isoform. However, partial match and partial mismatch sometimes result in only transient fusion, depending on the degree of mismatch, the time course with which the alleles are expressed and, thus, the current densities of the different CAM's on the surface of both counterparts.

Also within one and the same colony that is heterozygous at its *arl,* recognition of self, and therefore self-tolerance and fusibility, are dependent on permanent codominant expression of both alleles. Only codominant expression enables ectodermal cells to present at least one matching CAM isoform to other ectodermal cells. In the autoaggressive strains, newly formed motile stolon tips sometimes express only one of the two alleles, the other allele being silent. A colony displaying a strong autoaggressive phenotype consists of a mosaic with areas or cell clusters expressing the paternal and other clusters expressing the maternal *arl*-allele. If a stolon tip equipped with only one of the CAM isoforms comes into contact with adjacent tissue expressing only the other allele, the mutually presented CAM's do not match (Fig. 6). The stolons are unable to recognize the autologous origin of autoaggressive tissue and attack it erroneously. According to this model *diss* mutants are always heterozygous at their *allorecognition* locus. If with time both alleles are expressed, correct recognition is possible and the stolon adopts or resumes compatible behavior. This explains the frequently observed spontaneous regression of the tumor-like stolon masses, and the subsequent rescue of the individual.

Not all phenomena associated with self-reactivity can be readily subsumed into the proposed hypothesis. In colonies displaying a strong phenotype frequently stolon tips raised away from the substrate and took an upright position without having direct contact with other tissue. Apparently, in these colonies a strong disposition to aggressive behavior broke through without being elicited by non-matching historecognition markers.

Parallels and Differences with respect to Autoimmune Diseases in Humans

It may be pointed out that in mammals autoreactive behavior of the immune system could likewise derive from episodic non-codominant expression of MHC molecules in various tissues and body parts. To my knowledge, this possibility has not yet been taken into account in the search for origins of autoimmune diseases. Moreover, the proposed mosaic-type pattern of *arl* expression parallels the mosaic-type expression pattern of X chromosome-linked alleles caused by random inactivation of one of the two X chromosomes in the embryonic development of females. Female mammals, including humans, consist of a mosaic of cell clusters expressing alternatively the maternal or the paternal X chromosome-resident alleles. While such chimerism in gene expression is immunologically tolerable in organisms able to learn and to become familiar with the molecular equipment characteristic of the individual body, chimerism can be fatal in an organism which has to rely on its innate immune system only.

Multi-Headed He-mh *Mutants*

The occurrence of multi-headed mutants is explainable in terms of conventional theories of biological pattern formation as applied to hydra. In Turing-type reaction-diffusion systems an aysymmetrical pattern, for instance a body column with a head at the one end and a foot at the other end, can be transformed into a periodic pattern by changing a few parameters. In model simulations a periodic emergence and arrangement of heads can be achieved if the body column is elongated and the production rate of the activating morphogen increased (Meinhardt and Gierer, 2000; S. Berking, pers. comm.). Alternative models of pattern control in hydra are being developed (A. Marciniak, pers. comm.).

Several of detached or artificially removed polyps continued to form supernumerary heads over weaks or months without forming stolons. They remained freely floating (e.g. Fig. 4D) and resembled the multiheaded green hydras described by Novak and Lenhoff (1981) or the multi-headed hydras and *Hydractinia* polyps produced by periodic application of PKC activators (Müller, 1985, 1989, 1990). Other multiheaded forms eventually formed stolons which attached to the substrate (Fig. 3, Typ1), especially when subjected to lipid-free diet.

The transformation of the former gastric region into stolon tissue between the series of heads in multi-headed chains such as shown in Fig. 3, can be attributed to long-range assistance of heads in decreasing positional value at distant positions (that is normally at the lower end of the body column). In *Hydra* this long-range assistance promotes foot formation (Müller, 1990, 1995, 1996a,b), in *Hydractinia* it may promote stolon formation.

In accordance with this interpretation, the transformation of the basal body region into stolon tissue in polyps of *basto!* mutants can tentatively be ascribed to an increased long-range promotion by the head of stolon formation in the most distant body region. In terms of this hypothesis, the frequent coincidence of the *mh* and the *bst* phenotypes may have not only a genetic background, but also an epigenetic cause, being supported by long-range interactions at the physiological level.

The meaning of the detachment of hydranths in the *bst* phenotypes, unknown in wild-type animals, remains an enigma. Is it a kind of atavism?

"Mutants", a Critical Appraisal

The term "mutant" in the present context refers to aberrant phenotypes and not to physically identified DNA sequences underlying these phenotypes. In spite of the rapidly growing genomic databases, the vast majority of mutants described in animals and plants still belong to this merely phenomenologically defined category. Loss-of-function or gain-of function mutations as cause of aberrant phenotypes are deduced from the behavior of the aberrant traits in consecutive crosses. In the present study the appearance of aberrant phenotypes in F2 offspring testifies to a decisive role of recessive alleles in causing or promoting their occurrence.

