SCALING OF INDIVIDUAL BEHAVIOR TO GROUP DYNAMICS: THEORETICAL AND EXPERIMENTAL CONCERNS WITH REGARD TO POLYP AND CLONE BEHAVIOR IN *ANTHOBLEURA ELEGANTISSIMA* DISSERTATION

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ABSTRACT

How individual group members behave and interact can have dramatic effects on group level patterns, behaviors, and group success. Here I explore the effects of constituent agent behavior on group level patterns and interactions using both theoretical and empirical means. I aim to understand how behavioral decisions by individual agents alter and shape patterns at the group level.

I first explore a breadth of behaviors in response to a variety of stimuli in the intertidal sea anemone, *Anthopleura elegantissima*. Using a series of behavioral experiments and observational studies I explore movement, aggregation behavior, and agonistic encounters in this species. I use these studies and data from the literature to inform an agent based model of clone behavior. These studies give a fuller understanding of how clones of this species utilize clone specific behaviors in competition with one another for space in the intertidal, specifically, how neighboring clonal mats of this species can mitigate differences in individual agent fighting ability through differential movement rates. In rare cases, neighbors using dissimilar behavioral strategies can demonstrate group level equivalence in competitive ability.

I further explore group level dynamics through a theoretical model of a system that demonstrates a division of labor like *A. elegantissima*. This model is inspired by nest construction in *Metapolybia* wasps, where groups are ephemeral and reproductive success is dependent on groups completing two distinct tasks. I explore the conditions and variables that favor the coexistence of generalists, and their ability to do multiple tasks, with specialists, that
are limited to performing only a single task. I find that despite generalists often erring and performing the wrong task they persist in the population.
Dedicated to my father (James), my mother (Margaret), and my brothers (Michael and Brian) for all the support, love, and encouragement they have given me over the years.
I would first like to thank my advisor, Meg Daly and, for all intents and purposes, my co-advisor Ian Hamilton. Without Meg’s interest in my out-of-the-blue email for an anemone project in 2006 I would never have been able to complete this work. Her knowledge and financial support have been invaluable. Moreover, her personal interest and support during the various hurdles that I encountered has given me the strength to finish. I cannot thank her enough for all that she has given me. Similarly, Ian Hamilton has been overly generous with his time and knowledge, especially with my unannounced visits to his office armed with a multitude of analysis or modeling questions. Additionally, I would like to thank the other members of my committee, Libby Marschall and Kevin Passino. I also give a big tip of my hat to Tom Waite for believing in me and my ideas during a time when no one else would. Without him I would not be where I am today.

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Friday Harbor Laboratories, Lisbeth Francis, Lindi Wahl, a University Fellowship from The Ohio State University, and a large interdisciplinary grant from the Research Office of The Ohio State University.

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CHAPTER 1
INTRODUCTION

A research area that has garnered long-term attention in the biological sciences is the interaction between hierarchical levels, such as the individual and the group, that emerges in social systems (Buss 1987; Bell and Mooers 1997; McShea 2001a, 2001b; Herron and Michod 2007; Michod 2007). Particularly of interest is how lower level agents are subjugated to the needs of the group. This is often studied under the context of how the unit of selection changes from the lower to higher hierarchical level and how subjugation of lower levels help mitigate conflicts between hierarchical units (e.g. Buss 1987; Smith and Szathmary 1995; Michod 2007; Hochberg et al 2008). This particular problem appears to be resolved, at least in some groups, through modifications to lower level agents and reduction of their fitness in such a way that group level fitness is increased (Michod 2006, 2007). In some cases, such as the volvocine green algae (Michod 2006, 2007), nest selection in honeybees (Passino and Seeley 2006; Seeley et al 2006; Passino et al 2008), flocking behavior in birds (Wood and Ackland 2007), and schooling in fish (Parrish et al 2002) the modification or behavior of lower level agents can lead to large changes in group level dynamics. How individual agent behavior and relatedness at this lower level influences and shapes the dynamics of the group in which the agents are operating is an important area of research in biology (e.g. Hamilton and Taborsky 2005; Hochberg et al 2008; Miramontes and DeSouza 2008; Nabet et al. 2009; Wright et al. 2009; Fisher and Hoekstra 2010). In this dissertation I explore the effects of individual agent behavior on group level
patterns and processes in a variety of contexts using both empirical and theoretical approaches. The first four chapters of this dissertation investigate this relationship in an intertidal invertebrate, the sea anemone *Anthopleura elegantissima*. I then expand my scope to a generalized theoretical group in my final chapter.

*Anthopleura elegantissima* (Cnidaria: Anthozoa: Actiniaria) is an intertidal sea anemone found along the Pacific coast of North America between Northern Mexico/Southern California and Alaska (Hand 1955). Reproduction in this species occurs through both sexual and asexual means. The former occurs during the late summer and early fall, while the latter is throughout the year (Sebens 1977) and gives them their conspicuous appearance on intertidal rock faces.

Asexual reproduction by individual anemones (polyps) leads to large mats of physically distinct but genetically identical polyps known as clonemates (Francis 1973a, b). These mats may consist of several to hundreds of individual anemones (Francis 1973a, b). Each mat of genetically identical polyps is referred to as a clone. Clones are observed to form an interclonal boundary of 1-5 cm that remains anemone-free when they abut on another (Francis 1973a; Ayre and Grosberg 2005). Across this boundary agonistic interactions often occur between the neighboring clones (Francis 1973b; Ayre and Grosberg 2005)(Fig. 1.1). These encounters involve the inflation of columnar sacs, called acrorhagi, filled with holotrich nematocysts that are then applied to non-clonemates in a stereotyped series of movements (Francis 1973b)(Fig. 1.1). *A. elegantissima* only gives such a response to the tissue of non-clonemates and shows no such reaction to predators, prey, or probing stimuli (Francis 1973b, 1988; Ayre and Grosberg 1996). The polyps that occur at these interclonal boundaries often differ in their morphology from those within the interior of the clone in such a way that these clones demonstrate a division of
labor (DoL) with respect to performance in agonistic encounters and sexual reproduction (Table 1.1)(Ayre and Grosberg 2005).

