

**Morphological Variability and Intraspecific Aggression in the Clonal
Anemone, *Anthopleura elegantissima***

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Abstract

Individuals of the aggregating anemone, *Anthopleura elegantissima*, occupy the rocky intertidal and form large clusters of polyps by splitting (fission). These organisms are known to attack neighboring, unrelated clone-groups and although genetically identical, fighting ability varies within a clone-group; individuals on the edge of the aggregation take the role of warriors, leaving those further inside the clone-group free to reproduce. This study examined morphological differences between successful and unsuccessful combatants, both within a colony and across different clone-groups. This may confer a competitive advantage to individuals within a colony or to the clone-group as a whole. Anemones were collected from three distinctly separate clonal colonies in a single bay and agonistic interclonal interactions were staged. Competitors were chosen at random within each functional class, with 15 reproducer and 15 warrior trials. Outcomes of the competitions were determined and variations in tentacle length, tentacle density and acrorhagi density were assessed between the winners and losers, as well as within clone-groups. Warriors and reproducers from Clone-group 1 had the highest frequency of wins, while individuals from Clone-group 3 had the least. No differences between tentacle density and acrorhagi density were observed. Tentacle length varied both across clone groups and between warriors and reproducers within colonies (warriors: $P < 0.001$, reproducers: $P = 0.004$; and clone-group (CG) 1: $P = 0.001$, CG 3: $P = 0.003$; respectively). Morphological characteristics measured do not appear to give a competitive advantage to individuals ($P = 0.262$). Aggressive ability seems to be conferred to the aggregation as a whole.

Introduction

Protection and expansion of territory is a common goal of many animals. This is particularly true in densely occupied areas such as the rocky intertidal. In this harsh environment, space is often the limiting factor for successful survival, growth, and reproduction of sessile invertebrates (Ayre and Grosberg, 1995). Organisms that can develop an effective method to obtain and secure their position are the ones that will live to pass on their genes to the next generation.

Conflict and competition for territory and resources are not always an interspecific occurrence as many animals must also contend with other conspecifics. Clonal organisms provide a particularly interesting case study in this regard, as they are genetically identical (Sebens, 1986). Clone-groups possess the ability to, theoretically, increase their numbers and spread their particular genotype without limit (Ayre and Grosberg, 1995). Therefore, it is nearly inevitable that different colonies will meet, and they must either tolerate one another, or vie for dominance.

The aggregating sea anemone, *Anthopleura elegantissima*, is an abundant and hardy clonally-reproducing intertidal animal broadly distributed on the west coast of North America. *A. elegantissima* is gonochoristic and usually reproduces sexually once per year (Francis, 1976; Sebens, 1980). Thereafter, the resulting polyp can reproduce asexually via fission to form a clone-group. Once the aggregation reaches more than 100 clonal individuals, it is considered an established colony (Ayre and Grosberg, 2005). Separate colonies frequently come into contact, increasing stress and typically resulting in aggression and fighting from one or both clone-groups (Rossi and Snyder, 2001). In

densely packed habitats, this can result in a characteristic band of anemone-free area between the two genotypes (Francis, 1973a; Sebens, 1983).

As a result of these antagonistic interactions, even genetically identical individuals within an aggregation can develop different morphologies and assume different roles, effectively separating defensive and reproductive responsibilities. Traditionally, these roles have been divided into two main categories: “warriors” and “reproducers” (Francis, 1976; Ayre and Grosberg, 1996). Warriors are found at the distal edges of the aggregation and tend to be smaller, allocating most of their energy to increased development of the nematocyst-filled fighting tentacles known as acrorhagi. Consequently, the warriors are not only smaller, but also experience little to no gonadal development (Francis, 1976). The reproducers live proximal to the center of the clonal group. Rather than growing many powerful acrorhagi, reproducers have larger body sizes, accommodating substantial gonads (Ayre and Grosberg, 1996).

