

Effects of social organization on inter-clonal dominance relationships in the sea anemone *Anthopleura elegantissima*

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Abstract. In the sea anemone *Anthopleura elegantissima* contact between non-clonemates often elicits inter-clonal fighting, involving the application of nematocyst-laden fighting tentacles (acrorhagi) and the locomotory retreat of losing polyps. This study confirms that isoclonal aggregations of *A. elegantissima* display an inducible polymorphism, such that polyps at the inter-clonal boundaries (warriors) are typically smaller and carry more fighting tentacles (acrorhagi) than more central (reproductive) polyps. Based on the outcomes of eight separate contests between each combination of like and unlike morphs for each of seven pairs of neighbouring clones (i.e. $N=224$ contests), this study provides the first direct evidence that warriors are aptly named. All sets of neighbouring warriors and reproductives were mutually aggressive, and warriors, despite their overall size disadvantage, won 41 out of 81 contests with the reproductive polyps of their neighbouring clone. In addition, warrior and reproductive polyps were almost equally likely to initiate an attack and to retaliate. Thus, the success of warriors against the typically far larger reproductive polyps primarily reflects the proportionately greater allocation by warriors of resources to acrorhagi. The outcomes of the inter-clonal contests further indicate that pairs of neighbouring clones are unevenly matched in terms of their fighting ability, and that inter-clonal dominance is again largely determined by relative allocation of resources to acrorhagi. In five of seven inter-clonal combinations the warriors of the apparently dominant clone defeated the majority of opposing warriors (in three cases winning all eight contests). This result represents an apparent paradox, as earlier work showed that inter-clonal borders may persist for several years. Further work is needed to determine the extent to which agonistic behaviour determines the outcomes of inter-clonal competition for space and whether the outcomes of contests between pairs of individuals reflect the true dominance relationships of entire clones.

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Many colonial invertebrates display inducible polymorphism, such that their component modules exhibit discrete morphological or behavioural variation (reviewed in Harvell 1994). In functionally integrated colonies, this polymorphism presumably increases the absolute fitness of a colony by allowing different individuals to perform a

limited set of complementary tasks. The clearest examples of such polymorphisms occur in invertebrates that form colonies of asexually generated, hence genotypically identical modules, in which phenotypic variation among members of the same clone must be environmentally produced (Hughes 1989). For instance, in clonal insects such as encyrtid wasps (Cruz 1981) and several species of aphid (Aoki 1979; Dixon 1985), as well as numerous species of benthic cnidarian (reviewed in Buss et al. 1984), polymorphism often includes the production of specialized morphs that appear to play a defensive role. The functions of such specialized morphs are often inferred from several kinds of circumstantial evidence. In the most

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extreme cases, these morphs may entirely lack a gut or gonads, and therefore depend on their clonemates to perform these functions. In other cases, so-called defensive morphs have functional guts and gonads, but possess highly modified tentacles (e.g. tentaculozooids and dactylozooids in some colonial hydrozoans), which can be especially well-armed with batteries of specialized nematocysts (stinging cells) used only in aggression and defence (Bigger 1980).

Fission clones of the sea anemone *Anthopleura elegantissima* (Brandt) consist of morphologically discrete and physiologically independent modular units (polyps), that often remain associated as dense, isoclonal aggregations (Sebens 1982; personal observations). Laboratory studies reveal that when polyps from two different clones initially come into contact, one or both individuals usually mounts an attack involving withdrawal of the feeding tentacles and inflation of a specialized set of non-feeding tentacles, the acrorhagi (Äbel 1954; Bonnin 1964; Francis 1973a, b; Ayre & Grosberg 1995). The distal end of an acrorhagus is covered by nematocyst-laden ectoderm. When an acrorhagus is applied to a non-clonemate, the ectoderm detaches onto the opponent, leaving a white 'scar.' The nematocysts in the detached tip discharge, inflicting local tissue necrosis. An agonistic encounter usually involves the application of one, or more, acrorhagi onto an opponent, which may, in turn, retaliate by applying its own acrorhagi onto the attacker. Eventually, one of the anemones retreats by pedal locomotion; if retreat is impossible, the attacked anemone may eventually die from its injuries (reviewed in Ayre & Grosberg 1995).

Francis (1973a, b) reported that when naturally occurring clones interact, stable (2–4 years), anemone-free inter-clonal boundaries can form. She distinguished polyps along inter-clonal boundaries from central members of the same clone, and dubbed them 'warriors' based on three lines of morphological evidence (Francis 1976). First, polyps along inter-clonal borders typically possessed more acrorhagi than clonemates that were not interacting with other clones. Second, for a sample of two clones, the mean size of acrorhagi was greater for polyps along inter-clonal borders than for other clonemates. Finally, polyps along inter-clonal borders usually lacked mature gonads, when the typically larger central individuals (here termed 'reproductives') were ripe. From

these comparisons, she inferred that (1) warrior polyps are specialized as defensive/aggressive units, with the principal reproductive functions of the clone accomplished by more central individuals; and (2) clones of *A. elegantissima* exhibit 'social organization' (Francis 1976). Nevertheless, it remains unknown whether morphological differences between warrior and reproductive polyps are associated with differences in agonistic behaviour and outcomes of agonistic encounters.

Francis (1976) also found considerable morphological variation among warrior polyps from different clones with respect to potential determinants of the outcomes of agonistic encounters, namely mean numbers of acrorhagi (range: 17–40) and mean polyp weight (range: 0.4–1.1 g). On the one hand, this inter-clonal variation in warrior morphology suggests that warrior polyps from different clones will not be evenly matched. On the other hand, the existence of stable inter-clonal boundaries suggests either that these regions are ill-suited to colonization by *A. elegantissima*, or that warriors along such boundaries are competitively equal or mutually non-agonistic. Just as nothing is known about the relative fighting abilities of warrior and reproductive polyps, the dominance relationships of naturally occurring pairs of clones remain unstudied.