Although differences in polyp morphology between polyp types have been well documented (Ayre and Grosberg 2005)(Table 1.1) less is known about differences in the behavior between these types. Ayre and Grosberg (1996) found no difference between warrior and reproductive types (see Table 1.1 for terminology) in their likelihood to initiate an attack or retaliate after being attacked. They did find that warriors performed as well as reproductives in agonistic encounters despite an inherent size disadvantage. They concluded this was likely because of the warriors’ increased investment in acrorhagi. Additionally, these authors showed that clones themselves differed in their propensity to attack non-clonemates (Ayre and Grosberg 1995). Overall, a full description of polyp behavior in specific circumstances is lacking. Because of this, it is unclear how polyp behavior shapes the group level patterns that are seen in the intertidal. These include the aggregation of polyps into clonal mats and the formation and maintenance of interclonal boundaries (Francis 1973a).

The DoL expressed by *A. elegantissima* has been well studied morphologically, and following the work in this dissertation, more extensively behaviorally. However, I do not address the DoL in this specific group from a theoretical perspective. Instead, I expand my scope to address DoL in a more general model that fills a large hole in the literature. DoL remains largely understudied from a theoretical perspective across biological systems outside of the social insects (but see Wahl 2002a, b). This is despite the ubiquity of DoL in taxa as diverse as: honeybees (Johnson, 2003), wasps (Wenseleers *et al.* 2003), cooperatively breeding noisy miners (Arnold *et al.* 2005), green algae (Kuhn 1971), in multicellular organisms (Buss 1987), slime molds (Bonner 1967, 1988), wolves (Murie 1944), bottlenose dolphins (Gazda *et al.* 2005),
and the sea anemones discussed here (Ayre and Grosberg 1996, 2005) among many others. How DoL is organized within the group is often dependent on the constituent members of the group and their behaviors (Bourke and Franks 1995; Arnold et al. 2005). In my final chapter here I build a generalized model for how DoL can be maintained in a population at equilibrium.

In this dissertation I present a series of five self-contained chapters, briefly summarized below, describing a series of experimental and theoretical inquiries I have made with a number of collaborators. Although these chapters can be read independently, they all explore the common theme discussed in this introduction: how actions and decisions by lower level agents shape the patterns and behaviors expressed by the groups these agents are a part of.

In Chapter 2 my collaborator and I test two elements of clone behavior in A. elegantissima. First, we test whether or not there is an advantage to polyps aggregating into groups by determining if such aggregations minimize water lost due to desiccation stress. Second, we test whether or not clonemates have a preference for one another over a similarly moist competing substrate, a sponge. This hypothesis has not been directly tested before to our knowledge, and its resolution will give insight into how clones of A. elegantissima form aggregations in the intertidal via individual polyp requirements.

In Chapter 3 my collaborator and I explore the behavioral aspects of A. elegantissima at the level of individual polyps in order to gain inference into the competitive equivalence observed between neighboring clones. Our goals here are threefold: (1) to repeat the experiments of Ayre and Grosberg (1996) to determine if one member of the pair demonstrates competitive dominance over the neighbor in single polyp-on-polyp agonistic interactions; (2) to determine if movement rates vary between these neighbors, and (3) to see if the differences between neighboring clones are consistent and therefore indicative of the use of different
behavioral strategies. This study is necessary to determine how neighboring clones vary in their behaviors, and if these differences are consistent at the level of the polyp, and more importantly, the clone. These insights are valuable to understanding how clones compete for space, and how interclonal boundaries remain intact.

In Chapter 4 my co-author and I perform a series of observational studies of clones we place in multi-polyp interactions. We use an extended observational period to investigate clone behavior in a group setting rather than with individual polyps. Specifically, we test whether or not randomly chosen clones, when placed adjacent to one another, will form a long lasting interclonal boundary. Across these observations we also examine if clone behavior with respect to movement and attack behavior are consistent over time for each clone and therefore indicative of behavioral syndromes being expressed by each clonal group.

In Chapter 5 I develop a theoretical model of clone behavior in A. elegantissima using agent based modeling techniques. I parameterize the model using the data from the previous chapters. I have three goals in this chapter: (1) to develop models that can account for the formation of stable interclonal boundaries between two competing clones, (2) to further develop the literature on how individual agent behaviors combine in order to create novel group level behavior, and (3) add to our understanding of how competition occurs in the intertidal.

In Chapter 6 my collaborator and I move outside of A. elegantissima and develop a generalized DoL model that we use to investigate evolutionary stable outcomes for task allocation. We utilize both analytical and numerical techniques to solve for the stable equilibria of generalist-specialist ratios in a population where success is dependent on the completion of two independent tasks. We examine this across multiple group sizes. Our goals here are to determine under what conditions both generalists and specialists can coexist within a
population for a range of costs paid by generalists, and a range of generalist ability to perform the correct task. This work is loosely inspired by Metapolybia wasps and a model developed by Wahl (2002a, b).