This genetic background does not exclude non-genetic influences on the strength with which the mutant phenotype is expressed, nor does it exclude the occurrence of phenocopies. Previously, multi-headed polyps have been detected in two situations: (1) in colonies that have deteriorated due to long-lasting intoxication by incompatible, aggressive conspecifics (video recording, Müller, 1996c), and (2) in several laboratory colonies overfed with freshly hatched, lipid-rich *Artemia* larvae (this effect may be mediated by polyunsaturated fatty acids activating protein kinase C, unpublished). The quality and quantity of food also affects the strength of autoaggression.

One might argue that mutations per definition have a genetically stable character. This argument applies to the DNA sequence of a mutated gene but not to its phenotypic expression. Most mutations do not cause a complete loss of function but are hypomorphic

exhibiting reduced functionality, or they display incomplete dominance. The classic genetics know innumerable hypomorphic and conditional alleles of which the penetrance (expressivity) depends on the genetic background and on environmental influences, even on local conditions within the body (e.g. Stansfield, 1991; Snustad *et al.,* 1997). For instance, in humans the mutation *polydactyly*, though classified as dominant, not only exhibits variable penetrance among different individuals (Snustad *et al.,* 1997, p70) but even within one and the same individual: the *polydactylous* condition may be penetrant in the left hand (6 fingers) and not in the right (5 fingers), or it may be penetrant in the feet and not in the hands (Stansfield, 1991, p27). This classic example finds its parallel in the autoaggressive colonies described here: in some areas the colonies are normal, in others they display the mutant phenotype.

This twofold dependence of the aberrant phenotypes from genetic as well as from external factors, advises caution regarding interpretations in terms of classic Mendelian rules, all the more embryonic mortality was not quantified and post-metamorphic survival rates were variable and in some crosses as low as 5%. (These objections, however, apply as well to all mutants in mammals, because early embryonic mortality rates in these animals are always unknown).

The frequency with which a particular mutant phenotype occurred in this study was fairly reproducible and the frequencies scored in replicate crosses could be compiled. The scored ratios did not contradict predictions based on Mendelian genetics. Up to 50% offspring are compatible with Mendelian rules if the phenotype is attributed to one decisive, deficient allele exhibiting variable expressivity, but is not well compatible with the notion of a rare combination of several 'normal', i.e. fully functional, equivalent genes which would cause an aberrant phenotype only in a particularly unfavourable combination of their alleles.

Materials and Methods

Raising and Sexual Propagation of the Animals

Wild-type male and female colonies growing on shells inhabited by hermit crabs were collected from near-shore shallows of the North Sea along the coast of the isle of Sylt, near the marine biological station (Alfred Wegener Institut, List/Sylt). After removal of the crabs, shells bearing mature colonies were shipped to Heidelberg and the colonies were maintained at 18°C in an 80-L aquarium, in a current of aerated, recirculating artificial seawater under a light/dark cycle of 14/10 h year-round. To collect fertilized eggs (F1), the colonies were transferred in the morning to aerated glass bowls. Spawning occurs 1-2 h after light onset. Released and fertilized eggs were collected during early cleavage and transferred into filter-sterilized seawater. Thereafter the colonies were fed with *Artemia* nauplii (see below), and about 6 h after feeding returned into the aquarium.

Sexes are separate in *Hydractinia*. As a rule, a shell accommodates only one, a male or a female colony. Colonies to be crossed were kept in separate, aerated or gently shaken containers. A cross of one female and one male wild-type colony was used to generate F1 planula larvae. If a male individual was to be crossed with several of his sisters or, inversely, one female with several of her brothers, the crosses were done in separate, consecutive setups. Each couple was kept strictly separate, preventing the uncontrolled occurrence of half-siblings.

Induction of Metamorphosis

Within 48-57 h zygotes develop into planula larvae competent to metamorphose. The standard method for triggering metamorphosis consists of a 3-h pulse treatment with 116 mM Cs^+, prepared by mixing artificial seawater with an iso-osmolar CsCl stock solution. (Events associated with metamorphosis are reviewed in Frank *et al.,* 2001, and Müller and Leitz, 2002). Metamorphosis of the planula into a primary polyp, the founder of a new colony, commences with the adhesion of the larvae onto a substrate and with flattening of its anterior pole. Subsequently, a longitudinal contraction transforms the spindle-shaped body into the disc-stage of metamorphosis. A disc just flattened can easily be removed from the substrate and transferred onto any new substrate such as a glass slide or coverslip. Here the planula completes its development into a primary polyp. 3-ml plastic dishes containing a coverslip with one primary polyp each, were placed on a gently moving shaker. When the size of colonies surpassed about 30 polyps, the coverslip was transferred into a 50-ml Falcon tube with a conical bottom.

