



## Behind anemone lines: factors affecting division of labour in the social cnidarian *Anthopleura elegantissima*

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When natural clonal aggregations of the sea anemone *Anthopleura elegantissima* expand and encounter other clones they can form distinctive anemone-free zones, several centimetres across. Contact between isolated pairs of nonclonemate polyps often triggers a directed, aggressive response via deployment of fighting tentacles (acrorhagi), suggesting that agonistic interactions between polyps arrayed along interclonal boundaries produce the anemone-free borders. Prior studies indicated that competing clones consist of at least two morphologically and functionally distinguishable castes: (1) small, well-armed 'warrior' polyps along borders and (2) larger 'reproductive' polyps that are more likely to carry ripe gonads. Here we show that patterns of division of labour are more complex, with clones potentially consisting of up to five intergrading castes: 'scouts' (small polyps along interclonal borders); 'warriors' (larger polyps in rows immediately adjacent to scouts); 'free-edge' polyps; and in the centres of clones, large 'reproductives' and small 'reserves'. Quantitative observations of interactions between intact clones further revealed the expression of a complex array of previously unknown behaviours, including (1) incursion of 'scouts' into border areas and their subsequent death or repulsion, (2) the occurrence of multipolyp conflicts and (3) stereotypical searching behaviour of heavily armed warriors immediately following tidal inundation. Field surveys of intact clones also corroborate the existence of substantial interclonal variation in patterns of division of labour. To distinguish environmental from genetic and ontogenetic contributions to this variation, we experimentally tested the capacity of two stimuli characteristic of interclonal contact (tentacular contact and acrorhagial attacks) to induce morphological characteristics typical of warrior castes. These stimuli significantly increased average numbers of acrorhagi per polyp for reproductive polyps, but the magnitude of response varied among clones. This clone-specific variation in responsiveness, together with the complexity of agonistic behaviours, may partially explain the expression and maintenance of interclonal variation in the frequencies of different castes and patterns of division of labour.

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The ability to form stable kin groups, and the capacity for communication between group members, together provide the foundation for the evolution of morphological and behavioural division of labour among the individual members of a group (Hamilton 1987). Many social organisms display extensive variation among groups in patterns of division of labour, arising from differences

in the frequencies of particular behavioural types or morphological castes that constitute the group or colony (e.g. Robinson 1992; Crozier & Pamilo 1996; Strassmann et al. 2000; Velicer et al. 2000; Crespi 2001; Holway & Case 2001; Page & Erber 2002). For example, honeybee, *Apis mellifera*, hives often differ with respect to allocation to foraging, brood rearing and other worker activities (Page & Peng 2001), as well as to the proportions of males and females (Crozier & Pamilo 1996).

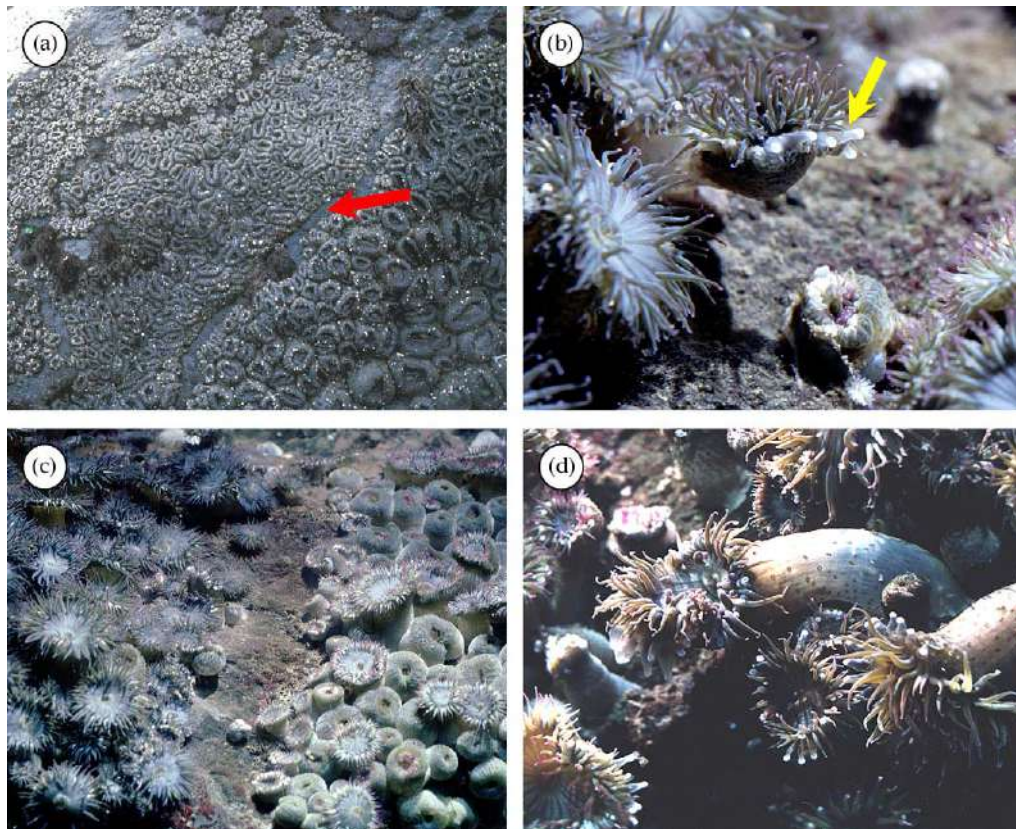
A critical first step towards understanding the evolutionary significance of this variation is to distinguish within- and among-group effects of genetic, developmental and environmental factors on the expression of division of labour (reviewed in Beshers & Fewell 2001). In the case of honeybees at least, colony-level differences

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in social organization arise from complex interactions between the state of a colony as a whole, and variation in the physiological condition, age, prior experience and genotype of individual workers (reviewed in Seeley 1995; Giray et al. 2000; Page & Erber 2002). In social organisms, such as Hymenoptera and Isoptera with obligate sexual reproduction and potentially complex within-group genetic structure (Robinson 1992; Fraser et al. 2000; Beshers & Fewell 2001; Page & Erber 2002), it therefore remains a major challenge to distinguish the contributions of genetic, developmental and environmental factors to the expression and maintenance of intraspecific variation in division of labour (Page & Erber 2002). From this perspective, facultatively asexual animals that form isoclonal social groups, including aphids and many clonal marine invertebrates, offer several important advantages over purely sexual species (reviewed in Ayre & Grosberg 1996; Knowlton 1996). First, the reproductive conflicts of interest that characterize the hives, nests and colonies of many social insects, and that can influence the expression and evolution of patterns of division of labour, are generally absent in isoclonal groups (Stern & Foster 1997). Second, it is straightforward to separate the contributions that clonal identity (genotype) and environment make to the expression of variation in patterns of division of labour.

Here, we characterize within- and among-clone variation in patterns of division of labour in the clonal sea anemone, *Anthopleura elegantissima* (Brandt 1835), and experimentally examine the effects of naturally occurring stimuli that may control the induction of different behavioural and morphological castes. Like many other social animals, *A. elegantissima* (Brandt 1835) lies in the middle of a continuum of physical integration of its parts: its functional units (individual polyps) lack direct morphological connections, but they often form dense aggregations of close relatives, in this case, clonemates (Francis 1973a, b; Sebens 1982). The establishment of these aggregations may enhance the ability of the clone to capture larger prey than individual polyps could subdue, as well as reduce desiccation and shear stress (Ayre 1984; Shick 1991), and may also limit the potential for invasion by inter- and intraspecific competitors (e.g. Buss 1981; Holway & Case 2001). For example, in some populations of *A. elegantissima*, stable (2–4 years) anemone-free borders, up to several centimetres wide, often separate clonal aggregations (Francis 1973a, b; Sebens 1982; Fig. 1a).