Overall, I find that A. elegantissima demonstrates behavioral consistency at both the clone and polyp levels for movement and attack rates. Neighboring clones show demonstrably different behavioral strategies with regards to movement, and this appears to be how neighboring clones that do not show competitive equivalence at the polyp level maintain an interclonal boundary and thereby demonstrate competitive equivalence on the scale of the clonal mat. Furthermore, I show that aggregations in this species are driven by desiccation stress, not a preference for self. In my final chapter I demonstrate that error-prone generalists may persist within a population when their mistakes do little to jeopardize group success. I show that incomplete DoL, where generalists and specialists both persist, can emerge even when generalists often err and must pay extra costs for their multitasking capacity. The work I present here expands our understanding of the behavioral syntax, context, and consistency for a group living Cnidarian that expresses DoL. Furthermore, it gives us insight into the dynamics and interactions of groups in both specific and generalized cases.
Table 1.1. The DoL in *A. elegantissima* is expressed via differential polyp morphology described in this table. Polyp types are also partially defined by their location in the clonal mat, whether it be at edges (interclonal or free) or towards the internal part of the clone. These polyp classes and locations come from a number of previous studies (Ayre and Grosberg 2005, 1996; Francis 1979, 1976) but I specifically use the language of Ayre and Grosberg (2005).

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<td>Warrior-Scouts</td>
<td>Interclonal boundary</td>
<td>Many acrorhagi; small; no gonads</td>
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<td>Warriors</td>
<td>Two rows from interclonal boundary</td>
<td>Many acrorhagi; medium sized; no gonads</td>
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<tr>
<td>Reproductives</td>
<td>Center of clone</td>
<td>Few acrorhagi; typically large; gonads present</td>
</tr>
<tr>
<td>Undefined</td>
<td>Center of clone</td>
<td>Few acrorhagi; small; gonads not present</td>
</tr>
<tr>
<td>Free edge</td>
<td>Non-interclonal boundary edges</td>
<td>Few acrorhagi; small; gonads not present</td>
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Figure 1.1. Photographs of *Anthopleura elegantissima* ecology. A) Photograph of an interclonal boundary, indicated by the arrows, between two clonal mats during a low tide in Bodega Bay, CA. Note that the two clonal mats can be discerned by their slightly different coloration. B) Photograph of a solitary polyp in the lab that has its tentacles retracted so that the pedal disk and column of the polyp are visible. The darker spots running the length of the column to the pedal disk are verrucae. The white spheres at the top of the column where the tentacles are contracted are acrorhagi. These structures are more evident when inflated as in C. where the polyp in the upper left has its acrorhagi fully extended, indicated by the upper arrow. The upper left anemone (bright pink tentacles) is attacking the polyp in the lower right as indicated by the arrow. Photograph taken at Cattle Pt. Friday Harbor, WA.
CHAPTER 2

DESICCATION STRESS MITIGATES DISTRIBUTION PATTERNS OF THE SEA ANEMONE

ANTHOOLEURA ELEGANTISSIMA

2.1. Introduction

The intertidal zone presents unique challenges to its inhabitants, including competition for resources with both con- and hetero-specifics (e.g. Ayre and Grosberg 2005), impact stress from incoming tides and debris (e.g. Dayton 1971; Denny 1988; Boller and Carrington 2006), changes in temperature between high and low tides (e.g. Miller et al. 2009; Szathmary et al. 2009), and alternately, flooding and desiccation during high and low tide (e.g. Hart and Crowe 1977). These ecological pressures are critical in shaping the biology of the inhabitants (e.g. Szathmary et al. 2009) and may often be the cause of the observed distribution patterns.

The sea anemone *Anthopleura elegantissima* inhabits the mid-intertidal region of the Pacific coast of North America and so encounters the stressors listed above. Reproduction in this species occurs asexually and sexually, with the former giving rise to large numbers of clonemates on boulders and rock faces (Francis 1973a). When not in tide pools, these clonemates often stay adjacent to one another, forming a cohesive mat on hard substrate (Francis 1973a, b; Sebens 1982). Clonal aggregations are especially conspicuous when clones encounter one another and then form a large, relatively stable, unoccupied boundary between one another across which agonistic interactions occur (Ayre and Grosberg 1996, 2005; Ferrell 2005).
The mechanisms supporting the formation and maintenance of these conspicuous aggregations are unknown. Members of the phylum Cnidaria are known to have the capacity for a strong division of labor (DoL) among constituent agents of a group (Harvell 1994; Cartwright et al 1999; Cartwright 2003; Ayre and Grosberg 2005; Dunn et al 2005; Dunn and Wagner 2006). However, how constituent agents are integrated into functioning, task-allocated groups is still under investigation (e.g. Dunn and Wagner 2006). Here we focus on two possible mechanisms promoting group formation in *A. elegantissima*: ecological pressures and self-preference. The former refers to the anemones’ reaction to ecological stressors of the intertidal, with the response to aggregate a means to alleviate these stresses. Self-preference refers to aggregations occurring as a result of clonemates having an innate attraction to one another. These mechanisms are not mutually exclusive and could reinforce on another or operate concurrently.

A preference for self requires an ability to recognize self. In *A. elegantissima*, capacity for self-recognition would be in addition to the demonstrated ability to recognize not-self at the clone-specific level (e.g. Francis 1973b, 1976; Ayre and Grosberg 1995, 1996, 2005; Turner et al. 2003). Although there is no observed behavioral response following contact with clonemates that differs from a polyp’s behavior when alone, we have observed polyps stopping a bout of movement when they come into contact with a clonemate (Personal observation). No neurophysiological mechanisms for clonemate recognition are known and the prevailing thought on self-recognition, although unconfirmed for most groups including *A. elegantissima*, is that it occurs passively (Grosberg 1988; Zeh and Zeh 1998): the neurophysiological reaction to a clonemate is no different than the reaction to a glass rod. In contrast, the anemone’s reaction to a non-clonemate is clearly different from its reaction to the rod (Lubbock 1979).
Contact with a non-clonemate elicits a dramatic response. Tentacle contact between non-clonemate (i.e. non-self) polyps triggers an attack, a movement response, or both (Francis 1973b; Bigger 1980; Ayre and Grosberg 1995, 1996, 2005; Westfall and Elliott 2002). In Anthopleura and some other members of Actiniidae, the attack constitutes the inflation and application of nematocyst laden structures (acrorhagi) to non-self polyps (Francis 1973b; Bigger 1980; Ayre and Grosberg 1995, 1996, 2005). Contact with non-self initiates local neural activity in the acrorhagus that results in the discharge of nematocysts into the non-self agent (Lubbock and Shelton 1981). These data clearly indicate that many members of the genus Anthopleura can recognize non-self individuals and perform complex behavioral patterns upon doing so. This indicates that at least some of the necessary components for recognizing self are present.