Feeding

In contrast to *Hydra, Hydractinia* does not tolerate freshly hatched, lipid-rich *Artemia*. Therefore, *Artemia* nauplii only 5-7 days old, hatched and maintained at 18°C, were used. The tiny young primary polyps were fed with a cut-off abdomen of the crustacean larva. Young colonies were fed 2-3 times a week, larger colonies 4-5 times. After 6 h the water was exchanged.

Checking Primary Polyps unable to Feed

A selection of primary polyps which failed to feed were fixed with paraformaldehyde, and subsequently immuno-stained to visualise in whole mount preparations nerve cells containing neuropetides of the widespread RFamide class (Grimmelikhuijzen, 1985). Additional examination with DIC optics allowed the nematocysts to be counted.

Clonal Replicates

The generation of subclones (clonemates), - that is clonal multiplication of colonies,- was achieved by a simple explantation procedure. The explants consisted of one or several feeding polyps with adhering stolon tissue. As a rule, from the peripheral, youngest area of the stolon mat rectangular pieces containing 1-6 feeding polyps were cut out with a fine scalpel, removed from the original substrate together with their chitin-containing, adhesive underlayer, and placed onto the substrate of choice. Initially, the pieces were gently held in place by putting glass splinters on the stolon tissue until the regenerating tissue resumed growth and adhered to the substrate by newly secreted adhesive.

Histocompatibility Assays

Histocompatibility was tested in pair-wise combinations of allogeneic colonies. An explant from one colony to be tested was placed in front of growing stolons of the second colony to be tested, or adjacent to the margin of its mat tissue. Over a period of several weeks or months, the pair was observed to determine whether they fused upon contact, forming a stable chimera, or did not fuse but rejected and attacked each other.

Statistics

For testing the significance of ratios, the Fisher-Yates χ^2 test was performed based on 2x2 contingency tables.

Conclusion

In summary, the study here points to a high genetic diversity in *Hydractinia* populations. This diversity is correlated with a high diversity in growth patterns, growth rates, final morphologies and in the allorecognition system. The results challenge studies on population biology and ecological adaptations in habitats so highly variable and demanding as are the tidal and subtidal shores of the North Atlantic Ocean.

Acknowledgements

I wish to thank Dr. Uri Frank, Regina Teo and unknown reviewers for critically reading the manuscript and making suggestions for improvements.

References

BOSCH, T.C.G. and DAVID, C.N. (1987). Stem cells of *Hydra magnipapillata* can differentiate into somatic cells and germ line cells. *Dev. Biol.* 121: 182-191.

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

BUSS, L.W., MOORE J.L. and GREEN D.G. (1985). Autoreactivity and self-tolerance in an invertebrate. *Nature* 313: 400-402.

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

DONALDSON, S. (1974). Terminal motility in elongating stolons of *Proboscidactyla flavicirrata. Amer. Zool.* 14: 735-744.

FRANK, U., LEITZ, T. and MÜLLER, W.A. (2001). The hydroid *Hydractinia*: a versatile, informative cnidarian representative. *Bioessays* 23: 963-971.

FUCHS, M-A., MOKADY, O. and FRANK, U. (2002). The ontogeny of allorecognition in a colonial hydroid and the fate of early established chimeras. *Int. J. Dev. Biol.* 46: 699-704 (2002).

GRIMMELIKHUIJZEN, C.J.P. (1985). Antisera to the sequence Arg-Phe-amide visualize neuronal centralization in hydroid polyps. *Cell Tissue Res.* 241: 171-182.

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

HAUENSCHILD, C. (1954). Genetische und entwicklungsphysiologische Untersuchungen über Intersexualität und Gewebeverträglichkeit bei *Hydractinia echinata* Flem. (Hydrozoa, Bougainvill.). *Roux's Arch. Entwicklungsmech.* 147: 1-41.

HAUENSCHILD, C. (1956). Über die Vererbung einer Gewebeverträglichkeit–seigenschaft bei dem Hydroidpolypen *Hydractinia echinata*. *Zeitschrift für Naturforschung* C11b: 132-138.

HOBMAYER, B., RENTZSCH, F., KUHN, K., HAPPEL, C.M., CRAMER VON LAUE, C., SNYDER, P., ROTHBÄCHER, U. and HOLSTEIN, T.W. (2000). WNT signalling molecules act in axis formation in the diploblastic metazoan *Hydra. Nature* 407: 186-189.

LANGE, R., 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.

LANGE, R.G. and MÜLLER, W.A. (1991). SIF, a novel morphogenetic inducer in Hydrozoa. *Dev. Biol.* 147: 121-132.

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.

MARCUM, B.A. and CAMPBELL, R.D. (1978). Developmental roles of epithelial and interstitial cell lineages in hydra; analysis of chimeras. *J. Cell Sci.* 32: 233-247.