These borders presumably reflect the outcomes of aggressive competition between clones. In laboratory studies of *A. elegantissima*, contact between individual polyps from different clones elicits agonistic behaviour (Francis 1973a, b; Ayre & Grosberg 1995, 1996). An



**Figure 1.** (a) Two adjacent *Anthopleura elegantissima* clones, separated by an anemone-free border, showing interclonal and intracolonial variation in polyp size (red arrow denotes interclonal border); (b) a polyp mounts a directed acrorhagial attack on a smaller nonclonemate (yellow arrow denotes inflated acrorhagus); (c) an interclonal border invaded by one 'scout' in the laboratory aquarium stud; and (d) a battle across a border, showing attacks by warriors several rows back from the edge of the clone.

attacking polyp inflates a set of nonfeeding tentacles, the acrorhagi, and applies one or more of them onto an opponent (Fig. 1b). The nematocyst-laden tips of the acrorhagi then detach from the opponent, leaving an ectodermal 'scar' or 'peel'. The nematocysts in the detached ectoderm discharge, inflicting local tissue damage. An opponent may then retaliate in similar fashion. In most cases, one of the anemones retreats by pedal locomotion; however, if retreat is impossible, the victim of an extensive attack may die from its wounds (reviewed in Ayre & Grosberg 1995, 1996). Laboratory studies further show that in pairwise trials, the polyp with the greater number of acrorhagi generally wins a fight (Ayre & Grosberg 1995, 1996). However, polyps with relatively large numbers of acrorhagi per unit body mass generally have smaller (if any) gonads than less well-armed clonemates, implying that there is an allocation trade-off at the level of individual polyps (Francis 1976; Ayre & Grosberg 1996).

The formation of aggregations also provides the opportunity for these allocation trade-offs to be expressed at the level of the entire social unit through morphological or behavioural specialization of units. Not surprisingly, therefore, clonal aggregations of *A. elegantissima*, like some flatworm parasites (e.g. Sapp et al. 1998) and colonies of many social insects, show division of labour, producing morphs (or castes) that appear to play specialized defensive/offensive roles and reproductive roles. Most strikingly, polyps positioned along interclonal borders have been described as warriors (Francis 1976), and these generally differ from their centrally located clonemates in at least three morphological respects: (1) they are smaller; (2) they possess more acrorhagi overall and on a per-gram basis; and (3) their acrorhagi are larger (Francis 1976; Ayre & Grosberg 1996). Limited evidence further suggests that warrior polyps are less likely to develop gonads than centrally located individuals (Francis 1976), prompting us to dub the midclone polyps as 'reproductives' (Ayre & Grosberg 1996). It remains unclear, however, whether these position-specific differences reflect energetic allocation trade-offs or simply differences in size. In any case, these morphological differences also represent functional differentiation: smaller, better-armed warriors can defeat much larger reproductive individuals (Ayre & Grosberg 1996). Francis (1976) recognized a third caste of polyp (free-edge), situated along clonal margins that do not abut other clones. These polyps are roughly the same size as warriors, but generally have fewer acrorhagi.

Our previous study of the characteristics of warrior and reproductive morphs of 14 clones of *A. elegantissima* further revealed substantial interclonal variation in patterns of caste allocation and division of labour (Ayre & Grosberg 1996). At one extreme, some clones appear to be nearly monomorphic with respect to acrorhagial armament, with both warrior and reproductive members of a clone being heavily armed. At the other extreme lie clones in which the reproductive polyps carry relatively few acrorhagi, with warriors bearing more acrorhagi than members of monomorphic clones. Aside from differing in their allocation to armament, clones also vary in their

propensity to attack first and to retaliate against non-clonemates (Ayre & Grosberg 1995).

The existence of dramatic interclonal differences in social organization and behaviour raises the question of how such diversity persists in the face of competition for an apparently simple resource, namely habitable space (Ayre & Grosberg 1995). To the extent that agonistic interactions between pairs of warrior polyps determine the outcomes of interclonal competition, our previous studies suggest that neighbouring clones are rarely competitive equals, and that interclonal boundaries should be unstable (Ayre & Grosberg 1996). However, these interclonal dominance trials involved replicated contests between pairs of individual polyps, isolated from the rest of their clonemates. Consequently, as in interactions between social insects (e.g. Hölldobler 1981; Fellers 1987; Adams 1990), the outcomes of these behavioural studies may better reflect the competitive abilities of individual members of an aggregation, whereas the dynamics and outcomes of competition in nature are likely to depend upon the collective morphologies and behaviours of the members of intact aggregations or colonies (Buss 1981; Holway & Case 2001).

In this context, four key aspects of interclonal competition that could account for the stability of interclonal expression of clone-specific variation in patterns of division of labour remain unexplored in *A. elegantissima*. First, the range of behaviours shown by individual polyps may vary according to whether they fight in isolation (as in our previous studies, and during the initial phases of clonal establishment), or in the company of their clonemates (also see Francis 1988; Gordon 1995; Herbers & Choiniere 1996). Second, predictions of competitive outcomes based on fights between isolated pairs of polyps assume that neighbouring clonemates play little, if any, direct role in fighting opposing clones. Third, because *A. elegantissima* typically lives in the turbid waters of the wave-exposed rocky intertidal, little is directly known about the nature, frequency, intensity and importance of behavioural interactions along borders between intact clones. Fourth, the outcomes of interactions at naturally occurring borders may reflect the ability of the 'clonal army' to generate and deploy replacement fighters from deeper within the clone. This should, in turn, depend upon the spatial distribution of castes, and how, or if, nonwarrior castes can be transformed into warriors.

In this paper, we expand the social context of previous studies of division of labour and interclonal aggression beyond the behaviours of individual polyps by documenting for the first time patterns and dynamics of behavioural interactions along a natural border between two intact clonal aggregations of *A. elegantissima*. We also quantify within- and among-clone patterns of variation in the morphology of polyps adjacent to, and distant from, naturally occurring interclonal boundaries. Finally, we experimentally show how intra- and interclonal variation in the effects of stimuli such as tentacular contact and acrorhagial scarring on the induction of acrorhagi in nonwarrior morphs can at least partially explain observed patterns of within- and among-clone variation in the expression of division of labour.

## METHODS

### Study Site

We conducted all of the field and laboratory studies using *A. elegantissima* clones colonizing two artificial jetties on the Doran Park and Bodega Head shores that protect the mouth of Bodega Bay Harbour (approximately 1.5 km south of the Bodega Marine Laboratory, subsequently abbreviated BML). The jetties, constructed in 1941, consist of large, quarried shale boulders (typically >2 m diameter).

### Agonistic Interactions along an Intact Interclonal Boundary

In September of 1998, we collected from the Doran Park jetty an entire boulder that carried two relatively extensive clones with a well-formed 35-cm-long interclonal boundary (Fig. 1c). We used heavy equipment to transport this boulder to the Bodega Marine Laboratory. We maintained the clones on the boulder in a flow-through aquarium ( $\approx 800$  litres), which we drained and filled on a daily tidal cycle. We allowed the polyps to feed daily for 30–60 min on dense cultures of adult *Artemia*, after which we drained the tank to flush off debris and simulate a low tide. We then refilled the tank. Between draining and filling cycles, we adjusted the flow to  $\approx 10$  litres/min.

We could readily distinguish the clones on the basis of the colours and pigment patterning on their tentacles and oral discs; in this case, one clone ( $N = 240$  polyps) was pink and the other ( $N = 370$  polyps) brown. Initially, there were 22 polyps of each clone along the interclonal boundary. Our preliminary observations of this border (within 2 weeks of collection) revealed that agonistic encounters occurred daily, but were especially intense after flooding from the previous simulated 'low tide'. We subsequently monitored these clones and the interclonal boundary for 1 week. During this third week, we made a total of 21 h of detailed daily observations and photographic records of the behaviours of all polyps (e.g. Fig. 1b–d) for 1-h periods between 0800 and 1100 hours and both before and after inundation (carried out between 1500 and 1700 hours).