Although the difference in fighting abilities between warriors and reproducers has been well established (Francis, 1976; Ayre and Grosberg, 1996), very little has been investigated with regard to the fighting abilities of these functional guilds across separate clone-groups. Warriors are known to be more aggressive and more successful combatants than reproducers due to their higher densities of acrorhagi (Ayre and Grosberg, 1996), but there has been no investigation into what possible morphological attributes may confer a competitive advantage upon a clone-group as a whole. It is the purpose of this study to determine if there are specific morphological features found in dominant individuals, and if those features remain consistent within and between clone-groups. For example, colonies with successful warriors may also contain similarly successful

reproducers. Although reproducers as a group are less effective fighters than warriors, they will still have an advantage when fighting other reproducers, if they have a genetic predisposition toward aggression (Sherman et al, 2008). Therefore, clone-groups and individuals will be more successful in agonistic interactions when they possess one or more of the following morphological features: (i) higher tentacle density, (ii) higher mean tentacle length, and (iii) higher density of acrorhagi.

Methods

Field Site and Organism Collection

Experimental organisms were collected from Eagle Cove on San Juan Island, WA (48°27'36" N, 123°01'53" W). Eagle Cove is a pocket beach with a sandy interior and rocky outcrops on either side, with *Anthopleura elegantissima* clone-groups found on the intertidal rocky outcrops. Twelve reproducer and twelve warrior individuals with similar oral disc diameter were collected from each of three distinct colonies, with the warriors collected from the periphery and the reproducers from the center-most portion of the group. Colonies were identified as groups that occupied a visually distinct area, such as an isolated tidepool, with the edges of each group selected at least 5 m away from the others to ensure that different clone-groups were sampled. Each clone-group was placed in contact with the other groups prior to the start of experimental trials to see if they would interact agonistically. This ensured that groups were distinct, as individuals from the same clone-group will not interact agonistically. All *A. elegantissima* were placed in holding tanks separated by clone-group and functional class and taken back to the laboratory. Specimens were settled on individual 3.5 x 3.5 cm acrylic plates and

acclimated to aquarium conditions for 96 hours. Subjects were placed in a sea table with running unfiltered flow-through seawater at 10-12°C. The sea table was shared with other organisms but the experimental organisms were isolated in small tanks with water from the sea table flowing through each individual tank.

Competitive Interactions

Staged agonistic interactions were conducted between individuals from different clone-groups, with 3-5 replicates of each agonistic clone-group pairing and 1-2 control trials for each clone group, for a total of 30 trials. Competitors were chosen at random within each functional class, as each class has different competitive abilities. Random selection included within-clone interactions, which serve as the control trials

An experimental acrylic tank, with eight 3.5 x 13 cm lanes, was used to conduct eight simultaneous trials. The experimental tank was also situated in the same sea table as the experimental organisms, with the inflow on the right edge of the raceway lanes and the outflow on the left (Figure 1). Lanes were used to allow for retreat while prohibiting lateral movement. Each plate containing an individual competitor was placed in a lane and the individuals were allowed to acclimate for 5 minutes while still separated. The plates were then slid together until the competitors' tentacles were in contact, with the starting position of each anemone marked. Observations of movement and aggressive behavior were recorded every 5 minutes for the first hour of each trial, and every following hour until the end of the trial period. Photographs were taken every hour. The first competitive interactions were allowed to continue for a 12-hour trial period, but observations of individual behavior supported an effective trial period of 6 hours, which

was used for all subsequent trials. At the end of a trial period, the outcome for each individual was determined. A win occurred if an individual forced the opposing polyp to retreat or killed it; a loss was classified by retreat or death; a draw occurred if there was an aggressive interaction between the individuals but neither death nor retreat was observed; and no interaction was when no aggression was observed (Figure 2).

After completion of the experimental trials, all individuals were placed in separate marked wells to facilitate identification. Each anemone will then be relaxed in 7% MgSO₄ for several hours and dissected. Oral disc diameter, tentacle number and number of acrorhagi will be counted, and other morphological features that may confer a competitive advantage to the individual will be noted.

Statistical Analysis

Numbers of wins, losses and draws was assessed for each functional class within discrete clone groups and frequency of wins was then calculated. Control trials were omitted from this analysis as no interaction was expected. Tentacle and acrorhagi density for each individual was calculated as a function of oral disc diameter. There was a weak correlation between oral disc diameter and tentacle length, so length was used as an absolute value rather than a ratio of oral disc diameter ($R^2 = 0.113$). Variability in tentacle length, tentacle density and acrorhagi density between warriors and reproducers within a clone group was examined. Each pairing was tested for normality, and as seven out of nine tested were normal, parametric statistics were used for all data. Variability in these three factors between all warriors and all reproducers was assessed using one-way ANOVAs with Tukey's Post Hoc Tests. Differences in tentacle length between winners

and losers of competitive interactions and those that participated in a draw was then analyzed using ANOVAs.