The presence of non-clonemates is one possible ecological mechanism that could lead to aggregation formation, as polyps typically move away from non-clonemates (Personal observation). Another ecological stress that could lead to association of clonemates with one another is desiccation. The limitation or avoidance of desiccation or heat stress has been invoked as an explanation for the adaptive value of gravel, shell pieces, or other debris attached to the polyp body wall (Hart and Crowe 1977). Thus, it may be that polyps are attracted to wet, inert substrates to relieve desiccation and heat stress (Hart and Crowe 1977; Francis 1979) although this has yet to be explicitly tested. Clonemates can fulfill this role as they are usually nearby. Non-clonemates or other sea anemone species, on the other hand, are interpreted as harmful and trigger the agonistic and retreat behaviors previously discussed.

Here we use two experiments to understand how and why polyps of A. elegantissima aggregate. In our first experiment, we test the effect of aggregating on water loss due to desiccation. In agreement with our initial hypothesis, we find that when polyps are adjacent to
one another there is decreased water loss, compared to that experienced by single polyps. Additionally, we use two desiccation treatments, to evaluate whether polyps of *A. elegantissima* are attracted to clonemates over a similarly wet substance (a sponge); this tests whether there is preference for self that might lead to aggregation formation. We find that polyps do not show a preference for either substrate over the other. Our results demonstrate a clear advantage for polyps to move adjacent to wet, benign substrates when faced with desiccation stress in the intertidal and gives an explanation for the aggregation behavior observed by this species.

2.2. Materials and methods

2.2.1. The effects of aggregation on water loss due to desiccation

We took groups of four polyps (in one case three polyps) from five clones and placed them into one of two treatments: aggregated or separated. In the aggregated treatment, we placed polyps on a thin piece of Styrofoam such that they were all in contact with one another in as tight a formation as possible. In the separated treatment, we placed polyps at the vertices of a rectangle, approximately three cm per edge. These distances varied slightly as polyps moved prior to the experiment beginning, but in no case did polyps in the separated treatment ever come within one cm of each other.

We selected polyps at random from those polyps that had attached to a plastic dish, having already eliminated extremely small polyps, expecting the experiment may not be sensitive enough to differentiate changes in mass between the two treatments for these small polyps. The clones used here had previously been used in a previous set of experiments (unpublished data) approximately one year prior. In the interim they had been kept in the
laboratory on a 13 h light: 11 h dark cycle, between 15.55-18.33°C and fed brine shrimp *ad libitum* approximately once a week except for the week leading up to the experiment, when they were fed three times to ensure they were in good condition.

The evening before an experimental trial, we gave polyps a 30 minute period without water, then pinned polyps to their Styrofoam boards and placed them under running water up until the following morning. That morning, 11.5–12.75 hours after pinning, we removed the pins and kept the polyps under running water for an additional 15 minutes to ensure they did not suffer any ill effects from being attached. To minimize the time spent pinned we shifted the light:dark cycle to 14 h: 10 h starting a week prior to the start of the experiment and kept it as such for the experiment’s duration.

In each treatment, after pin removal and the additional time under running water, we removed polyps and the Styrofoam piece they were attached to from their containers and blotted them three times on paper towels. We then placed the Styrofoam piece on one edge and dabbed for 5-6 seconds to allow any pooled excess water on top of the Styrofoam to run off before we took our initial mass measurement. The polyp-Styrofoam combination was then massed and placed 58 cm from a fan set on low. After 1 h, polyps were removed from under the fan and the final mass was recorded. At the end of the first run, we placed polyps in an ice cube tray with artificial sea water and an aerator. Polyps were kept in the container until the following night (~33 h later) when they were re-pinned, as we did in their first run, for a second run under the other experimental treatment. At the end of their second run we removed polyps from the experimental pool of polyps.
We analyzed data using linear mixed models using the lme4 program in R version 2.13.0 (R Development Core Team 2005) (Pinheiro & Bates 2000). We treated clone and grouping as random effects in the model. Our only fixed effect in the model was treatment.

2.2.2. Preference for clonemates versus a similarly wet substance

We used a macroscopic behavioral experiment to test whether or not polyps preferred clonemates over a similarly wet substance. We collected polyps from four clones at Cattle Pt. and Eagle Cove on the west side of San Juan Island, WA, USA on May 1st (Clones A and B) and 13th (Clones C and D), 2010. Collected polyps were transported in sea-water filled plastic bags and transferred to plastic finger bowls upon arrival at Friday Harbor Laboratories.

Polyps used in the experiment were chosen at random from among those polyps that had attached to the plastic bowl since collection the day prior. Each chosen polyp was lightly squeezed to remove excess water and massed before being pinned to cork board that was then attached to a plastic dish using hook and loop fasteners. Fresh sea water was added and the polyps were allowed to attach overnight. The following day we randomly assigned each polyp to either a low or high desiccation treatment. The two treatments were identical except for polyps under the high desiccation treatment experienced their low tide under a fan whereas those in the low desiccation treatment did not.

We took each of the pinned polyps (focal polyps) and the small square of cork they were attached to and placed them in the center of an arena bordered by desiccation relief substances. In the experimental treatments, two sides of the arena were composed of clonemates that were pinned into cork, and the other two were composed of sponge (XL Pro Sponge from Qep, Boca Raton, Fl. USA) (Fig. 2.1). Clonemates and sponge were placed
equidistant from the focal polyp, at a distance of ~1.5-2 cm (Fig. 2.1). A control arena composed only of sponge was used to test whether polyps had an agonistic reaction to the sponge and to demonstrate that they were willing to move toward it. The corresponding control of clonemates on all four sides was not performed as we did not see a need to test whether or not they would have such a reaction to clonemates.