### Transect Studies of Morphological Variation Within and Among Clones

To characterize within- and among-clone patterns of morphological division of labour with respect to distance from interclonal boundaries, we collected 100 polyps per clone from each of 10 clones with extensive (80–120 cm) interclonal boundaries (e.g. Fig. 1a). For each clone, we positioned 10 approximately evenly spaced transects, each aligned perpendicularly to the boundary. Each transect included 10 contiguous rows (with respect to the orientation of the interclonal boundary) of polyps, with the first row of polyps situated on the interclonal boundary. We collected anemones from clones 5 and 11 on the Doran Park jetty in October 1998 and from clones

14–21 on the Bodega Head jetty in May 1999. We placed all sampled anemones in individually labelled bags for transportation to BML, where we relaxed and dissected them within 18 h of collection. We weighed each polyp and counted the number of acrorhagi. We determined the gender of polyps by examining the basal portion of mesenteries using a dissecting microscope. Male polyps display cream gonads with white foci; the presence of numerous brown ova distinguishes ripe females (Francis 1976).

### Acrorhagial Induction Experiments

To determine whether interclonal contacts could induce the production of acrorhagi, we carried out two experiments, each of which mimicked one of the principal interactions that we observed between the polyps arrayed along the interclonal border. We collected all polyps for these induction experiments in August and September of 1998 from the Doran Park and Bodega Head jetties. We gently removed anemone polyps from the rock surface using a flat head screwdriver and immediately discarded animals with ruptured pedal discs or columns. We then used a scalpel blade to scrape their pedal discs clean of algae and other foreign material. So that we could easily move individual animals in the stimulation experiments described below, we allowed the detached and cleaned polyps to reattach to small, dry pebbles ( $\sim 2$ –4 cm diameter) that we collected from nearby beaches. We submerged polyps that had attached within 4–8 h in the BML flow-through sea water system (at 14–16°C), using them within 48 h of collection. In subsequent experiments we maintained anemones in aquaria for 16 days and fed them every fourth day with adult *Artemia*. To count acrorhagi, we relaxed polyps by immersion in 10% MgCl<sub>2</sub> in deionized water. Polyps behaved normally soon after reimmersion in sea water. At the conclusion of all trials we weighed all of the polyps after first slicing open their coelenterons and blotting off excess water.

#### *Effects of repeated acrorhagial stimulation*

We tested the hypothesis that, for both free-edge and central polyps, the receipt of acrorhagial attacks from nonclonemate warriors would increase the number of acrorhagi per polyp. We collected polyps from the interclonal boundaries (the external row of warrior polyps abutting the interclonal border), free edges (the external row of polyps abutting anemone-free space), and centres (reproductive polyps located at least 10 polyp diameters distant from the nearest interclonal boundary or free edge) of seven large clones that each displayed both an extensive interclonal boundary and free edge.

On both days 1 and 8 we exposed 8–10 of the reproductive and free-edge polyps of each of the seven clones to acrorhagial attacks. We left a comparable number ( $N = 8$ –13 per clone) of reproductive and free-edge polyps from each clone unstimulated as controls. To standardize the intensity of stimulation we used two additional highly aggressive clones, both with large and relatively uniform acrorhagi, as attackers. We used

alternate clones as attackers on days 1 and 8. Thus, all polyps in all seven experimental clones received attacks from the same stimulatory clone on a given day. We first induced these attacking polyps to inflate their acrorhagi by stimulating them with the tentacles of polyps of a third clone. We then allowed the stimulated attackers to peel the ectoderm of two of their acrorhagi onto the column of each recipient polyp. Because each attacking polyp displayed approximately 40 inflated acrorhagi, we used each polyp to attack several victims.

One day 1 of the experiment, prior to the first acrorhagial stimulation, we relaxed all of the polyps in both the experimental and control groups and counted their acrorhagi. We then allowed all of the polyps to recover, and initiated stimulations on the experimental groups. On day 15 we counted the acrorhagi and weighed all of the experimental and control animals. To estimate the relative impacts of the attacks on the conversion of nonwarrior (i.e. free-edge and central polyps) to warrior castes for each clone, we also weighed 10–13 warrior polyps that we had collected at the same time as the reproductive and free-edge polyps and maintained in the aquaria for an identical period.

#### Effects of repeated tentacular stimulation

We used reproductive polyps from six pairs of clones that shared an interclonal border to test the hypothesis that allogeneic (i.e. nonclonemate) tentacular (as opposed to acrorhagial) stimulation would increase the number of acrorhagi per polyp. Preliminary trials involving prolonged contact of polyps from each interclonal pairing elicited acrorhagial attacks by polyps of one or both clones within each pair, indicating that all pairwise interactions were, in fact, allogeneic.

For each pair of clones, we randomly allocated 10 polyps from each clone to a treatment and a control group. Except during the stimulation periods, we normally maintained all of the polyps within each treatment and control group in separate aggregations with their similarly treated clonemates. We reciprocally stimulated all polyps within each treatment group by haphazardly pairing polyps from neighbouring clones, and allowing tentacular contact for up to 10 min per day for 12 days within a 14-day period. If one, or both, of the polyps inflated its acrorhagi before the end of the 10-min interval, we separated polyps to prevent acrorhagial fighting and terminated the stimulus for the day. On the 15th day we compared the blotted wet weights and number of acrorhagi per polyp of stimulated polyps and controls.

## RESULTS

### Agonistic Interactions along an Intact Interclonal Boundary

Throughout the 3-week observation period, the two clones remained separated by a clear and stable anemone-free zone, approximately two polyp diameters across (Fig. 1c). Nevertheless, we observed high levels of agonistic behaviour at the interclonal boundary. These

behaviours featured both an unexpected diurnal periodicity and previously undescribed behavioural sequences.

During the 21 h of detailed observation, we noted 68 cases of acrorhagial inflation (involving complete inflation of some or all acrorhagi by 21 pink polyps and 47 brown polyps) and 22 acrorhagial attacks that resulted in the scarring of an opponent (3 by pink polyps and 19 by brown polyps) (Table 1). There was a strong association between inundation following a simulated low tide and acrorhagial inflation: 77% of inflations occurred in the hour immediately following reimmersion compared with 4% and 19% in the morning and immediately prior to draining, respectively.

Previous observations of acrorhagial inflation and acrorhagial attacks in isolated pairs of polyps revealed a directed response to stimulation by a specific nonclonemate (reviewed in Ayre & Grosberg 1995). In particular, only acrorhagi on the stimulated side of a polyp fully inflated. Our observations of interactions along this intact border substantially differed. Although there were some cases of both directed (and often reciprocated) attacks involving pairs of nonclonemate polyps that had recently made tentacular contact across (or within) the interclonal border, many polyps that inflated their acrorhagi and completed attacks were located up to three polyp diameters (rows 2–4) from the interclonal boundary and were surrounded by quiescent clonemates. Soon after resubmergence, many polyps within rows 1–4 simultaneously lengthened their bodies and inflated acrorhagi around their entire crown before initiating a stereotypical searching behaviour. Searching involved a twisting and rotation of the now elongated column. The behaviour typically ceased either within the hour if the polyp failed to make contact with a nonclonemate, or when the behaviour led to an attack on a nonclonemate in or across the interclonal border (Fig. 1b, d). In several cases (although none of these was captured by our formal observation periods), agonistic interactions at the border involved simultaneous attacks and reciprocation by a number of polyps within the first 3–4 rows and so gave rise to ‘battles’ (Fig. 1d).

Although the position of the interclonal border remained almost completely stable over the 3-week study, polyps directly adjacent to the boundary (i.e. the first row) made a total of 24 incursions into the border within the final week of close observation (12 pink and 12 brown polyps). Direct observation of fighting or the presence of

**Table 1.** Summary of agonistic and locomotory behaviour, recorded during 21 h of observation of a pair of adjacent clones of the sea anemone *Anthopleura elegantissima*

Behaviour	Pink clone	Brown clone
Acrorhagial inflation	21	47
Acrorhagial attacks	31	9
Polyp deaths	3	0
Border incursions	12	12

We scored acrorhagi as inflated only when they were fully expanded. Attacks entailed application of acrorhagi onto the body of an opponent and subsequent peeling of acrorhagial ectoderm. Border incursions involved movements of up to 3 cm.

new scar tissue on these polyps showed that on every occasion, the opposing clone attacked these 'scouts'. Several distinct outcomes followed these incursions into the border. Nineteen scarred polyps retreated back to the margins of their own clone after initiating fights or eliciting attacks that they failed to reciprocate; at least one of these retreated some seven rows into its own clone. In addition, three badly scarred polyps (all pink) remained in the interclonal border for up to 4 days before they died and were dislodged from the boulder by water movement (Fig. 1b, c, Table 1). At least one of these polyps, Stumpy, was repeatedly attacked and scarred by polyps from both clones (we also observed similar behaviour during periods of more casual observation).