Results

Competitive Interactions

Out of 30 trials run, no death was observed so all wins were determined by the retreat of a competitor. No agonistic interactions occurred between individuals from the same clone-group, although some individuals did move away from their clonemate. Clone-group 1 had the highest proportion of wins for both warriors and reproducers, with clone-group 2 having the second highest and clone-group 3 with the lowest. Warriors had a higher proportion of wins than reproducers for both clone-group 1 and 2, but the opposite trend was observed in clone-group 3 (Figure 3; Table 1). Warriors in clone-group 1 never lost an interaction, whereas warriors from clone-group 3 never won.

Morphology

Within clone-groups, no differences in tentacle density or acrorhagi density were observed between warriors and reproducers, with tentacle length as the only characteristic that was significantly different. Clone-group 1 and 3 warriors had shorter tentacles than their corresponding reproducers ($P = 0.001$ and 0.003 , respectively), while there was no difference in tentacle length observed between the warriors and reproducers in clone-group 2 ($P = 0.375$; Figure 4).

When comparing tentacle length, tentacle density and acrorhagi density across all warriors, differences were observed between clone groups ($F = 13.315$, $P < 0.001$).

Clone-group 1 had significantly shorter tentacles than both groups 2 and 3 ($P = 0.001$ and <0.001 , respectively) while clone-groups 2 and 3 did not have different tentacle lengths ($P = 0.811$). There were no significant differences in tentacle or acrorhagi density between warriors across all clone groups ($F = 2.158$, $P = 0.135$ and $F = 1.942$, $P = 0.163$, respectively; Figure 5).

Reproducer tentacle length also varied by clone-group ($F = 6.743$, $P = 0.004$). Clone-group 3 reproducers had significantly longer tentacles than groups 1 and 2 ($P = 0.004$ and 0.045 , respectively), while tentacle lengths between clone-groups 1 and 2 did not vary ($P = 0.558$; Figure 3). However, no differences were observed in reproducer tentacle or acrorhagi density between clone-groups ($F = 0.806$, $P = 0.457$ and $F = 1.227$, $P = 0.309$, respectively; Figure 5).

No differences in tentacle length were seen between the winners, losers and those that resulted in a draw of any competitive interaction ($F = 1.380$, $P = 0.262$).

Discussion

Warrior polyps in this study collectively had a higher rate of successful agonistic interactions than the reproducers, in support of previous literature (Francis, 1976; Ayre and Grosberg, 1995). As a whole, clone-group 1 displayed the most aggression and most successful attacks. Conversely, clone-group 3 experienced the most frequent losses, with the warriors from that colony never winning a match. Although warriors generally won more competitions, that was not true for group 3. Therefore, increased competitive potential appears to be stronger at a clone-group level rather than merely a functional group level.

Despite finding clear disparities in fighting ability across clone-groups, there were no statistically significant morphological variations with the exception of tentacle length. However, the results for tentacle lengths were actually the opposite of what was expected. Warriors from clone-group 1, the most competitive colony, had the shortest tentacles, while reproducers from clone-group 3 had the longest tentacles despite their relative inadequacies in antagonistic encounters. More studies involving larger sample sizes may clarify any possible morphological differences between successful and unsuccessful polyps. This approach is recommended as the result that acrorhagi density was not a significant factor is in conflict with several past studies, which found a correlation between number of acrorhagi and combat success (Francis, 1973b; Ayre and Grosberg, 1995; Zeh and Zeh, 1997).

Another factor observed in these trials was the degree of movement between the competitors, although these data were not quantified. In situ, those polyps near the center of the colony, namely the reproducers, are unable to move away from potential competitors. Accordingly, less movement and more draws were observed during the reproducers' trials. Warrior polyps located on the periphery of the clone-group may have more options for movement, which may be reflected in the tendency for more warrior interactions to end in substantial individual movement and retreat from conflict. Future studies in which movement is restricted would provide a more accurate determination of fighting ability, rather than allowing immediate retreat from the confrontation.