After focal polyps were in position, water was drained from the dish to simulate a low tide and photographs were taken to mark polyp position. Bowls were placed next to a closed, north-facing window either 37 cm from a desk fan set on low (high desiccation treatment), or simply placed next to the window without the fan present (low desiccation treatment). Air temperatures were ~18-20C for the month. Bowls were left in place for 4.5 h. At the end of this time period, the side and corresponding tentacles of the focal polyp were stroked three times, concurrently with sponge on the sponge-ward side of the focal polyp and a clonemate on the other side to give the focal polyp exposure to both substrates.

At the end of the low tide period, the dish was placed under running sea water for two hours. At the 0.5 hour and one hour mark of this simulated incoming tide, focal polyps were stroked with sponge and clonemate concurrently for a total of three touch bouts. After these stimulations, water flow ceased, and the container with the submerged polyp sat in approximately 1-2 cm of sea water until the following morning, when photographs were taken to evaluate polyp position. The experimental protocol was repeated (low tide, stimulation, introduction of water) for a second day. The experiment was completed the following morning (~48 h after experiment start), when final photographs were taken.
On six pseudo-randomly selected days, sponges from both treatments were massed at the start of the low tide and immediately before it ended. The change in mass was recorded as a relative proxy for desiccation in each treatment.

Data were analyzed using PASW Statistics 18. Six trials for each of two clones (C and D) were run for only a single day, not the two days as described above. We left these data in the analyses, having observed that no polyp changed its direction of movement between the first and second days. Thus, the choice exhibited in the first day should be indicative of the final choice for direction of movement. Additionally, analyses with these data removed did not change any qualitative conclusions or results.

2.3. Results

2.3.1. The effects of aggregating on water loss due to desiccation

We did not note any ill effects of our treatment on the polyps, either during the course of the experiment or after the experiment had ended. Polyps did not show any dis-inclination to attach to the Styrofoam pieces. Qualitatively, it appeared that polyps retained more moisture in the aggregated experiment than in the separated treatment, as they appeared wetter. In 25 of the 32 groups of polyps the separated treatment case lost a greater percent of its mass than the aggregated case; the inverse was true in five cases; and twice the percent mass lost was the same. Our LMMs for these data, used percent mass lost as the response variable and included both individual grouping, run and clone identity as random effects with treatment as the only fixed effect. Treatment was found to be a significant factor (T-test, $T_{31}= 3.61$, $P < 0.002$) as was
the particular grouping of polyps (likelihood ratio test, $P < 0.001$). Neither run nor clone appeared to have any effect on the model.

### 2.3.2 Preference for clonemates versus a similarly wet substance

In total, we tested 114 polyps from 4 clones, ranging from 24 polyps for clone D to 34 for clone B. Of these 114 polyps, 19 were controls, leaving 95 experimental trials. Of the experimental trials, 10 were dismissed because focal polyps never attached to the cork board, resulting in 85 usable experimental trials. In the low desiccation treatment, 32 of 45 experimental trials evinced movement. In the high desiccation treatment, 22 of 40 experimental trials evinced movement (Table 2.1). In the control treatment (sponge only), 8 of 10 polyps moved for the low desiccation treatment, and 9 of 10 for the high desiccation treatment. In all of the control replicates, the focal polyp moved next to the sponge. We did not observe any negative reaction of the polyps to the sponge; in fact, in some cases, the focal polyp climbed onto the sponge.

If we remove the cases in which it was unclear which substrate the focal polyps moved toward (ambiguous cases, e.g. they moved toward the intersection of the two substrates) we find no evidence for a preference toward either the clone or sponge (Table 2.1) under either treatment (Low desiccation: $\chi^2_{1} = 0.15$, $P > 0.60$; High desiccation: $\chi^2_{1} = 1.67$, $P > 0.15$; Pooled data: $\chi^2_{1} = 0.22$, $P > 0.60$). This lack of preference prevails if we split the ambiguous cases evenly between sponge and clonemate tallies (Low desiccation: $\chi^2_{1} = 0.13$, $P > 0.70$; High desiccation: $\chi^2_{1} = 1.14$, $P > 0.20$; Pooled data: $\chi^2_{1} = 0.17$, $P > 0.60$). The lack of evidence for a preference occurred also for the pooled treatment data for each individual clone, for those replicates that
saw the focal polyp move. No clones preferred one substrate over the other (all $\chi^2 \leq 2$, $P > 0.15$).

The two treatments did cause differing levels of desiccation as sponges lost a greater amount of mass in the high desiccation treatment. Sponge in the high desiccation treatment lost significantly more weight (-9.1 g) than sponge in the low desiccation treatment (-6.9 g) (T-test for unequal variances, $T_{68} = 2.57$, $P = 0.012$).

2.4. Discussion

2.4.1. General conclusions

Overall, we find a clear benefit for polyp aggregation in *Anthopleura elegantissima* with respect to desiccation relief. However, it appears that polyps have no active preference for clonemates over a benign substrate that also offers desiccation relief. These data suggest that aggregation in *A. elegantissima* is beneficial and driven by ecological pressures rather than an inherent preference for self.

2.4.2. The effects of aggregating on water loss due to desiccation

Unsurprisingly, our data indicated that aggregated polyps lost less water than solitary polyps. This likely explains the observation that individuals of *A. elegantissima* are more likely to occur in aggregations in the intertidal than are those individuals living in more permanent tide pools (Sebens 1982; Personal observation). Although we did not find clone identity to be a significant factor in the model, we believe that clone identity will affect the absolute mass lost because polyp size varies with clone identity (Ayre and Grosberg 1996, 2005). Large polyps are
able to hold a greater mass of water than small polyps and, given our results here, should be more resistant to desiccation.