In total, four undescribed behaviours featured prominently in these interactions: (1) movements of small peripheral polyps into the anemone-free space of the interclonal border; (2) retreat of damaged polyps into the matrix of clonemates; (3) cyclical acrorhagial inflation of the several rows of polyps adjacent to the border; and (4) columnar extension and searching behaviour of these polyps. The latter two behaviours closely followed inundation.

### Patterns of Division of Labour Within and Among Clones

Of the 10 clones that we used to characterize patterns of intraclonal morphological variation, we sampled two (5 and 11a) after the normal reproductive season of *A. elegantissima*. Consequently, their polyps lacked mature gonads. Among the eight remaining clones, 77% of the 800 polyps that we examined were reproductively mature, and seven of these consisted exclusively of male or immature polyps (clones 14, 15 and 17–21). Only one clone (16) was female.

This suggested that male clones successfully occupy larger areas of shore than female clones. We therefore estimated the sex ratios of established clones (i.e. continuous aggregations represented by >100 polyps) by collecting a large, presumably mature, polyp from the approximate centre of each of 100 separate aggregations, spaced at ~2-m intervals, along almost the entire length of each jetty at the entrance to Bodega Harbour. In the two sampled populations, there was a slight, although not significant, excess of males on the Bodega Head jetty (58 males:41 females; chi-square test:  $\chi^2_1 = 2.919$ ,  $P = 0.088$ ) and similar numbers of males and females on the Doran Park jetty (47 males:45 females). In each case, a minority of the sampled polyps were immature.

On average, the 10 *A. elegantissima* clones that we surveyed had significantly smaller, better-armed polyps closer to interclonal boundaries and larger, less well-armed, polyps deeper within clones (Fig. 2). Indeed, we detected highly significant effects of row number (i.e. distance from an interclonal border) on the mean weight of polyps ( $F_{9,81} = 2.09$ ,  $P < 0.05$ ), number of acrorhagi per polyp ( $F_{9,81} = 17.73$ ,  $P < 0.0001$ ), and number of acrorhagi/g ( $F_{9,81} = 20.81$ ,  $P < 0.0001$ ) (Table 2). All clones showed a trend towards decreasing investment in

weaponry (number of acrorhagi/g) with increasing distance from interclonal borders, although we did detect striking variation among clones (Fig. 2, Table 2). Furthermore, a log-linear analysis of the relationship between row number and reproductive condition for the eight clones sampled during the 1999 breeding season showed that the proportion of sexually mature polyps increased with distance from an interclonal boundary ( $P < 0.001$ ).

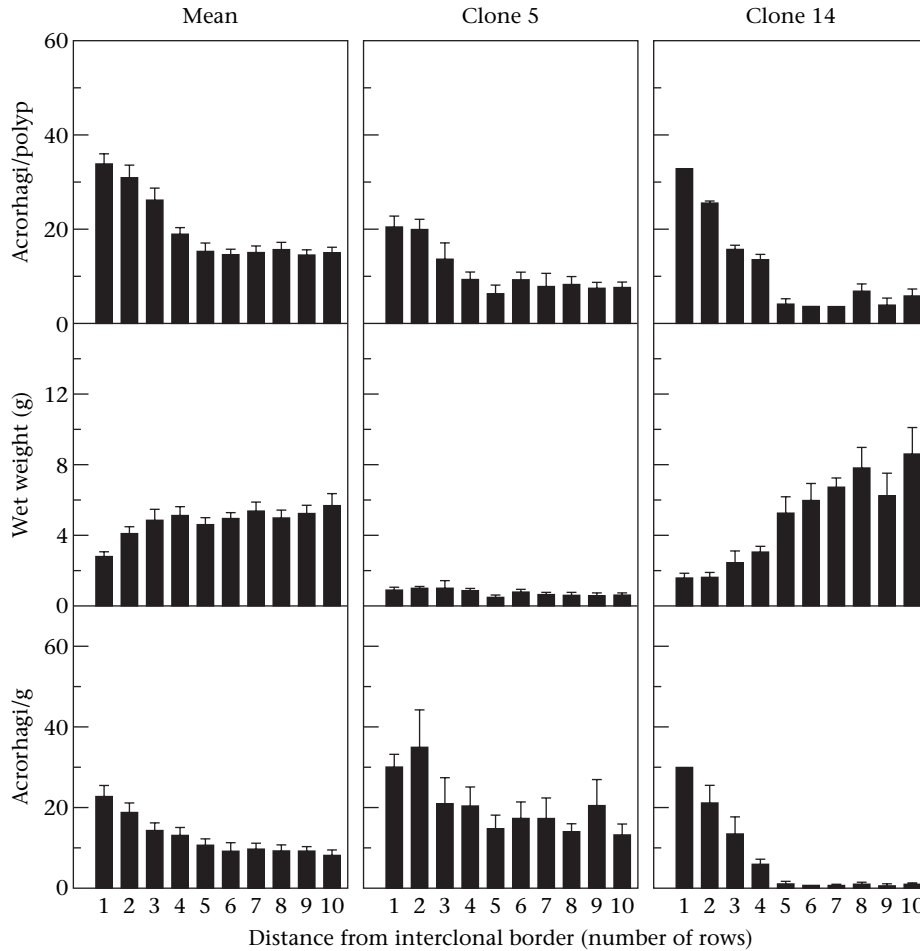
Although averaging our transect results across the set of 10 surveyed clones revealed striking variation in patterns of resource allocation, that pattern was most apparent for clones such as number 14, in which polyps close to the interclonal boundary displayed an investment in acrorhagi/g that was 20 times greater than more central polyps, and polyp size dramatically increased with distance from interclonal boundaries (Fig. 2). Clones such as number 5 represented the other extreme, with no significant variation in polyp size or armament with distance from the interclonal boundary (Fig. 2). Unsurprisingly, ANOVA revealed a significant interaction between clonal identity and distance from a boundary for mean weight, number of acrorhagi per polyp, and number of acrorhagi/g, indicating that these attributes of social organization differ from clone to clone (Table 2).

For many clones, polyp size covaried with position within a clone. To determine whether intraclonal variation in armament and sexual maturity vary according to position within a clone (rather than according to polyp size alone), we compared for nine of the 10 clones the mean weight, mean number of acrorhagi, mean number of acrorhagi/g, and proportions of sexually mature polyps for all small polyps (<2 g) within rows 1–4 (peripheral) and rows 7–10 (central). We excluded clone 14 from these analyses because no polyps from rows 7–10 weighed less than 2 g. We did not make comparable comparisons using only large polyps, because many clones lacked peripheral polyps weighing more than 2 g. In fact, clone 5 lacked any polyps weighing more than 2 g (Fig. 2). These ANOVAs revealed that small peripheral polyps displayed significantly more acrorhagi ( $F_{1,8} = 20.45$ ,  $P < 0.005$ ) and more acrorhagi/g (two-factor ANOVA:  $F_{1,8} = 20.3$ ,  $P < 0.005$ ), and were significantly less likely to be reproductively mature (log-linear model:  $P < 0.001$ ) than small central polyps (Fig. 3). Although mean weight also differed significantly between central and peripheral polyps ( $P < 0.02$ ), this could not explain the persistence of the morphological gradients seen in the analysis of all polyps (Fig. 2), since with a single exception (clone 5) central polyps were on average larger than peripheral polyps.