Reactions to aggressive interactions, whether it is to fight or retreat, may be an artifact of genotypic relationships. Genetic analyses are beyond the scope of this study, but future research should look at genetic variability and how that may relate to

aggression level during conflicts. In this particular study, it was noted that competitions between clone-groups 1 and 3 had the most obvious and aggressive encounters, while interactions between clone-group 2 and any other group often resulted in a draw. This suggests there may be some genetic component causing group 2 to both initiate and experience less intense antagonistic behaviors. Genetic similarities and relatedness between clone-groups may be responsible for these differences in aggressive response. *Anthopleura elegantissima* possess the ability to differentiate between clonemates and non-clonemates (Ayre and Grosberg, 1995; Zeh and Zeh, 1997). Differences in amount of multilocus homozygosity correspond to whether a non-clonemate initiates an attack, and possibly to how antagonistic the interaction becomes (Zeh and Zeh, 1997). Future analyses of the genetic relationships between clone-groups may reveal more complex interactions, such as the possibility that clone-group 2 is in some way more genetically similar to groups 1 and 3 than they are to each other. This would then lead to more agonistic encounters between the dissimilar groups than the more similar group 2.

Genetic studies may also elucidate how genetically identical individuals can still differentiate into highly specialized and morphologically dissimilar functional guilds. The relative simplicity of the anemone body plan makes *A. elegantissima* an excellent candidate as a model organism. The phenotypic and behavioral plasticity exhibited by this animal provides a unique opportunity to evaluate the relative effects of genotype and environment. In addition, the concept of biological tradeoffs and their impacts upon an organism's fitness and evolutionary capacity can be analyzed using the differing functional classes. *Anthopleura elegantissima* can provide science with many interesting and novel studies in the field of genetics over the coming years.

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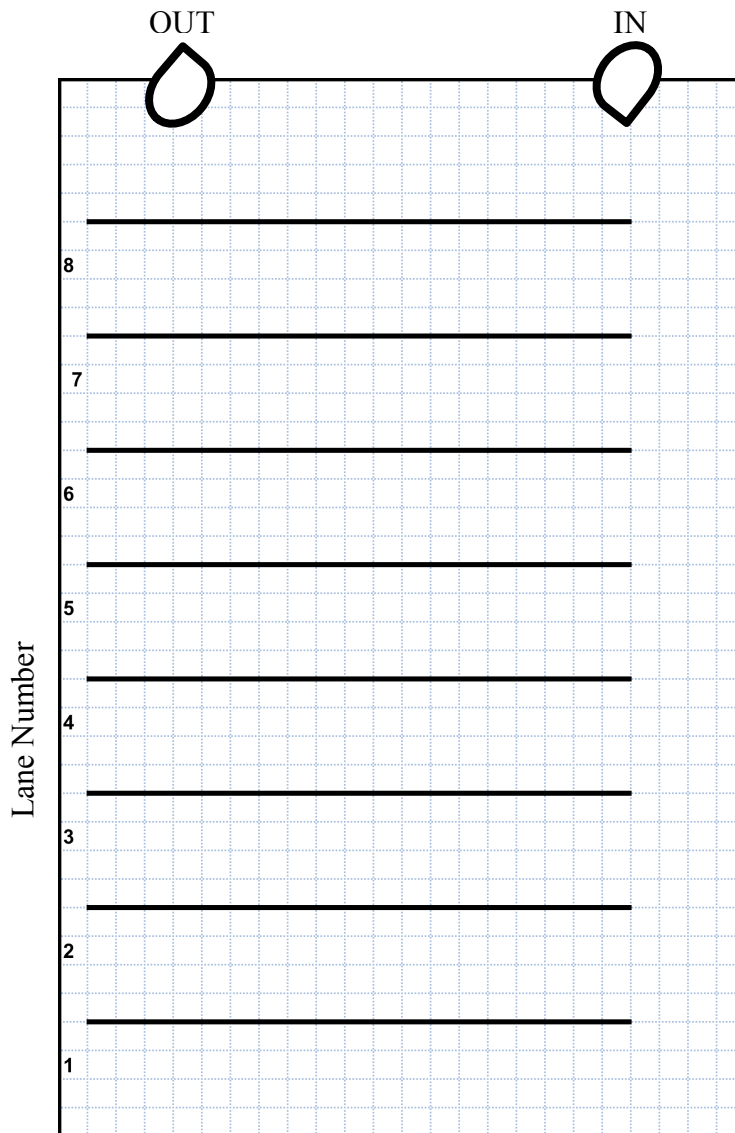


Figure 1:

A diagram of the experimental flow-through tank, with 8 lanes measuring 3.5 x 13 cm. The inflow of seawater runs along the right edge of the raceway lanes with the outflow on the left.