In this paper, we first show for seven naturally occurring pairs of clones that (1) polyps along inter-clonal borders differ morphologically (in terms of polyp size and number of acrorhagi) from other clonemates and (2) so-called warriors from adjacent clones are not necessarily evenly matched in terms of polyp size and numbers of acrorhagi. We then assess whether these morphological differences between the warrior polyps of naturally occurring pairs of clones can account for differences in agonistic behaviour and outcomes of pair-wise contests. Finally, given that warriors from neighbouring clones differ in fighting ability and the implication that inter-clonal boundaries should not persist, we examine the circumstances in which the fighting ability of individual warriors in pair-wise contests may or may not reflect the competitive ability of entire clones.

METHODS

Collection and Maintenance of Animals

We sampled three pairs of neighbouring clonal aggregations of *A. elegantissima* from Doran

Rocks, an inter-tidal boulder field approximately 4 km south of the Bodega Marine Laboratory, Bodega Bay California (31°18'N, 123°03'W). We collected an additional four pairs of neighbouring clones from the moles at the entrance to Bodega Harbour, 1.5 km south of the Bodega Marine Laboratory. Both populations consist of many hundreds of genetically distinct clones confined to boulders of varying size (personal observations). We chose only pairs of moderate to large aggregations (>1600 cm²). Each clone consisted of a continuous mat of identically coloured and patterned polyps. Each pair of clones shared a single boulder, but a distinct, anemone-free zone (inter-clonal border) approximately 3–5 cm wide separated the two clones.

From each of the 14 clones, we collected a minimum of 60 central polyps (more than two anemone diameters from the edge of an aggregation) and 30 warrior polyps (arrayed along the inter-clonal border). We gently levered individual anemones from the rocks, using a small spatula or screwdriver. We then scraped their pedal discs clean of algae and other foreign material before transporting groups of warriors and central anemones from each clone back to the laboratory in 41 separate plastic bags, one-third filled with seawater. We removed any anemones with damaged disks or columns, and placed the remaining anemones in recirculating seawater aquaria (13–14°C with a 12:12 h light:dark cycle). We allowed the anemones 24–48 h to attach to small (2–4 cm diameter), flat pebbles gathered from the Doran Rocks site.

Genetic Identities of Aggregations

We used horizontal starch gel electrophoresis to confirm that each aggregation was genetically distinct from all others used in this study, and to ensure that members of each aggregation were clonemates. At the conclusion of the behavioural experiments described below, we electrophoresed tissue extracts from six randomly chosen anemones (three warrior and three reproductive polyps) per putative clone, and stained for polymorphic enzymes (methods and electrophoretic phenotypes are described in Smith & Potts 1987; Ayre & Grosberg 1995). All clones differed electrophoretically for at least one locus, and all polyps from the same aggregation were electrophoretically identical.

Inter-clonal Contests

To determine whether neighbouring clones were mutually aggressive, and to compare the agonistic behaviour of warrior (W) and central reproductive (R) polyps, we paired single anemones from each clone (1–8) with members of their neighbouring clone. For each pair of clones, we created eight replicate pairings of each of the four possible morphological combinations of polyps (i.e. W_x versus W_y; R_x versus R_y; W_x versus R_y; W_y versus R_x). We used each polyp in only one contest. We allowed pairs to remain in tentacular contact for 24 h, or until one anemone attacked and defeated its opponent. We observed these contests at 1-min intervals during the first hour of contact, but less frequently after that. At each observation we recorded whether (1) one or both individuals behaved aggressively; (2) an acrorhagial peel occurred (i.e. a completed attack); and (3) one, or both, polyps moved out of range of attack. We also noted which anemone attacked first and whether retaliation (a reciprocated attack) occurred. In cases where we failed directly to observe an attack(s), we inferred the sequence of these behaviours from the order in which scars were first observed. Four contests (all involving pairings of warriors and reproductives) could not be scored as anemones became dislodged during the trial. At the end of all other contests (*N*=220), we judged an anemone to be the victor if its opponent was both scarred and had retreated onto the floor of the aquarium, or if the opponent was scarred and moved out of the tentacular reach of its aggressor by changing its posture. We scored three other outcomes as 'unresolved': (1) cases where animals remained in contact for the duration of the trial; (2) cases where both anemones moved apart; and (3) cases where one animal moved away, but showed no acrorhagial scarring. At the end of each contest, we relaxed all polyps in 10% MgSO₄ for 1–2 h to allow counts of their acrorhagi. We then dissected the anemones to expose their coelenterons, blotted off any excess water, and weighed them.

RESULTS

Morphological Characteristics of Polyps

The number of acrorhagi per polyp was neither strongly, nor consistently correlated with wet