2.4.3 Preference for clonemates versus a similarly wet substance

These data demonstrate that focal polyps have no preference for clonemates over a similarly benign wet substance. In both the high and low desiccation treatments, polyps effectively chose randomly between the two (Table 2.1). These data suggest that focal polyps will move to the closest benign wet substrate, presumably to alleviate desiccation stress.

Surprisingly, the high desiccation treatment saw a larger proportion of polyps not move as compared to the low desiccation treatment, although this difference was not statistically significant (32 of 45 in the low desiccation, 22 of 40 in the high desiccation). Despite not being significant, this finding is intriguing, as it runs contrary to the expectation that greater desiccation stress will result in more movement: if relief of this stress is paramount to polyp survival, increased movement would be predicted for the high level treatment. The answer for why these polyps did not move as often may come from our observations of the polyps themselves at the end of the low tide. The focal polyps, especially smaller ones, often appeared extremely desiccated, and their epidermises had become leathery and lacked moisture. Although no focal polyp died, it appeared as though there was a recovery period for these polyps that exceeded the two days of the experiment. We hypothesize that it is this recovery period or inability for polyps to move brought on by the high desiccation treatment which caused the difference in the proportion of polyps that moved between the two treatments. Furthermore, polyp movement may be inhibited on very dry substrate, as it makes gliding or attaching more difficult.
We observed during the simulated low tide that non-focal polyps would often leak water, especially the larger non-focal polyps. In contrast, water evaporated evenly from the sponge. This leaked water may act as an attractant for heavily stressed, desiccated polyps, explaining the slight skew in movement toward clonemates in the high desiccation treatment (Table 2.1). Under the increased stress of this treatment anemones may be more sensitive to signals that would indicate a possible respite from desiccation. Leaked water could act as such a signal and thereby draw anemones toward the clonemate side of the experimental setup.

Our results refute preference for clonemates as an explanation for aggregation formation in *A. elegantissima*. Our methods preclude assessment of whether polyps can identify clonemates (can “self-recognize”), although our data suggest that it is unlikely, or at the very least not a trait that seems to be relevant to the ecology of this species. Because this behavioral assay cannot give a definitive answer on this topic, this matter will likely remain ambiguous until a neurophysiological response is tested for in clonemate-clonemate contact.

2.4.4. Final conclusions

Our results fail to refute ecological stress, particularly desiccation stress, as an explanation for aggregations. The occurrence of clonal aggregations in nature (Francis 1973b; Hart and Crowe 1977; Ayre and Grosberg 2005), or polyps ceasing movement when they contact clonemates (Personal observation) is presumably the result of polyps identifying a benign substrate. This means that large, monoclonal groups found in the field are likely maintained by ecological pressures rather than a preference of members of the group.

Although our results attest to the importance of desiccation in the formation of clonal aggregations, this is not the only relevant ecological factor. Hart and Crowe (1977) showed that
under increased temperatures and wind conditions, water loss from anemones increased and survival decreased. Their data suggest that in the field, desiccation, UV, and heat stress are likely to co-occur. UV and heat stress may amplify the intensity of the response in the field. Another ecological pressure is that imposed by non-clonemates. Polyps are likely to move away from non-clonemates and, presuming that clonemates are in the opposite direction as non-clonemates, this behavior could also lead to an association of clonemates with one another. Furthermore, the intertidal zone is spatially heterogenous and abounds with potentially space-limiting boundaries. Other organisms or substrates that polyps cannot or will not traverse will also serve to limit polyp movement and spread and thereby promote the observed clustering of clonemates of *A. elegantissima*. 
Table 2.1. Summary of data for polyps that moved over the course of the experiment (omitting run 10). Sponge indicates the focal polyp moved toward the sponge, clonemate indicates the focal polyp moved toward clonemates and ambiguous is the label for polyps whose movement did not indicate a clear direction (e.g. they moved toward the intersection of clonemates and sponge).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sponge</th>
<th>Clonemate</th>
<th>Ambiguous</th>
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</thead>
<tbody>
<tr>
<td>Low desiccation</td>
<td>14</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>High desiccation</td>
<td>5</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Pooled data</td>
<td>19</td>
<td>22</td>
<td>13</td>
</tr>
</tbody>
</table>
Figure 2.1. Diagram of the setup for an experimental run. Dark blocks represent sponge, gray circles clonemates pinned in place and the light gray X is the location of the focal polyp at the start of the experiment – equidistant from all sides.
3.1. Introduction

*Anthopleura elegantissima* is a clonal sea anemone native to the Pacific coast of North America (Hand 1955) that engages in conspecific aggression between clonal mats (hereafter referred to as either clonal mats or clones) (Francis 1973b; Ayre & Grosberg 2005). Polyps of different genotypes (non-clonemates) that encounter one another use nematocyst-laden structures called acrorhagi (Daly et al 2003) to attack one another (Francis 1973b; Ayre & Grosberg 2005). This is often most clearly visible when large clonal mats of allospecific anemones abut each other and an interclonal boundary forms (Francis 1973a; Ayre & Grosberg 2005). In such cases it is common that the polyps of each clone along this border are specialized for agonistic encounters (Francis 1976; Ayre & Grosberg 1996, 2005). This specialization is part of a larger division of labor (DoL) employed by clones, generally between the tasks of agonistic encounters and sexual reproduction (Ayre & Grosberg 2005).