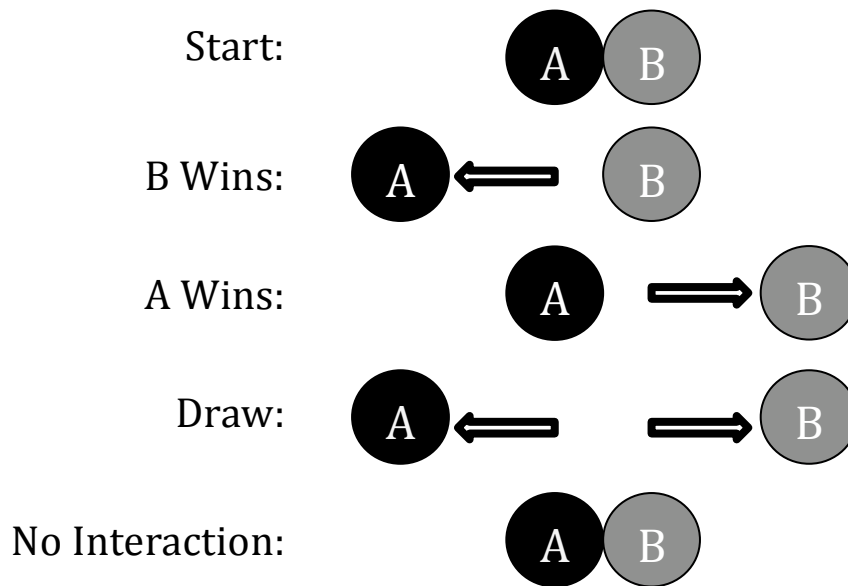


Figure 2:

A categorization of trial outcomes relative to individual starting positions. Polyp B wins if A moves away, and vice versa, a draw occurs if both polyps move away from each other, and no interaction is characterized as no movement from either individual.

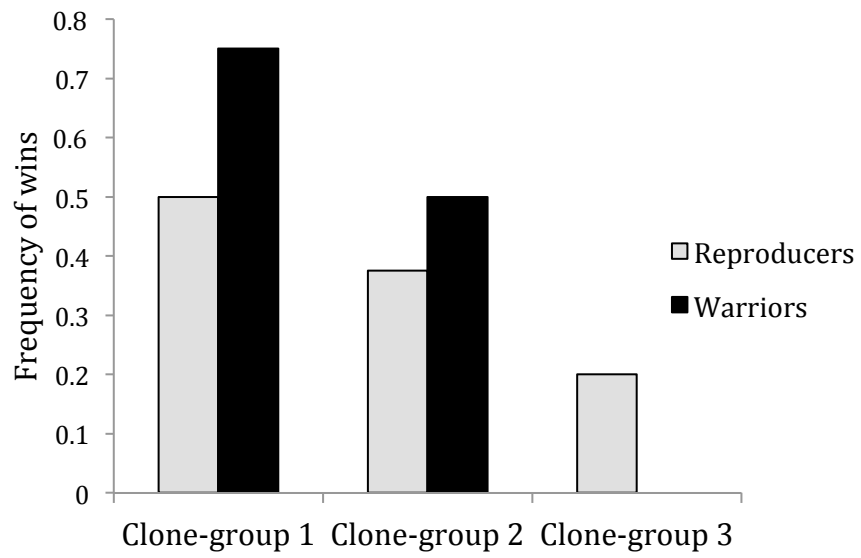


Figure 3:

Frequency of wins compared between reproducers and warriors within clone-group 1, clone-group 2 and clone-group 3.

Table 1:

Frequencies of wins, draws, losses and no interaction for reproducers and warriors within each of three clone groups.