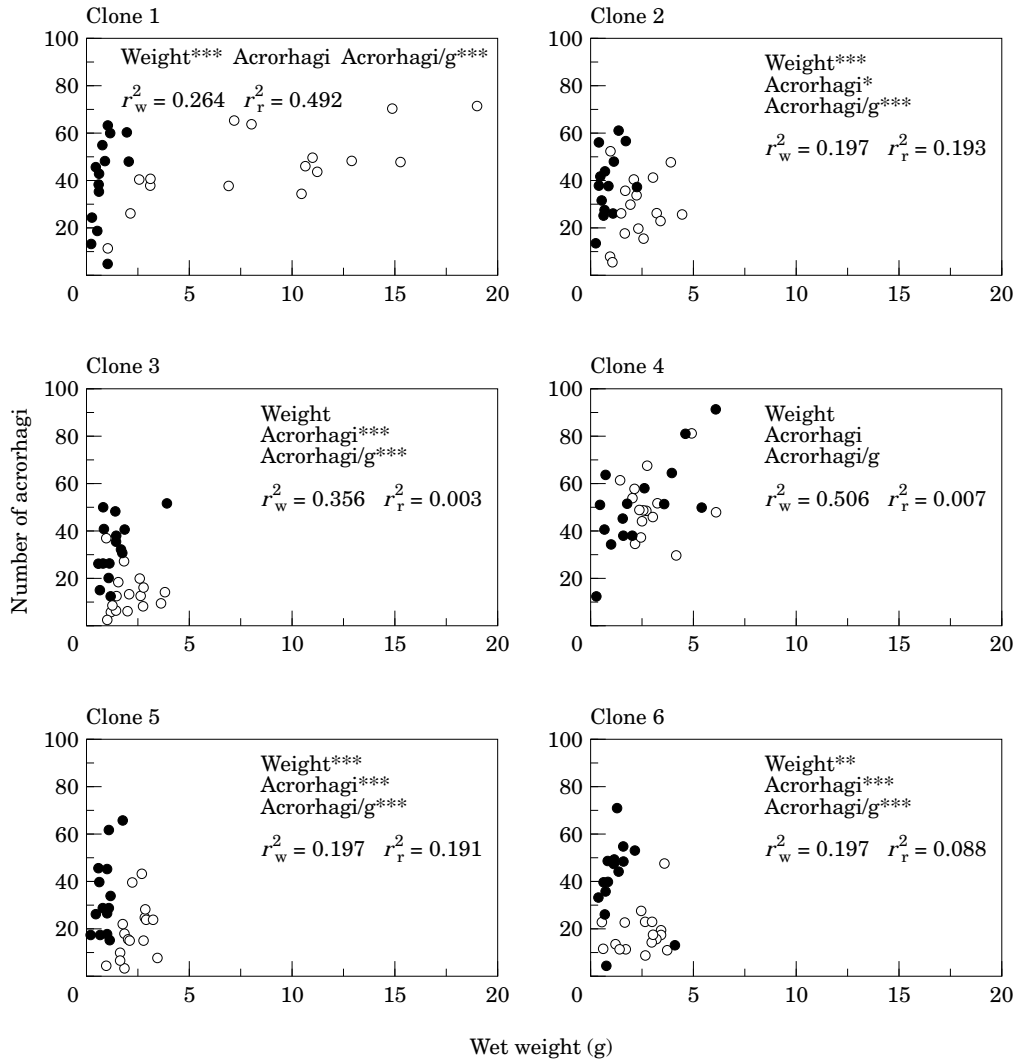


Figure 1. (a)

Figure 1. Relationships between wet weight and number of acrorhagi for peripheral warrior polyps (●) and central reproductive polyps (○) in 14 clones of the sea anemone *A. elegantissima*. Statistical comparisons (Student's *t*-test) between the warrior and reproductive polyps of each clone for mean polyp weight, number of acrorhagi per polyp, and number of acrorhagi per g wet weight are shown. The r^2 values are Pearson product-moment correlations for warrior and reproductive morphs, respectively (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$).

weight among either warrior or reproductive polyps in any of the 14 clones (Fig. 1). Overall, the number of acrorhagi per polyp was weakly positively correlated with wet weight in 12 of 14 groups (i.e. clones) of warrior polyps, and 10 of 14 groups of reproductive polyps (Pearson product-moment correlation: $r^2 > 0.1$; Fig. 1); however, only two

(warrior polyps of clone 7 and reproductive polyps of clone 8) of these relationships were statistically significant (Pearson product-moment correlation: $P < 0.05$) and r^2 values were typically < 0.3 .

Warrior polyps were typically morphologically distinct from their reproductive clonemates in terms of wet weight, numbers of acrorhagi per