### Acrorhagial Induction Experiments

#### *Effects of repeated acrorhagial stimulation*

Our initial examination of morphological variation within and among the seven clones used to test the effects of two mild acrorhagial attacks on the armament of polyps suggested that, with the possible exception of the free-edge polyps of clone S1B, both central (i.e. reproductive) and free-edge polyps of all remaining clones could dramatically increase their level of armament



**Figure 2.** Patterns of division of labour in *Anthopleura elegantissima* with respect to distance from an interclonal border. Division of labour is expressed in terms of mean  $\pm$  SE number of acrorhagi per polyp, wet weight and mean number of acrorhagi/g of polyp wet weight. We surveyed 10 transects within each of 10 clones. For each transect, we collected a single polyp from each of 10 contiguous rows of polyps, extending from an interclonal border (row 1) towards the centre of the clone (row 10). Histograms show the overall means for all 10 sampled clones (left), and the means for a clone showing relatively low levels of division of labour (clone 5, centre) and a clone showing a strong pattern of division of labour (clone 14, right).

(Fig. 4). Two-factor ANOVAs revealed significant effects of both clonal identity and location of polyps within clones on both the weight of polyps and number of acrorhagi per polyp, reflecting the smaller size of warrior and free-edge polyps and the greater armament of warrior polyps ( $P < 0.001$ ). Reproductive polyps, on average, weighed at least 1.7 times more than both warrior and free-edge polyps (Fig. 4b). Nevertheless, warrior polyps averaged at least twice as many acrorhagi per polyp as both central and free-edge individuals (Fig. 4a).

Analyses of variance showed that acrorhagial attacks significantly increased the number of acrorhagi per polyp ( $P < 0.001$ ), but only for reproductive polyps (Fig. 5a). Although the effect of acrorhagial stimulation varied significantly among clones ( $P < 0.0001$ ), stimulated central polyps displayed on average 15% more acrorhagi than unstimulated controls. Problems of non-normality potentially confounded these analyses, reflecting the fact that many polyps showed no change in acrorhagial numbers. We therefore reanalysed the data, using both absolute

numbers of acrorhagi and ranks, and omitting cases in which stimulated polyps showed no change in acrorhagial numbers. The reanalyses did not differ qualitatively from comparable analyses that included zero values. In contrast to responses of central polyps, acrorhagial stimulation had no detectable effect on mean numbers of acrorhagi of free-edge polyps ( $F_{1,6} = 0.62$ ,  $P = 0.92$ ; Fig. 5b). Acrorhagial stimulation also had no significant effect on the weights of either free-edge ( $F_{1,6} = 1.98$ ,  $P = 0.40$ ) or central polyps ( $F_{1,6} = 1.51$ ,  $P = 0.53$ ).

#### Effects of repeated tentacular stimulation

Twelve days of repeated short-term tentacle contact with nonclonemates increased the average level of armament in almost all of the 14 stimulated clones (Fig. 6a). As was the case with stimulation by acrorhagi, the magnitude of the response varied among clones. The average number of acrorhagi was 12.5% greater for stimulated versus control polyps (Fig. 6a). Two-way ANOVAs on square-root

**Table 2.** Two-factor ANOVAs testing the effects of clonal identity (random) and distance from interclonal boundary (fixed) on the mean weight of polyps, number of acrorhagi per polyp and number of acrorhagi/g for 10 *Anthopleura elegantissima* clones

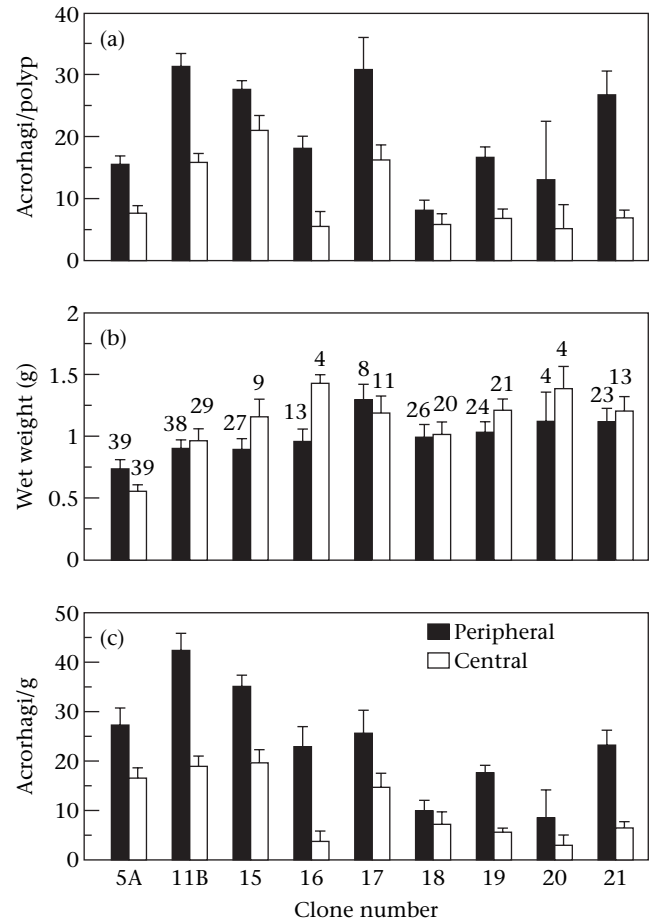
	df	Sum of squares	Mean square	F	P
<b>Effects on mean weight of polyps</b>					
Distance	9	600	67	2.09	<0.05
Clone	9	6594	733	44.74	<0.0001
Clone*distance	81	2588	32	1.95	<0.0001
Residual	899	14719	16		
<b>Effects on mean number of acrorhagi per polyp</b>					
Distance	9	54101	6011	17.73	<0.0001
Clone	9	59522	6613	37.85	<0.0001
Clone*distance	81	27495	339	1.93	<0.0001
Residual	899	157084	175		
<b>Effects on mean number of acrorhagi/g</b>					
Distance	9	36892	4099	20.81	<0.001
Clone	9	51817	5757	54.31	<0.0001
Clone*distance	81	15939	197	1.85	<0.0001
Residual	899	95597	106		

Values for each of 10 clones were obtained from 10 approximately evenly spaced transects per clone, perpendicular to an interclonal border, consisting of 10 adjacent polyps.

transformed data revealed significant effects of both clonal identity ( $F_{11,193} = 24.84$ ,  $P < 0.0001$ ) and stimulation ( $F_{11,193} = 11.17$ ,  $P < 0.02$ ) on the number of acrorhagi per polyp, respectively, but no significant clone\*stimulation interaction ( $F_{11,193} = 0.51$ ,  $P = 0.893$ ; Fig. 6a). Importantly, these differences between treatments were not attributable to biased allocation of larger polyps to stimulation treatments. Within each clone we allocated polyps at random to each of the stimulated and control groups: two-way ANOVA showed that at the conclusion of the experiment the wet weight of polyps varied significantly across clones ( $F_{11,193} = 12.91$ ,  $P < 0.0001$ ). However, there was neither a significant treatment effect on wet weight ( $F_{1,11} = 0.04$ ,  $P = 0.89$ ), nor a significant clone\*treatment interaction ( $F_{11,193} = 0.68$ ,  $P = 0.98$ ; Fig. 6b).

## DISCUSSION

Division of labour may arise when individual members of a social group differ in their responses to stimuli that induce morphological and behavioural specialization, or when they experience different stimuli (Oster & Wilson 1978; Robinson 1992; Crozier & Pamilo 1996; Frank 1999; Pankiw & Page 2001). In turn, if conspecific social groups differ with respect to the frequencies of individuals with a particular response, or the stimuli they receive, then patterns of division of labour will likewise vary across social groups. Among-group variation in patterns of division of labour appears to be widespread in social organisms, raising the question of how, or if, natural selection maintains this variation. The answer depends, in part, on distinguishing environmental from genetic sources of within- and among-group variation in patterns of division of labour. This remains a major hurdle in sexually