Group	Win	Draw	Loss	No Interaction
Reproducers 1	4	2	2	2
Reproducers 2	2	3	3	2
Reproducers 3	2	5	3	0
Warriors 1	6	2	0	2
Warriors 2	2	3	1	4
Warriors 3	0	1	7	2

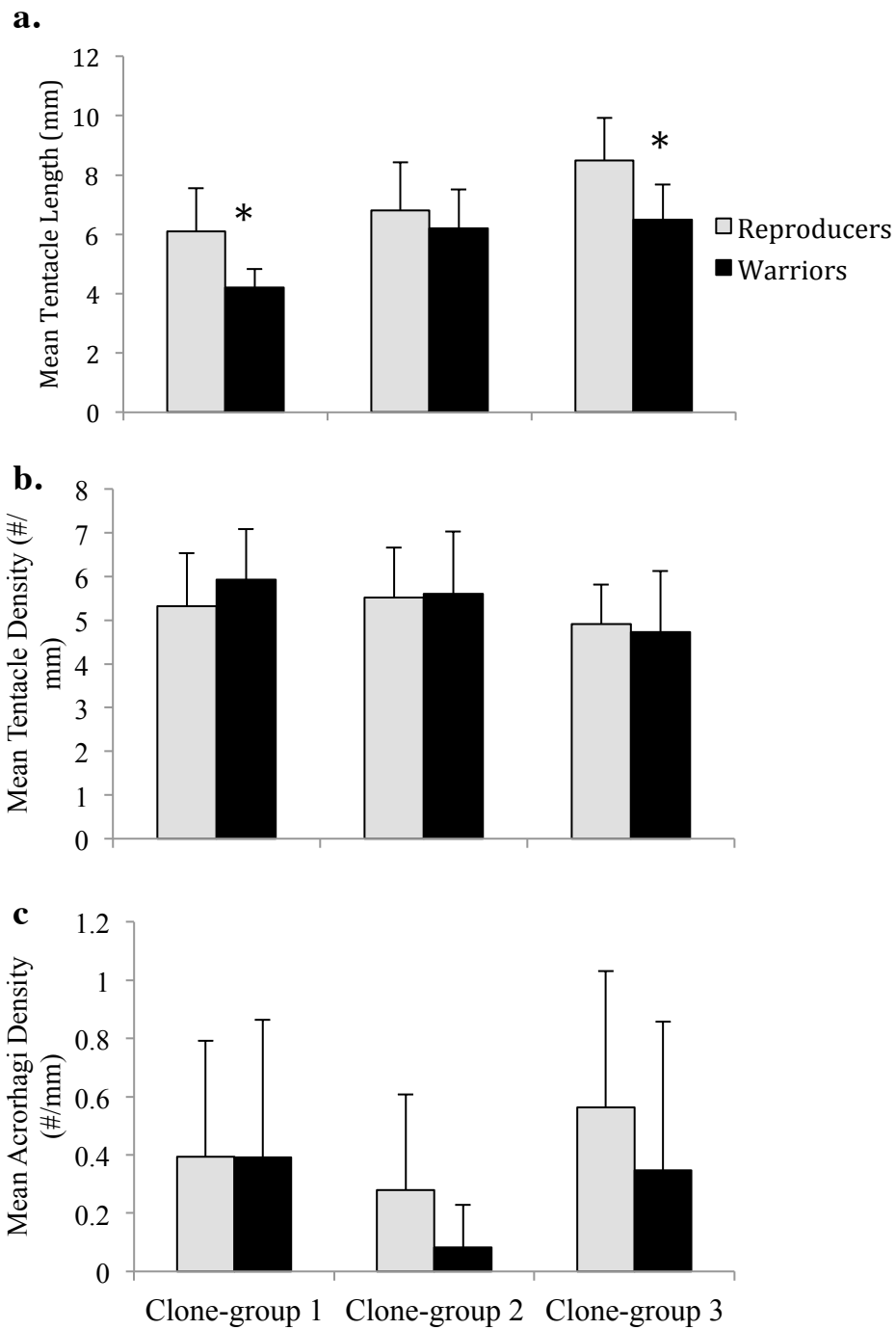


Figure 4:

A comparison of **(a) mean tentacle length** (*clone-group (CG) 1*: $P = 0.001^{**}$; *CG 2*: $P = 0.375$; *CG 3*: $P = 0.003^{*}$); **(b) mean tentacle density (#/mm)** (*CG 1*: $P = 0.69$; *CG 2*: $P = 0.89$; *CG 3*: $P = 0.30$); and **(c) mean acrorhagi density (#/mm)** (*CG 1*: $P = 0.88$; *CG 2*: $P = 0.076$; *CG 3*: $P = 0.34$); between reproducers and warriors within clone groups.