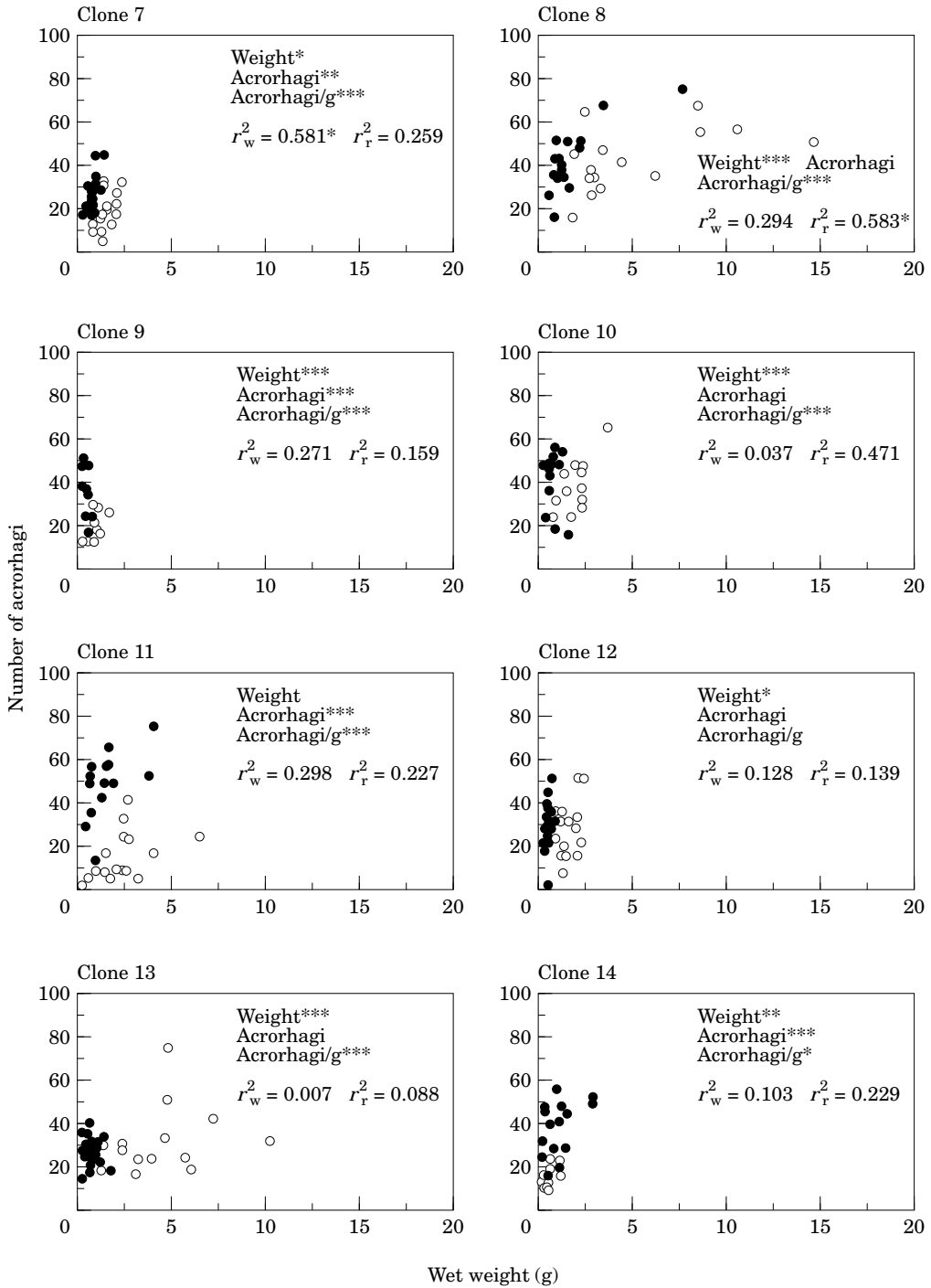


Figure 1. (b)

polyp, and numbers of acrorhagi/g of polyp wet weight. For 11 of 14 clones, warriors were on average significantly lighter than reproductives (one-tailed, unpaired Student's *t*-test: $P < 0.05$; Fig. 1). Clones 1 and 8 showed the most differentiation between warrior and reproductive polyps, with warriors averaging respectively only 12% and 8.4% of the weight of their reproductive clone-mates. Although reproductives were generally considerably larger than their clonemate warriors, in eight clones the reproductive polyps carried significantly fewer acrorhagi per polyp than warriors (one-tailed, unpaired Student's *t*-test: $P < 0.05$; Fig. 1). At the extreme, reproductive polyps of clones 9 and 11 bore 18.0 ± 1.3 ($\bar{X} \pm \text{SE}$) and 14.0 ± 2.9 acrorhagi, compared with 40.0 ± 4.2 and 48.8 ± 3.7 for their respective warriors. Both reproductive and warrior polyps of the remaining six clones displayed relatively large numbers of acrorhagi; however, for each clone differences between warriors and reproductives were all non-significant (one-tailed Student's *t*-test: $P > 0.05$). Most strikingly, warriors carried significantly more acrorhagi/g wet weight for 12 of the 14 clones (one-tailed, unpaired Student's *t*-test: $P < 0.05$; Fig. 1).

Agonistic Behaviour

Some 215 of the 220 (97.7%) scoreable inter-clonal pairings elicited agonistic behaviour by at least one contestant. All seven pairs of neighbouring clones were mutually agonistic (i.e. at least one warrior polyp and one reproductive polyp of each clone attacked its opponent), although aggressiveness as judged by the proportion of contests in which a clone mounted either first or reciprocated attacks ranged from 94% (clone 6 versus clone 5, and clone 12 versus clone 11) to 34% (clone 13 versus clone 14). Overall, reproductive and warrior polyps completed initial or reciprocated attacks on their opponents with almost equal frequency (69.9% versus 72.9%), and both groups completed 50% or more of initial attacks within 30 m of initial contact. Retaliation occurred in 109 of 215 contests. In contests where we could identify the order of attack, approximately equal numbers of reproductive polyps (46) and warrior polyps (44) completed reciprocated attacks.

Contests between like morphs

We characterized interactions between pairs of clones as (1) dominance: the same clone won a

statistically significant majority of resolved pair-wise warrior versus warrior and reproductive versus reproductive contests; (2) reversal: the dominant clone differed between pairs of warriors and pairs of reproductives; and (3) equality: neither clone won a significant majority of contests in either type of pair-wise interaction. Only one pair of clones (clones 5 and 6) was approximately evenly matched. The win:loss record of clone 5 was 3:2 for contests between pairs of warriors and 2:3 for pairs of reproductives (Table I).

In three of the seven inter-clonal combinations (clones 1 versus 2; 7 versus 8 and 9 versus 10), one clone consistently dominated its neighbour (Table I). In all three of these combinations, one clone won a significant majority of all contests between pairs of warriors and pairs of reproductives ($\geq 90\%$; binomial test: $P < 0.011$). In two cases (clone 1 versus 2 and clone 9 versus 10) the dominant clones (1 and 10, respectively) won all eight contests between warriors (binomial test: $P < 0.004$), and seven of the contests between reproductives (clones 1, 7:1; binomial test: $P = 0.035$ and clone 10, 7:0; $P = 0.008$).

The identity of the dominant clone switched between morphological combinations for contests between clones 3 and 4, 11 and 12, and 13 and 14 (Table I). The most dramatic reversal of dominance involved clones 13 and 14, in which the warriors of clone 14 defeated all eight warriors of clone 13, but their reproductives lost all eight encounters with the reproductives of clone 13 (Fisher's exact test: $P = 7.7 \times 10^{-5}$). Similarly, the warriors of clone 11 won the majority of their resolved encounters with the warriors of clone 12 (4:2); however, their reproductives lost all of their seven resolved encounters with the reproductives of clone 8 (Fisher's exact test: $P = 0.021$).