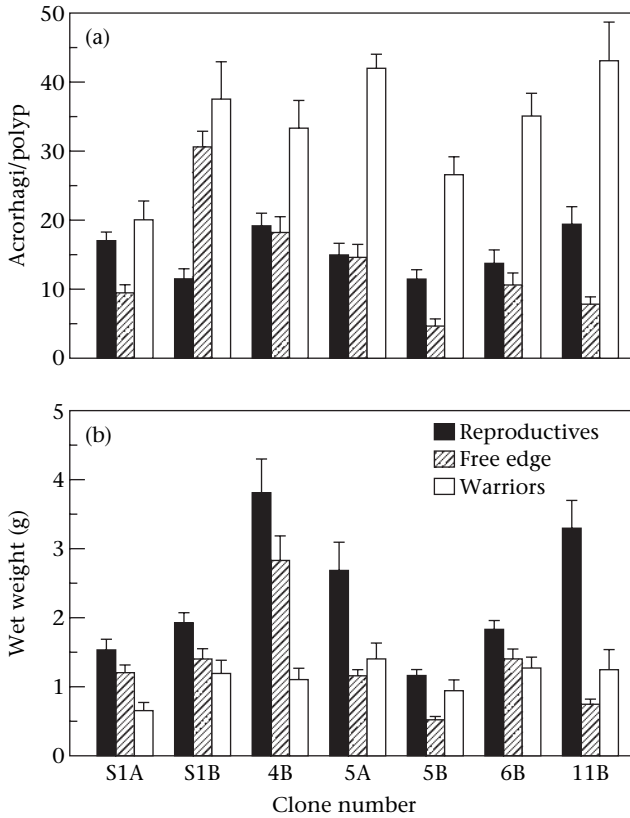
**Figure 3.** Morphological characteristics of nine clones of *Anthopleura elegantissima* with respect to mean  $\pm$  SE (a) number of acrorhagi per polyp, (b) wet weight and (c) acrorhagi/g for small polyps (<2 g), categorized according to distance from an interclonal border. Peripheral polyps were located within four rows of an interclonal border; central polyps were sampled from rows 7–10. The values above each bar represent the number of polyps sampled from each clone.

reproducing organisms displaying division of labour, such as many social insects, where there is potentially both within- and among-group genetic variation (Beshers & Fewell 2001). Social clonal organisms, such as the anemone *A. elegantissima*, simplify the task, by essentially eliminating the within-group component of genetic variation.

Earlier studies showed that most clones of *A. elegantissima* minimally consist of morphologically distinct (Francis 1976; Ayre & Grosberg 1996) and functionally specialized (Ayre & Grosberg 1996) reproductive and warrior castes. Moreover, our previous work, focusing on the behaviour of isolated individual polyps of *A. elegantissima*, revealed both a highly repeatable, transitive interclonal dominance hierarchy, underlain by a complex array of behavioural diversity (Ayre & Grosberg 1995), and consistently uneven fighting abilities for sets of warrior polyps from adjacent clones.

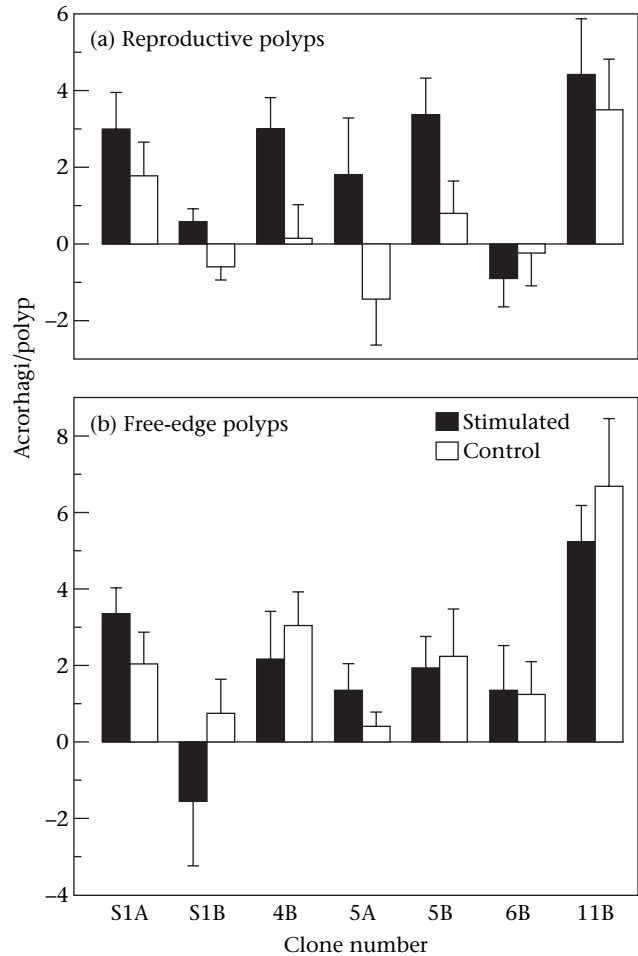
The present study extends this work into a broader social context, focusing on patterns of division of labour





**Figure 4.** Initial (a) numbers of acrorhagi per polyp and (b) weight weights for the reproductive, free-edge and warrior polyps in the seven *Anthopleura elegantissima* clones used in the acrorhagal stimulation trials (see Fig. 5).

and behavioural interactions among intact clones, and the stimuli responsible for inducing variation in patterns of division of labour within and among clones. Specifically, the field and laboratory observations, combined with the experimental studies on the induction of different morphological castes, show that (1) members of intact clones show novel social behaviours, previously unknown from studies of isolated polyps, that facilitate communication and functional integration of an aggregation; (2) in most clonal aggregations there is a gradual transition in the morphology of polyps, from smaller, well-armed, non-reproductive warrior morphs along interclonal borders to larger, poorly armed, reproductive morphs away from the interclonal border; (3) clones can dramatically differ with respect to (a) the distinctiveness of warrior and reproductive castes and (b) relative allocations to warrior, reproductive and intermediate castes; and (4) castes and clones differ in their phenotypic responsiveness to stimuli capable of inducing caste-specific morphological changes. As we discuss below, these results suggest that, despite the absence of physical connections among polyps, clonal integration is an important component of fighting in this species, affecting the expression of behaviour, the costs (and presumed benefits) of aggression and caste distinctions. Moreover, both environmental and genetic factors influence the expression of among-group variation in division of labour in *A. elegantissima*.

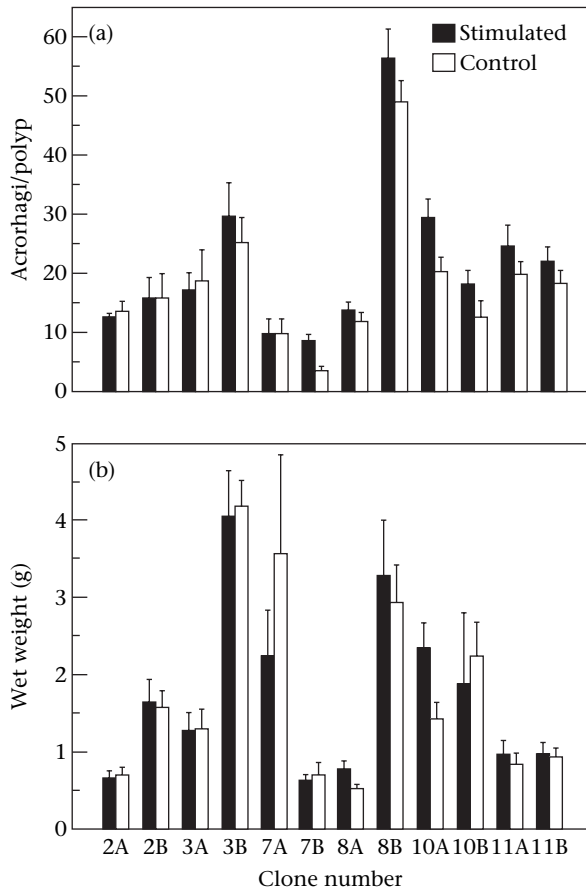


**Figure 5.** Effects of acrorhagal stimulation (attacks by a non-clonemate) on the change in mean  $\pm$  SE numbers of acrorhagi per polyp for groups of (a) reproductive and (b) free-edge polyps (see text for definitions) in seven clones of *Anthopleura elegantissima*.

### From Individuals to Intact Clones: Communication and Clonal Integration

Our laboratory observations of interactions between intact clones revealed formerly undescribed behavioural sequences that clarify how clones operate as functionally integrated units. Specifically, these behaviours could promote coordinated attacks on adjacent clones and expansion of clonal territories, permit the recycling of damaged warriors, ensure that the interclonal boundary is simultaneously defended by groups of clonemates rather than individual warriors, and transfer information about the status of an interclonal fight to members of the clone that are not adjacent to the interclonal border.