In *A. elegantissima* DoL is achieved through differential polyp morphology (Ayre & Grosberg 2005). The degree of intra-clone differentiation differs among clones (Ayre & Grosberg 2005). Polyps in the interior of the clonal mat are referred to as either reproductive or undifferentiated polyps. The former are the largest type of polyp within the clone, contain
gametes and have fewer and smaller acrorhagi than polyps at interclonal boundaries (Francis 1976; Ayre & Grosberg 1996, 2005). Undifferentiated polyps are smaller than reproductives, but also have relatively fewer and smaller acrorhagi compared to polyps at the interclonal boundary. Moving out toward the interclonal boundary, approximately 3-4 rows from the boundary is another polyp class: warriors (Ayre & Grosberg 2005). This class is characterized by polyp sizes larger than those polyps at the boundary but with similar number of acrorhagi, typically many more than reproductive polyps (Ayre & Grosberg 1996, 2005). The polyps in the first two rows adjacent to an interclonal boundary are called warrior-scouts, are small and acrorhagi-laden (Ayre & Grosberg 1996, 2005). Ayre & Grosberg (1996, 2005) identified one other polyp type located at the edge of the clonal mats, specifically non-interclonal edges. These free-edge polyps are warrior-scout sized but have fewer acrorhagi.

Ayre & Grosberg (1996) investigated agonistic behavior between neighboring clones of _A. elegantissima_ and found that when polyps of neighboring clonal groups were pitted against one another in one-on-one contests, polyps of one clone consistently won the encounters (in 5 of 7 pairs tested). In contrast, a follow-up study using both _A. elegantissima_ and three species of the genus _Hydractinia_ Ferrell (2005) concluded that neighbors that share a boundary are evenly matched. Ferrell compared the observed number of aggressive structures (acrorhagi in _A. elegantissima_ and hyperplastic stolons in _Hydractinia_) between neighbors to what one would expect if the distribution of these structures was distributed randomly among neighbors. His results showed that neighbors are more equally matched than would be expected by chance. He concluded from this that when dominant clones encounter overmatched clones the former quickly outcompetes the latter and a boundary is never formed (Ferrell 2005).
While it is clear that clones that share a stable boundary are competitively equivalent, by definition, Ferrell’s (2005) study does not directly address the inequality in fighting ability between neighbors found by Ayre & Grosberg (1996). This apparent paradox between the results of the anatomical and the combat studies remains to be explained: how can a stable border persist when clones have predictable and significant differences in fighting ability?

One possible explanation for competitive equivalence at the level of a clone but competitive inequality at the level of an individual polyp is the use of different behavioral strategies employed by neighbors. Clones show differences in the degree of DoL they express (Ayre & Grosberg 2005), suggesting that each clonal group either partitions tasks adaptively to its local environment, is genetically pre-disposed to a specific level of DoL, or some mixture of the two. This variation, either in terms of a clonal group’s response to the environment or its genetic predisposition suggests underlying variation in how the clonal group may behave in contexts outside of agonistic encounters.

Polyp movement may mitigate differences in individual polyp fighting behavior into an equivalent, or nearly so, group interaction. For example, a clonal group whose polyps are less able to fight but more likely to move might compete effectively for space by filling in gaps in the clonal boundary before a member of the opposing clone can colonize them. However, there is no a priori reason that this should be the case between clones mismatched at the polyp level. Still, if movement is critical to boundary stability, and there is variation in this behavior in the population, we would expect to see differences in movement strategy between neighboring clones. This could be expressed as differences in distanced moved, in the frequency of movement, or both. Furthermore, these differences should be most pronounced when comparing warrior and warrior-scout (hereby collectively referred to as warrior castes) polyps
on either side of the border because these are the polyps that engage in the agonistic encounters between clones. If differences in movement and other behavioral traits (e.g. aggressiveness) persist in multiple contexts, it would be indicative of not only clone-specific strategies, but behavioral syndromes (see reviews: Sih et al. 2004; Bell 2007, shown in Cnidarians by Briffa & Greenaway 2011).

An alternative, but not mutually exclusive, hypothesis is a change in behavior by one or both of the neighbors following the initial agonistic encounter. Although Ayre & Grosberg (1995; 1996) did not test the effects of multiple agonistic interactions on the outcome of agonistic encounters, they did test the effects of repeated stimulation of one clone on another (Ayre & Grosberg 1995) and found that reactivity decreased after repeated stimulation.

Here we tested these predictions in a series of three experiments using three pairs of neighboring clones collected from two locations along the California coast of North America. We first duplicated the agonistic encounters used by Ayre & Grosberg (1995, 1996) to determine the relative polyp-on-polyp fighting ability of each clonal mat. We then added an additional agonistic encounter to test for the effects of repeated contests. Following this experiment we tested the response of polyps to contact by either a clonemate or non-clonemate. Lastly, we tested the response of polyps from a seventh, standard opponent clone to attacks by members of our six focal groups. By recording the behavioral response by polyps of this standard opponent clone, we obtained a qualitative estimate of the attack strength of each study group. In agreement with Ayre & Grosberg (1995, 1996) we found that neighboring groups differed in their individual level polyp fighting ability and the additional result that these differences declined with repeated interactions. Neighboring clonal groups also differed in their rates of polyp movement. Groups with polyps that did poorly in one-on-one agonistic encounters
typically moved more often than their stronger neighbor. We concluded that clonal groups use different behavioral strategies in order to mitigate differences in polyp-on-polyp fighting ability.