Contests between unlike morphs















A summary of all warrior versus reproductive contests suggests that the two morphs do not obviously differ in terms of contest outcomes, propensity to attack first, and propensity to retaliate. Overall, warrior polyps won 41 of 81 resolved contests with reproductive polyps, and defeated larger reproductives in 35 of 41 cases. Of the 86 contests in which we could identify a first attacker, warriors attacked first in 41 cases. Finally, in the 32 contests featuring retaliatory attacks, warriors

Table 1. Outcome, dominance patterns and possible determinants of success (mean number of acrothagi per polyp \pm se, and order of attack) for inter-clonal contests between like morphs (i.e. Warriors (W) versus Warriors (W) and Reproductives (R) versus Reproductives (R)) of seven pairs of neighbouring clones of the sea anemone *A. elegantissima*

Clones	Wins		Number of acrothagi				Attack first			Dominance
	W versus W	R versus R	W _A	W _B	R _A	R _B	W _A :W _B	R _A :R _B		
1 versus 2	** 8 ↑	** 7 ↑	40.1 \pm 7.0	39.8 \pm 4.9	43.3 \pm 6.4	26.5 \pm 4.7	5:2	8:0	1 > 2	
3 versus 4	2 6 ↓	1 4 ↓	40.4 \pm 4.8	43.5 \pm 5.4	14.0 \pm 3.8	52.0 \pm 3.6	5:2	4:2	Reversal	
5 versus 6	3 2 ↓	3 2 ↓	35.9 \pm 5.2	41.5 \pm 7.2	24.0 \pm 3.3	18.3 \pm 1.9	3:3	4:3	1 = 2	
7 versus 8	4 4 ↓	5 1 ↓	20.0 \pm 1.9	38.0 \pm 4.7	19.0 \pm 3.2	45.0 \pm 7.0	4:1	3:3	7 > 8	
9 versus 10	** 8 ↓	** 7 ↓	37.5 \pm 3.6	37.3 \pm 5.7	16.0 \pm 1.1	35.5 \pm 3.7	0:7	1:6	10 > 9	
11 versus 12	2 4 ↓	** 7 ↓	49.4 \pm 5.3	24.0 \pm 3.9	10.0 \pm 2.5	29.8 \pm 3.9	2:3	2:5	Reversal	
13 versus 14	** 8 ↓	** 8 ↓	28.5 \pm 3.0	40.0 \pm 3.4	37.8 \pm 6.7	14.3 \pm 1.7	1:7	7:1	Reversal	

For each pair of clones, we established eight replicated 24-h contests using both pairs of warriors and pairs of reproductive polyps. Vertical arrows indicate victories to the first clone in each pair; horizontal arrows indicate victories by the second. The relative size of the arrows indicates the proportion of contests won by each clone. The values to the top right, bottom left and bottom right of each arrow denote respectively the number of victories to the first clone in a pairing, the second clone in a pairing and the number of unresolved encounters. One contest between warriors of clones 9 and 10 was not completed as polyps became dislodged. Asterisks reflect whether the margin of victory by the dominant clone in a pairing was statistically significant by a binomial test: * $P < 0.05$; ** $P < 0.01$.

Table II. Outcomes and possible determinants of success (mean number of acrorhagi per polyp \pm SE, and order of attack) for inter-clonal contests between unlike morphs (i.e. Warriors (W) versus Reproductives (R) and Reproductives (R) versus Warriors (W)) of seven pairs of neighbouring clones of the sea anemone *A. elegantissima*

Clones	Wins		Number of crorhagi				Attack first	
	W versus R	R versus W	W _A	W _B	R _A	R _B	W _A :R _B	R _A :W _B
1 versus 2	 5 1	**  8	41.1 \pm 5.8	37.1 \pm 4.5	48.6 \pm 5.1	30.3 \pm 4.9	4:3	7:1
3 versus 4	 3 3	 5	28.6 \pm 3.6	58.0 \pm 7.3	11.6 \pm 2.9	50.1 \pm 5.7	4:0	1:6
5 versus 6	 3 3	 2 5	34.1 \pm 6.0	40.0 \pm 4.5	14.0 \pm 4.4	21.4 \pm 2.9	0:8	4:2
7 versus 8	 3 2	**  7 1	28.8 \pm 3.9	47.8 \pm 5.7	16.8 \pm 3.0	39.0 \pm 4.6	4:2	4:1
9 versus 10	 3 2	 4 2	40.4 \pm 6.5	48.0 \pm 1.2	18.9 \pm 2.6	42.4 \pm 6.3	3:1	5:1
11 versus 12	 2 5	*  6 6	48.1 \pm 5.6	39.4 \pm 5.6	18.0 \pm 5.0	26.0 \pm 4.9	5:1	4:3
13 versus 14	 5	*  6 6	25.0 \pm 3.1	41.6 \pm 4.9	23.9 \pm 1.9	16.8 \pm 1.0	0:5	0:7

Procedures and symbols correspond to those shown in Table I. Three contests between warriors of clones 9 and reproductives of 10 were not completed as polyps became dislodged. Asterisks reflect whether the margin of victory by the dominant clone in a pairing was statistically significant by a binomial test: * $P < 0.05$; ** $P < 0.01$.

retaliated 17 times, and reproductives retaliated 15 times. However, pooling the outcomes for all contests not only precludes statistical analysis of the data (because replicate contests within each of the seven inter-clonal combinations are potentially non-independent with respect to the entire data set), it also masks striking differences within inter-clonal combinations in both morph-specific outcomes and relative aggressiveness (Table II).