MCFADDEN, C.S., MCFARLAND, M.J. and BUSS, L.W. (1984). Biology of hydractiniid hydroids. I. Colony ontogeny in *Hydractinia echinata* (Flemming). *Biol. Bull.* 166: 54-67.

MEINHARDT, H. and GIERER, A. (2000). Pattern formation by local self-activation and lateral inhibition. *Bioessays* 22: 753-760.

MOKADY, O. and BUSS, L.W. (1996). Transmission genetics of allorecognition in *Hydractinia symbiolongicarpus* (Cnidaria: Hydrozoa). *Genetics* 143: 823-827

MÜLLER, W.A. (1964). Experimentelle Untersuchungen über Stockentwicklung, Polypendifferenzierungen und Sexualchimären bei *Hydractinia echinata*. *Wilhelm Roux Archiv Entwicklungsmech.* 155: 181-268.

MÜLLER, W.A. and PLICKERT, G. (1982). Quantitative analysis of an inhibitory gradient field in the hydrozoan stolon. *Roux's Arch. Dev. Biol.* 191: 56-63.

MÜLLER, W.A. (1985). Tumor-promoting phorbol esters induce metamorphosis and multiple head formation in the hydroid *Hydractinia*. *Differentiation* 29: 216-222.

MÜLLER, W.A., PLICKERT, G. and BERKING, S. (1986): Regeneration in Hydrozoa: distal versus proximal transformation in *Hydractinia*. *Roux's Arch. Dev. Biol.* 195: 113-518.

MÜLLER, W.A., HAUCH, A. and PLICKERT, G. (1987). Morphogenetic factors in hydroids. I. Stolon tip activation and inhibition. *J. Exp. Zool.* 243: 111-124.

MÜLLER, W.A. (1989). Diacylglycerol induced multihead formation in *Hydra*. *Development* 105: 306-316.

MÜLLER, W.A. (1990). Ectopic head and foot formation in *Hydra:* Diacylglycerol-induced increase in positional value and assistance of the head in foot formation. *Differentiation* 42: 131-143.

MÜLLER, W.A. (1995). Competition for factors and cellular resources as a principle of pattern formation in *Hydra*. II. Assistance of foot formation by heads and a new model of pattern control. *Dev. Biol.* 167: 175-189.

MÜLLER, W.A. (1996a). Pattern formation in the immortal *Hydra*. *Trends Genet.* 12: 91-96.

MÜLLER, W.A. (1996b). Head formation at the basal end and mirror-image pattern duplication in *Hydra vulgaris*. *Int. J. Dev. Biol.* 40: 1119-1131

MÜLLER, W.A. (1996c). Defense of conspecific habitat competitors in *Hydractinia echinata*. VHS-Video C 1907, IWF (Institut fur den Wissensch. Film), D 37075 Goettingen, Germany.

MÜLLER, W.A. and LEITZ, T. (2002). Metamorphosis in the Cnidaria. *Cand. J. Zool.* 80: 1735-1754.

NISHIMIYA, C., WANEK, N. and SUGIYAMA, T. (1986). Genetic analysis of developmental mechanisms in hydra. XIV. Identification of the cell lineages responsible for the altered developmental gradients in a mutant strain, reg-16. *Dev. Biol.* 115: 469-478.

NOVAK, P.L. and LENHOFF, H.M. (1981). Asexual reproduction and regeneration properties of a nonbudding mutant of *Hydra viridis*. *J. Exp. Zool.* 217: 213-223.

SNUSTAD, D.P., SIMMONS, M.J. and JENKINS, J.B. (1997): Principles of Genetics. John Wiley & Sons, Inc. New York

STANSFIELD, W.D. (1991). Theory and problems of Genetics. 3d ed., MacGraw-Hill, Inc., New York

SUGIYAMA, T. and FUJISAWA, T. (1977). Genetic analysis of developmental mechanisms in hydra. I. Sexual reproduction of *Hydra magnipapillata* and isolation of mutants. *Dev. Growth. Differ.* 19: 187-200.

SUGIYAMA, T. and FUJISAWA, T. (1979). Genetic analysis of developmental mechanisms in hydra. VI. Cellular composition of chimera hydra. *J. Cell Sci.,* 35: 1-15.

SUGIYAMA, T. (1982). Roles of head-activation and head-inhibition potentials in pattern formation of hydra: Analysis of a multi-headed mutant strain. *Amer. Zool.* 22: 27-34.

VAN WINKLE, D.H. and BLACKSTONE, N.W. (2002). Variation in growth and competitive ability between sexually and clonally produced hydroids. *Biol. Bull.* 282: 156-165.

Received: August 2002
Reviewed by Referees: November 2002
Modified by Authors and Accepted for Publication: November 2002