The expression of at least some of these agonistic behaviours depends on social context. Unlike the usually brief interactions between isolated pairs of polyps, almost all attacks between members of intact clones involved reciprocated fights that typically continued for several days. However, once warrior polyps crossed into the interclonal border and lost physical contact with clonemates, they behaved like losing individual polyps in our



**Figure 6.** Effects of short-term (10 min/day) tentacular stimulation by a nonclonemate on (a) the mean  $\pm$  SE number of acrorhagi per reproductive polyp and (b) mean wet weights (g) for groups of 10 control and 10 stimulated polyps, from 12 *Anthopleura elegantissima* clones.

earlier studies (Ayre & Grosberg 1995), and retreated after being attacked by the opposing clone. Other behaviours expressed by polyps in intact clones, such as undirected acrorhagial inflation and acrorhagial sweeping and searching were conspicuously displayed by polyps several rows distant from interclonal borders soon after submersion by the incoming 'tide' and without direct stimulation by the opposing clone, but never occurred in contests between isolated polyps. Similarly, the mobility of polyps arrayed along the interclonal border may represent a genuinely increased level of locomotory activity in these polyps (although similar behaviour might be expected of free-edge polyps); however, directed locomotion towards the opposing clone also probably reflects two features of the social context, namely the proximity to open space along the border and the presence of a solid mass of clonemates elsewhere.

Interindividual communication via direct morphological connections, or through visual, auditory, electrical, mechanical, or chemical means, is a key organizing element within most animal societies (reviewed in Bradbury & Vehrencamp 1998), and may be essential for the evolution of reproductively altruistic castes such as warrior-scouts in colonies and aggregations. In the case of

*A. elegantissima* aggregations, communication between clonemates appears to be critical for maintaining or developing the preparedness of clones for the defence or acquisition of habitable space. Although members of a clonal aggregation lack physical connections, several of the behaviours observed in this study may serve to transfer information between *A. elegantissima* polyps. In particular, when pairs of nonclonemate polyps interact, their nematocysts discharge. In so doing, the polyps deposit potentially antigenic material on the columns or tentacles of their opponent, which can, in turn, be transferred directly or indirectly to other polyps.

The most extreme situation arises when polyps are heavily scarred and so carry numerous 'peels' of acrorhagial ectoderm from other clones. In our laboratory observations of the interactions occurring at an interclonal border, clonemates sometimes recognized badly scarred polyps as nonself and attacked them. We therefore anticipate that the mobile, peripheral polyps play an important role in communicating the presence (or absence) of a neighbouring clone before the majority of polyps come into direct contact with the competitor. Similarly, the searching behaviour of polyps near to, but not on, an interclonal border could transfer allogeneic (i.e. conspecific nonself) nematocysts to clonemates deeper within a clone. These behaviours may have both immediate and longer-term effects on the expression of aggression. For example, in an earlier study, we demonstrated that some clones display 'immune memory', and respond more rapidly to clones that they have previously encountered (Ayre & Grosberg 1995). Depending on the frequency of interclonal contact, or, conversely, the responsiveness of a given clone, this information transfer has the potential to increase the aggressiveness of warriors towards their neighbour and may be the key proximal factor that induces morphological polymorphism (see below).

### Caste Variation and Division of Labour

Like many social insects such as honeybees in which behavioural and morphological caste boundaries are indistinct and subject to variation in age, experience, or social context (Page & Peng 2001; Shingleton & Foster 2001), the castes of *A. elegantissima* appear to represent a developmental series that is typically more fluid than caste distinctions in species that show discrete morphological variation among the members of a social group (e.g. Oster & Wilson 1978). Our transect surveys of morphological variation within *A. elegantissima* clonal aggregations, together with examination of polyps used in the induction experiments, revealed up to five intergrading castes, each corresponding to a specific location within a clone and an apparently specialized function.

We now recognize morphologies that match the previously described groups of small, poorly armed free-edge polyps; similarly sized but typically much more heavily armed warrior-scouts at interclonal boundaries (previously dubbed 'warriors'; Francis 1976; Ayre & Grosberg 1996); and larger central polyps, or reproductives, which are

most likely to be sexually mature, but have relatively few acrorhagi. In addition, the relatively large and heavily armed polyps immediately adjacent to interclonal borders form a less clearly defined warrior caste that may be the principal source of clonal defence (such polyps with relatively large numbers of acrorhagi occurred in eight of the 10 clones surveyed).

Without the benefit of our behavioural observations of interactions between intact clones, we might have considered these polyps as part of a simple transitional series ranging from the large, relatively poorly armed reproductives to small, well-armed warrior-scouts. However, the specialized searching and attacking behaviours displayed by warrior polyps in our aquarium study suggest that these polyps play an active role in clonal defence and aggression. Moreover, although several clones showed a relatively smooth transition from warrior-scouts to reproductives in terms of polyp size, the transition in terms of acrorhagial numbers was often far more abrupt, suggesting that the stimulus that induces the production of acrorhagi in warriors has a similar distribution.

We also observed a fifth type of arguably unspecialized polyp in clones with relatively large reproductives. These small polyps (typically  $< 2$  g) are intermingled with the much larger reproductive caste. At low tide, these small central polyps are usually invisible on casual inspection, lying hidden beneath the contracted columns of surrounding larger reproductives. However, polyps  $\leq 2$  g compose 36% of all central (rows 7–10) polyps. Neither the origin, nor function, of this category of polyp are obvious, although these polyps are (1) minute relative to typical reproductives, (2) significantly more likely to possess gonads, and (3) more poorly armed than equivalently sized warriors. The presence of these polyps may reflect unusual episodes of asexual reproduction (i.e. they may be the product of recent episodes of fission). Indeed for the eight clones with multiple large and small central polyps, the small polyps possessed on average almost exactly half the number of acrorhagi displayed by their larger neighbours (57%), as would be expected following fission. Alternatively, these small polyps may be recycled scouts that have retreated deep into the clone following fighting (we observed this once in the laboratory). However, such polyps should carry acrorhagial scars or greatly elevated numbers of acrorhagi. In either case, these small polyps may potentially be a multipotent 'reserve' caste that can be transformed into any type of polyp as required. Until the longer-term fates of these polyps can be monitored, their role will remain uncertain.

### Patterns of Division of Labour: Allocation Trade-offs and Competition for Space

A major goal of this and related studies of division of labour is to document the extent of within- and among-group variation in the production of different morphological and behavioural castes, and to understand the factors that maintain this variation. All but one (clone 5) of the clones surveyed in this study showed some facets of division of labour. With the exception of clone 20,

warrior-scouts arrayed along interclonal borders were generally better armed on both an absolute and per-mass basis than reproductive polyps.

Nevertheless, no single pattern of allocation to different castes characterized the 10 actively competing clones that we surveyed on the relatively uniform large boulders that constitute the Bodega Harbour jetties. Most notably, clones varied with respect to the size and armament of polyps across the different castes and in the proportion of each clone that could be classified as warrior-scouts, warriors and reproductives. However, all of the large and apparently successful clones that we surveyed in both this and our earlier study (Ayre & Grosberg 1996) showed both (1) evidence of substantial investment in weaponry at interclonal borders and (2) consistently high levels of investment in production of gametes, especially through central reproductive polyps.