Outcomes within inter-clonal combination for pairings of unlike morphs were typically less consistent than those involving like pairings of either warriors or reproductives. We identified only four of 14 inter-clonal combinations between unlike morphs in which either the reproductives or the warriors of a clone won a significant majority of contests (R₁ versus W₂, 8:0; binomial test: $P = 0.004$; R₇ versus W₈, 7:0; binomial test: $P = 0.008$; R₁₁ versus R₁₂, 6:0; binomial test: $P = 0.016$; R₁₃ versus R₁₄, 6:0; binomial test: $P = 0.016$), compared to seven of 14 combinations between like morphs. However, in five of the seven inter-clonal combinations between unlike morphs, both the warrior and reproductive polyps of a single clone won the majority of contests with both the reproductive and warrior polyps of its neighbouring clone. Two of these five

clones (clones 1 and 7) were also the most successful in pairings of like morphology: clone 1 (versus clone 2) and clone 7 (versus clone 8) won respectively 13 of 14 (binomial test: $P = 0.009$) and 10 of 12 (binomial test: $P = 0.019$) resolved encounters between morphologically dissimilar polyps.

Patterns of morph-specific aggressiveness (judged by the proportion of first attacks) also varied across inter-clonal combinations between unlike morphs. In some cases, both warrior and reproductive polyps of a particular clone appeared to be comparably aggressive. For example, the reproductive and warrior polyps of clone 9 initiated attacks on five out of six and four out of five clone 10 warriors and reproductives, respectively, in contests for which the order of attack was known (cf. one initial attack by clone 10). In contrast, within two inter-clonal combinations (clones 3 versus 4 and clones 5 versus 6), warriors and reproductives, regardless of clonal identity, differed in their propensity to initiate a first attack. The warriors of clone 3 and clone 4 were consistently more aggressive than the reproductive polyps of their opponent, attacking first in 10 of 11 cases (binomial test: $P = 0.018$). The reverse was true for pairings of clone 5 with clone 6, in which reproductive polyps attacked first in 12 of 14 cases (binomial test: $P = 0.006$).

Morphological and Behavioural Correlates of Dominance

Over all contests between like and unlike morphs, two attributes, relative aggressiveness (propensity to attack first) and possession of the relatively larger number of acrorhagi, appear to account for the outcomes of most contests. In 106 of the 215 contests featuring agonistic behaviour, the first attack was decisive and the opponent did not retaliate. In a further three cases the initial attack did not provoke retaliation but also failed to yield a clear victory. We were able to identify a clear winner in 67 of the remaining 106 cases featuring reciprocated attacks. In these cases the anemone that attacked first had no apparent advantage, winning only 26 of 50 (52.0%) resolved contests for which the order of attack was known. In contrast, the polyp with the larger number of acrorhagi won 48 of 66 (72.7%) resolved contests where the contestants had different numbers of acrorhagi.

In contests between like morphs, aggressiveness and possession of the larger number of acrorhagi appears to explain the dominance relationships within six of seven inter-clonal combinations. That is, the most successful group of warrior or reproductive polyps typically attacked first and possessed the larger number of acrorhagi. Most strikingly, the consistently dominant clones 1 and 10 showed little morphological or behavioural differentiation between morphs. Both reproductive and warrior members of these clones possessed relatively large numbers of acrorhagi ($R_1 = 43.3 \pm 6.4$ ($\bar{X} \pm SE$); $R_{10} = 35.5 \pm 3.7$; $W_1 = 40.1 \pm 7.0$; $W_{10} = 40.0 \pm 3.4$; Table I), and were highly aggressive. The reproductive polyps of their neighbouring clones (2 and 9) carried far fewer acrorhagi ($R_2 = 26.5 \pm 4.7$, $R_9 = 16.0 \pm 1.1$), and in contests between pairs of reproductives, attacked first only once, losing all 14 resolved contests (Table I). In partial contrast, the warriors of clones 2 and 9 had roughly the same number of acrorhagi as their opponents ($W_2 = 39.8 \pm 4.9$, $W_9 = 37.5 \pm 3.6$), but in contests between pairs of warriors they again rarely attacked first (2 of 16 contests), and lost all 16 pairings (Table I).

The reversal of dominance that occurred between pairs of warriors and pairs of reproductives of clones 3 versus 4, 11 versus 12 and 13 versus 14 may also reflect differences between morphs in both the number of acrorhagi per

polyp, as well as the relative aggressiveness of the clones (Table I). For example, the reproductive polyps of clones 11 and 14 (1) possessed on average <40% the number of acrorhagi of their dominant clone 12 and 13 opponents (Fig. 1); (2) attacked first in only three pairings; and (3) lost all 15 resolved contests (Table I). In contrast, the warriors of clones 11 and 14 were significantly more successful, winning 12 of 14 resolved encounters. These warriors carried greater numbers of acrorhagi than their clone 12 and 13 opponents (Table I), and attacked first in 10 of the 13 cases in which we could determine the order of attack.

In the single inter-clonal combination in which we judged the clones to be evenly matched (5 versus 6; Table I), the numbers of acrorhagi carried by their respective groups of warriors and reproductives were statistically indistinguishable (although warrior polyps of both clones bore approximately twice as many acrorhagi as the reproductive polyps), and each clone attacked first approximately 50% of the time (Table I).

The interaction between clones 7 and 8 is an exception to the pattern described above. In contests between like morphs, the reproductive and warrior polyps of clone 7 defeated their clone 8 opponents in nine of 10 resolved encounters. However, on average, clone 7 displayed approximately half the number of acrorhagi and attacked first in only seven cases, compared to four initial attacks by clone 8 (Table I).