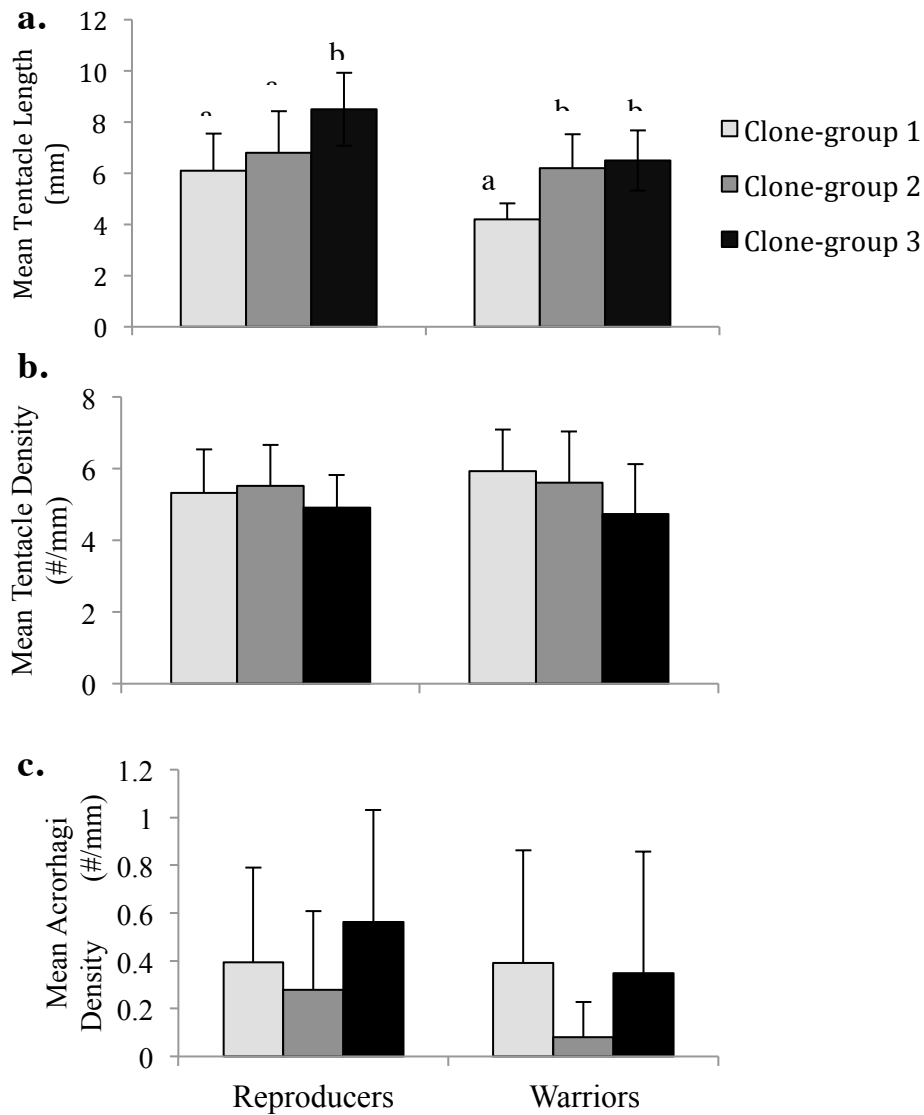


Figure 5:

A comparison of **(a) mean tentacle length** (*reproducers*: $F = 6.743$, $P = 0.004^*$; clone-group (CG) 1 v. CG 3: $P = 0.001^{**}$, CG 1 v CG 2: $P = 0.558$, CG 2 v. CG 3: $P < 0.001^{**}$; *warriors*: $F = 13.315$, $P < 0.001$; CG 1 v. CG 3: $P < 0.001^{**}$, CG 1 v CG 2: $P = 0.001$, CG 2 v. CG 3: $P = 0.811$); **(b) mean tentacle density (#/mm)** (*reproducers*: $F = 0.806$, $P = 0.457$; *warriors*: $F = 2.158$, $P = 0.135$); and **(c) mean acrorhagi density (#/mm)** (*reproducers*: $F = 1.227$, $P = 0.309$; *warriors*: $F = 1.942$, $P = 0.163$), between reproducers and warriors across clone groups.