Given the inevitable allocation trade-offs among sexual reproduction, asexual proliferation or somatic growth, and armament and defence that generally underlie caste differentiation and social organization (Wilson 1971; Harvell 1994; Crespi & Choe 1997), there are several ways that a sessile, clonal organism such as *A. elegantissima* could enhance its ability to compete for space. For example, because *A. elegantissima* polyps are phenotypically plastic both with respect to the expression of sexual maturity (Francis 1976; this study) and rates of asexual fission (Tsuchida & Potts 1994), *A. elegantissima* clones could dramatically decrease their investment in sexual reproduction, increasing resources available for asexual proliferation and clonal expansion. Nevertheless, several lines of evidence suggest that, at least within these populations on relatively young, artificial structures (constructed in 1941, and since augmented with additional boulders), established clones appear committed to making a significant investment in sexual reproduction. First, we found consistently high proportions of sexually mature polyps within the eight clones that we surveyed in detail during the reproductive season (77% of all 800 polyps and 86% of the 320 polyps in rows 7–10). Furthermore, all of the 191 central polyps, each from a separate aggregation, collected for our analysis of sex ratios were reproductively mature. It remains to be seen whether clones in their initial stages of establishment, prior to filling free space, invest substantially in sexual reproduction (see Harvell & Grosberg 1988).

The present study also highlights the complexity of trade-offs between investment in gonads and weaponry within individual *A. elegantissima* clones. Our data support Francis' (1976) observation that the large central (reproductive) polyps make a greater contribution to sexual reproduction than the smaller and yet more heavily armed polyps along interclonal borders. Because the probability of reproductive maturity is positively correlated with polyp size in other anemones (e.g. Ayre 1984; Hunt & Ayre 1989), within-clone variation in allocation of resources to gonads probably reflects, to some extent, the contrasting mean sizes of central versus marginal polyps. However, acrorhagial numbers are not typically inversely related to polyp size within groups of marginal or central polyps (Ayre & Grosberg 1996). Moreover, our

comparisons of the characteristics of small polyps at interclonal borders, and small and large polyps deep within clones, show that both the expression of sexual maturity and number of acrorhagi vary independently of polyp size. Thus, in concert with the results of the acrorhagial induction experiments, these observations suggest that a key determinant of the number of acrorhagi borne by a polyp is its proximity to an interclonal boundary.

### Determinants of Interclonal Variation in Patterns of Division of Labour

Within insect societies and many polymorphic colonial aquatic invertebrates, the formation of, and allocation to, morphological and behavioural castes is proximately determined both by epigenetic influences during development and by genetic variation for inducibility (reviewed in Harvell 1994; Crozier & Pamilo 1996; Beshers & Fewell 2001; Evans & Wheeler 2001). For example, in some social insects, particular lineages may have a genetic predisposition to perform specific tasks (social insects: e.g. Fraser et al. 2000; Pankiw & Page 2001; reviewed in: Page et al. 1992; Beshers & Fewell 2001). *Anthopleura elegantissima* aggregations are genetically homogeneous; consequently, our data suggest that caste formation in *A. elegantissima* entails a series of epigenetically inducible changes, and that clones may differ with respect to their inducibility. Specifically, both acrorhagial scarring and tentacular contact, events that naturally occur at interclonal borders, can induce the transformation of reproductive polyps to a more warrior-like form (analogous to the induction of catch or sweeper tentacles in other cnidarians; reviewed in: Francis 1988; Harvell 1990; Williams 1991).

It is important to note that these outcomes fell far short of complete conversion from reproductive to warrior castes. In particular, we did not detect any consistent effect on polyp size, and the numbers of acrorhagi displayed by stimulated polyps were far fewer than those of clonemate warriors. However, our laboratory observations of the interactions between intact clones clearly showed that polyps at interclonal borders are likely to experience higher frequencies and greater intensities of stimulation than we employed, and that several forms of stimulation can occur simultaneously.

The simplest explanation for the observed within-clone variation in the number of acrorhagi per polyp assumes that all polyps in a clone have the same innate capacity to produce acrorhagi, and that the variation reflects differences in the level of stimulation by direct interclonal contact and indirect nematocyst transfer. However, our induction experiments using acrorhagial peeling as a stimulus did not increase acrorhagial numbers in free-edge polyps, suggesting that the mechanism of formation of scouts and warriors is complex. This was unexpected, since free-edge polyps are more likely than reproductives to encounter another clone, and therefore appear the obvious source of the first new scouts and warriors produced by each clone (also see Francis 1976). The lack of response by the small free-edge polyps could reflect

either a difference in the induction threshold for re-productive versus free-edge polyps, or developmental canalization (Waddington 1957), perhaps mediated by the resource limitations of the smaller free-edge polyps. Alternatively, if warriors and scouts are typically formed from more centrally located polyps, then the initial process of transforming a clone from a relatively undifferentiated and poorly armed state to one with multiple rows of further differentiated defenders may be prolonged and involve the formation of distinct lineages.

If asexual lineages become committed to a particular pattern of resource allocation during the development of a clonal aggregation, then interclonal variation in, for example, the number of rows of warriors may reflect differences in history rather than differences in responsiveness or plasticity. A key issue is the way in which phenotypically plastic characters, including numbers of acrorhagi, are transmitted through asexual divisions. A system in which large reproductive polyps change into warriors, within realistically short time periods, would require that reproductive polyps must both allocate proportionately increased energy to the production of acrorhagi and become smaller through fission. Each daughter polyp must then regenerate an elevated number of acrorhagi, while recovering from fission; or, in contrast to the outcome that we observed for similarly small free-edge polyps, retain the capacity to produce additional acrorhagi in response to interclonal contact. Given the time frame over which large clonal aggregations develop (we suspect decades), and the technical difficulties of marking individual polyps (e.g. Sebens 1976) and following the fates of their asexual offspring, obtaining this critical information will remain a serious challenge to our understanding of caste formation, division of labour, and social organization in *A. elegantissima*.

### Conclusions: Spatial and Temporal Dynamics of Interacting Clones

The work of Francis (1973a, b, 1976, 1988) focused attention on the role of interclonal competition and agonistic behaviour in the formation and maintenance of apparently stable interclonal borders in populations of *A. elegantissima*. However, our observations of interactions between *A. elegantissima* clones suggest a basis for both rapid changes in the size of clonal territories (and hence interclonal borders), as well as periods of prolonged stasis. Clones could rapidly expand when surrounded by vacant space (or a subordinate clone), as scout polyps form the nuclei for new clonal aggregations that may eventually coalesce. Stasis may be an inevitable consequence of the capacity of clones to defend established territory, or, alternatively, may occur only when an expanding or contracting clone reaches a physical contour that alters the nature of competition. Our earlier comparisons of the fighting ability of pairs of individual polyps from sets of opposing clones suggest that clones, at the level of contests between individual polyps, are rarely evenly matched (Ayre & Grosberg 1996). However, borders between large and established clones may approach

equilibria because an advantage gained by the superior fighting ability of the individual polyps of one clone may be offset by the combined effects of a defending clone's scouts and warriors in rows 1–4, coupled with the defender's capacity to generate or provision new warrior recruits.

Among successful clones, the tactics used to acquire and maintain territories probably reflect a clone's history of interaction with other clones, along with the fitness trade-offs among the many characters that affect the outcomes of interclonal aggression. At the extremes we expect that low initial settlement densities favour clones that have small innate numbers of acrorhagi and that devote most energy to asexual reproduction. At very high settlement densities, selection may favour aggressive clones with a fixed heavy investment in armament. Superimposed upon this selective framework of the likelihood and intensity of intraspecific competition must be (1) the magnitude and spatial and temporal dynamics of physical disturbance, which opens habitable space, (2) the incidence and density of heterospecific competitors and predators (e.g. Dayton 1971), and (3) temporal and spatial variation in thermal regime and the availability of food and light (for photosynthesis by symbionts; see Saunders & Muller-Parker 1997; Bates 2000; Secord & Augustine 2000). To the extent that patterns of division of labour and inducibility displayed by the majority of clones that we have closely examined reflect the population of *A. elegantissima* as a whole, the outcome of this complex selective regime generally appears to be some measure of phenotypic plasticity in patterns of allocation at the level of individuals and clonal aggregations. Nevertheless, some clones appear to be morphologically invariant, and nearly all clones differ in their patterns of division of labour and their responsiveness to inducing stimuli. Other fitness-related traits, such as oxygen consumption, lipid concentration and chlorophyll concentration, also show clone-specific variation in *A. elegantissima*, as well as other anemones (Shick & Dowse 1984). It remains to be seen whether these interclonal differences are primarily the result of underlying genetic variation, the varying histories of clones during their ontogenies, or some combination of genetic and environmental factors.

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