DISCUSSION

Polymorphism and Social Organization

Our data confirm Francis' (1976) description of polymorphism and social organization within *A. elegantissima* clones: the peripheral, warrior polyps of most clones were on average smaller than their central, reproductive clonemates, and, in nearly every case, possessed greater numbers of acrorhagi. Such morphological differentiation has not been described in other clonal cnidarians, although it occurs in other clonal organisms in which clonemates are physiologically independent (e.g. Aoki 1979; Cruz 1981; Dixon 1985). Our analysis of morph-specific behaviour also provides the first direct demonstration that the more heavily armed warrior polyps are aptly named.

Although warriors were typically far smaller than reproductive polyps, they won half their contests with the reproductive polyps of their neighbours (41 versus 40, Table II). At first sight, this result appears surprising because some studies of other anemone species (which apparently do not exhibit intra-clonal polymorphism) report size-dependent dominance hierarchies, in which larger polyps are more aggressive and better fighters than smaller polyps (e.g. Brace et al. 1979; Brace 1981; Sebens 1984). The present data, together with our earlier study of inter-clonal contests between reproductive polyps (Ayre & Grosberg 1995), imply that much of the success of *A. elegantissima* warriors reflects their greater proportional allocation of resources to acrorhagi.

Inter-clonal Borders and Dominance Relationships

If indeed the inter-clonal borders separating the seven pairs of clones were persistent (as described by Francis 1973a, b), then the simplest explanation would require that neighbouring clones were not mutually aggressive, either because (1) naïve polyps lacked sufficient allogeneic disparity to elicit a 'non-self' agonistic response or (2) polyps became habituated to the genotype of their neighbour after repeated or prolonged contact. Previous work suggests that both of these hypotheses are credible. Seemingly non-allogeneic interactions have been reported for pairs of cnidarian clones, including those of several species of coral (Heyward & Stoddart 1985; Resing & Ayre 1985; Willis & Ayre 1985) and in *A. elegantissima* (in one of 21 inter-clonal combinations between non-neighbouring clones; Ayre & Grosberg 1995). In addition, under laboratory conditions, repeated interactions between *A. elegantissima* clones may initially produce an enhanced agonistic response, followed by clone-specific habituation (Ayre & Grosberg 1995). Similarly, in two other anemones, *Anthopleura xanthogrammica* (Sebens 1984) and *Metridium senile* (Purcell & Kitting 1982), long-term neighbours within otherwise aggressive populations often behave passively.

Lack of aggression may account for local maintenance of clonal diversity; however, it should also lead to intermixing of clones, hence it does not readily explain the persistence of stable inter-clonal boundaries in *A. elegantissima*. Moreover, our results suggest that neither the lack of allogeneic disparity, nor clone-specific habituation,

underlies the long-term maintenance of inter-clonal boundaries in *A. elegantissima*. Although some neighbouring pairs of *A. elegantissima* may not be mutually agonistic, all seven pairs of clones used in this study were. Moreover, the behaviour of warriors and reproductives was similar, both in terms of their initial aggressiveness and tendency to retaliate if attacked. If habituation and tolerance following repeated contact were a common feature of the behaviour of neighbouring *A. elegantissima* clones, then it should be most apparent in the behaviour of warriors from adjacent clones, because these polyps ought to have experienced the most recent and frequent inter-clonal contact. It remains possible that warrior polyps were, at the time of collection, habituated to their neighbours, but lost their tolerance by the time we used them in the laboratory trials. However, our previous study (Ayre & Grosberg 1995) showed that habituation continued for at least 24 h (and probably considerably longer), and the laboratory trials reported in the present study took place within 24–48 h of collection. Consequently, we believe that (1) warriors did not have time to lose a habituated response; (2) habituation does not generally feature in natural inter-clonal interactions in this species; and (3) opposing warriors should fight whenever they make tentacular contact.

Stable inter-clonal borders could also form if neighbouring clones were mutually agonistic, but competitive equals. Several studies of spatial competition between colonies and clones of a range of benthic invertebrates suggest that unresolved contests, or standoffs (sensu Karlson 1980), can contribute to coexistence in space-limited species assemblages (e.g. Karlson 1980, 1981; Kay & Keough 1981; Russ 1982; Sebens 1986, 1987). However, our laboratory trials suggest that most neighbouring clones of *A. elegantissima* are grossly mismatched. In five of seven warrior versus warrior inter-clonal combinations, the warriors of the dominant clone defeated the majority of the opposing warriors, including three cases in which the dominant clone won all eight contests (Table I).

To the extent that agonistic interactions between paired polyps determine the outcomes of inter-clonal competition for space, this study implies that most neighbouring clones are not competitive equals, and that the dominant clone should eventually displace its neighbour. In so

doing, the study fails to provide a simple and general behavioural explanation for the persistence of stable inter-clonal boundaries. However, the inter-clonal dominance trials involved replicated contests between pairs of individual polyps, isolated from their clonemates. This design allowed us to determine that aggressive behaviour and the outcomes of contests were often consistently repeatable for particular clones and inter-clonal combinations. None the less, these outcomes may better reflect the interference competitive abilities of pairs of individual polyps rather than those of entire clones.

There are at least three reasons why a clone could appear to consist of a team of star attackers, but not function effectively as a winning team. First, the behaviour of individual polyps may vary according to whether they fight in isolation (as in this study, and during the initial phases of clonal establishment), or in the company of their clonemates (also see Francis 1988). Second, these trials allowed defeated polyps to move away from their opponent, potentially over-emphasizing the importance of initial aggressiveness as a determinant of clonal dominance. Nothing is yet known about natural interactions at inter-clonal borders; however, it seems unlikely that scarred warriors could easily retreat from a fight, because they are surrounded on three sides by warrior and reproductive clonemates. If retreat is unlikely then contests may be prolonged and could involve a higher frequency of retaliatory attacks. We found that in reciprocated contests the order of attack did not appear to affect outcomes. Thus, if contests were more prolonged in nature than in the laboratory, as seems likely along inter-clonal borders, then the potential for standoffs could increase, and dominance rankings could shift. Finally, the outcomes of prolonged contests may depend not only on the fighting behaviour of individual polyps, but also on clone-specific patterns of allocation to replacement warriors. In other words, clones with inferior warriors in single one-on-one contests may also have at their disposal a greater stock of reinforcement warriors to replace damaged warriors on the front line (see next section).

The Evolution of Social Organization

Because the effects of an array of stimuli can be tested independently for large numbers of

genetically identical individuals, clonal organisms offer a unique but rarely exploited opportunity to isolate the effects of genotypic and environmental variation on the expression and evolution of behaviour. This study, along with our previous analysis of aggression in *A. elegantissima* (Ayre & Grosberg 1995), reveals a previously unsuspected range of inter-clonal variation in agonistic behaviour, associated with clone-specific variation in morphology, genotype specific enhancement or habituation of agonistic behaviour, and the level and nature of social organization. To the extent that these phenotypic patterns of inter-clonal variation reflect genetic differences, *A. elegantissima* provides an experimental system in which it should be possible to analyse the strength and nature of selection on variation in morphological and behavioural characters associated with the outcomes of inter-clonal agonistic encounters. Similarly, it should also be feasible to isolate the effects of variation in the genotypic identity and diversity of opponents on the expression of these characters.

From a microevolutionary perspective, one of the most intriguing features of the agonistic biology of *A. elegantissima* revealed by this study is the existence of distinctive clone-specific variation in the pattern of allocation to acrorhagi in warrior versus reproductive polyps and the association between this variation and inter-clonal dominance. Among established, interacting clones two patterns appear to be common: (1) monomorphism, in which both warrior and reproductive members of a clone are heavily armed and display similar numbers of acrorhagi and (2) polymorphism, in which reproductive polyps carry relatively few acrorhagi, but warrior members of a clone bear as many or more than members of monomorphic clones. Thus, under the conditions at our study site, some clones show more phenotypic plasticity than others which may reflect varying histories of inter-clonal interactions.

Among those clones that exhibit polymorphism, there is also substantial clone-specific variation in the magnitude of morphological disparity between warrior and reproductive polyps. Nevertheless, warrior polyps are, on average, typically smaller, and never larger, than reproductive clonemates. Although our data show that small warriors are on average very effective fighters, their relatively small size does not appear to be the result of selection to maximize interference

competitive ability in one-to-one combat (Lanchester 1916). At the level of clones or colonies, however, selection may favour the production of small warriors, particularly if their inferior fighting ability can be balanced by their lower energetic cost, and the potential to produce more warriors (Morse & Kimball 1951). For example, relatively small and energetically cheap soldiers are a common feature of many polymorphic colonies of social Hymenoptera and Isoptera (Wilson 1972; Hölldobler & Wilson 1990; reviewed in Franks & Partridge 1994). Furthermore, models of warfare involving replacement of injured or dead warriors may not only favour the production of large numbers of suboptimal warriors, but also lead to the stable coexistence of clones with different patterns of allocation to warrior numbers and armament (Morse & Kimball 1951).

The relatively small size of warriors, and inter-clonal variation in size disparity between warriors and reproductives, may be explained by factors unrelated to their role in interference competition. Indeed, Francis (1976) reported that all peripheral polyps (and not just those at inter-clonal borders) were on average smaller than those at the centres of aggregations. Two aspects of the physical environment of peripheral polyps that may lead to small size are (1) elevated rates of fission at the 'free edges' of a clone and (2) a phenotypically plastic response to increased drag. Polyp size is inversely related to wave exposure in many sea anemone species (Ayre 1984; Shick 1991) and a graded increase in size from peripheral to central polyps may be a general adaptation that allows *A. elegantissima* clones to reduce drag and the risk of dislodgement by wave action in exposed locations.

A general explanation for the evolution of social organization in *A. elegantissima*, and the persistence of inter-clonal variation in the expression of social organization, necessarily involves consideration of temporal and spatial heterogeneity in other physical and biotic attributes of the environment. For example, several models predict that at high densities and when rates of resource renewal are low, selection should favour highly competitive clones (reviewed in Sebens & Thorne 1985). In contrast, in highly disturbed habitats where interference competition for space is expected to be less intense, selection should favour clones that are superior colonizers (Sebens & Thorne 1985). These contrasting conditions are

likely to occur within many populations of *A. elegantissima*. At Doran Rocks where we conducted this study, *A. elegantissima* inhabits isolated large boulders which are often dominated by a single clone, extensive stable rock platforms in the mid-low inter-tidal occupied by multiple clones (personal observations), and the highly disturbed upper shore mussel beds, where clonal diversity is maximal (Sebens 1982; personal observations). During the initial phases of clonal establishment and expansion, which are likely to involve encounters with numerous other clones and high levels of interference competition, selection should favour the production of heavily armed, monomorphic polyps. Once a clone becomes established, it is likely to face a lower diversity of conspecific competitors, but may remain involved in long-term interactions with one, or a few other, clones. Under these circumstances, phenotypic plasticity, in which polyps of the same genotype may differentiate into either warriors or reproductives, may become particularly important, as central polyps in a clone are unlikely to encounter other conspecific genotypes.

The extent to which any, or all, of these environmental characteristics influence the evolution of variation in the genetic and ontogenetic expression of social organization minimally requires an understanding of (1) the temporal dynamics of changes from one morph to the other; (2) allocation trade-offs at the level of the clone between the production of warriors and reproductives; and (3) the natural circumstances in which clones with different allocation patterns can attain competitive dominance. The next steps in our analysis of the evolution of social organization in *A. elegantissima* entail field transplants in which we characterize how, and if, clone-specific patterns of allocation vary in different habitats and competitive regimes, and whether the dominance relationships inferred from laboratory pair-wise contests can be extended to entire clones in the field.

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