3.2. Materials and Methods

3.2.1. Clone collection, acrorhagi counts, and massing

Clones 1, 2 and our standard opponent clone were collected in August 2010 from in front of the Scripps Institution of Oceanography, La Jolla, CA, USA. Clones A-D were collected from the jetty at the entrance to Bodega Bay at Bodega Bay, CA USA in October 2010. For all clones except the standard opponent clone we specifically targeted clonal mats that shared a clear interclonal boundary with a neighboring clone. Although these borders are often quite obvious in the field, we verified that the two clones were distinct by gently pressing clones at the border to render the acrorhagi more visually distinct. Heavy armament was interpreted as a signal that the polyps on either side of the boundary were from distinct clonal groups. From each clonal group we collected polyps from specific representatives of the polyp types identified in prior work (Ayre & Grosberg 2005). Clones from the first and second row at the border represented warrior-scouts; polyps from rows three and four represented warriors; polyps on edges of the clonal mat not bordering another mat represented free edge polyps; and polyps taken from the center of the clone (at least five rows from an interclonal boundary) represented interior polyps. Although we tried to collect only large reproductive type polyps from the interior, some smaller polyps were also collected, presumably these belong to the undifferentiated class described by Ayre & Grosberg (2005). Because neither undifferentiated polyps nor reproductive polyps interact with the neighboring clone and because both have
relatively light investment in acrorhagi, we did not differentiate between them, considering reproductive and undifferentiated interior polyps as a single ‘interior’ class. We used 490 polyps spread across our six focal clones for use in the following experiments.

In the laboratory, we kept polyps in finger bowls of artificial salt water at between 32 and 35 PPT at 20° C when not in an experiment and for the experiment on agonistic polyp encounters. Polyps were kept at 15-17° C for the movement experiments and for attacks on the standardization clonal group. Polyps were fed twice weekly with brine shrimp (eggs from Brine Shrimp Direct) unless the polyps were being used in an experiment that day. Anemones were kept on a 13h:11h light:dark cycle through all experiments. Before experiments commenced we recorded mass and acrorhagial number for each polyp. Acrorhagial counts were made using a dissecting microscope on polyps anesthetized with isosmotic MgCl₂. Wet mass was assessed after gently hand-squeezing water out of the polyp; for consistency, only author 1 performed mass assessments. We allowed polyps to recover for at least a day in isolation before being using them in an experiment. Due to experimenter oversight we did not record masses for ten of our 490 polyps.

During the course of the experiment clones C and D became sick with what we believe was a bacterial infection. These two clones seemed to be uniquely affected, with warriors of each of these clones more affected than other types, possibly as a result of the infection originating in those containers. This infection caused a great deal of mortality and led to a complete loss of warrior polyps for clone C and a large loss of warriors for clone D. As a result, we were unable to test the effects of clonemate and non-clonemate contact on the former group. We were able to complete all other experiments with a similar number of replicates to the other clones tested.
3.2.2. Individual competitive ability between neighboring clones and repeated agonistic interactions

We tested the individual polyp fighting ability of each clone using the methods of Ayre & Grosberg (1995, 1996) with the addition of a second agonistic interaction for a subset of polyps that participated in an initial agonistic encounter. Since we were specifically interested in how neighbors interacted with one another, members of each clonal mat were tested against members of their neighboring clonal mat with whom they shared an interclonal boundary (as in Ayre & Grosberg 1996). For each set of neighbors, we used warrior (W) and reproductive (R) polyps from both clones to test fighting ability. We placed polyps in one-on-one encounters with a polyp from the neighbor using a full factorial design (i.e. Wx versus Wy; Wx versus Ry; Rx versus Wx; Wx versus Ry; Rx versus Wx) to observe which clone, if any, had an advantage in such contests. As in Ayre & Grosberg (1996), we ran eight replicates for each pairing type. For the duration of this experiment, warrior and warrior-scout polyps not being used in an experiment that day were stimulated with a warrior from the opposing clone by gently rubbing the competing warrior polyp across the focal polyp’s tentacles to simulate the daily exposure the two clones have with one another at the natural interclonal boundary (Ayre & Grosberg 2005).

Polyps were pinned to cork board, either a movable piece or a piece affixed to a small plastic container, without water present, the day before the experiment began. Our work with this species has shown that this treatment has no discernible effect on polyp behavior. Water was added as the polyps were allowed to attach overnight. The following day, to mimic more closely the polyps’ natural environment, we simulated a low tide by removing the sea water from the containers. One hour after the start of the low tide, polyps on the movable piece of
cork were moved into contact with a polyp affixed to the container. The moveable cork square was affixed in place within the container using hook-and-loop adhesive tape. Polyps remained pinned to the cork to limit movement of polyps during this initial phase. At approximately two hours into the low tide, the pins were removed. At two and a half hours from the start of the low tide new sea water was added to each container. Containers were observed at one minute intervals for the next two hours and then occasionally after that. Attacks and acrorhagial displays were recorded for each interaction. The following day, at between 23 and 24 hours after the start of the initial low tide, polyps were photographed. Winners of contests were discerned (as discussed below), and distance either of the polyps had traveled was measured using the photographs taken and standards that had been placed on the cork.

To test the effects of repeated interactions and possible winner-loser effects, we ran a second fighting interaction for polyps that had been pinned to the fixed piece of cork board (retained polyp) and had been in either a Wₓ:Wᵧ and Rₓ:Rᵧ pairing. These polyps were given a day to rest in their container while their opponent (on the movable piece of cork) was removed from the experiment. The retained polyp was then paired with a new opponent of the same polyp class as the original moveable polyp (either a W or R) and had not been used in a prior contest. This second run was performed in the same manner as the first run.

We designated two different, non-mutually exclusive, types of victories: movement victories and battle victories. In a movement victory, the loser moved away from the winner, literally backing away from the interaction. In a battle victory, the loser retracted its oral disc after having been attacked, ending its engagement with the winner by withdrawing its tentacles. We scored cases where there was no clear winner as a draw, however in some of these one of the polyps may have shown signs of losing (e.g. leaning away from the opponent, had begun to
move away but had not moved its full body yet). In these cases we gave an edge to the polyp that appeared to have the upper hand in the contest. Since we bias ourselves against wins in these cases, we analyzed these edges as both their own distinct category and also as wins (thereby reducing our number of classes to only wins and losses in the latter case). In no case did we see a movement victory by one polyp and a battle victory by its opponent. We therefore counted both battle and movement victories as wins.

3.2.3. The effects of contact with a clonemate or non-clonemate

In our second experiment, we tested whether there was variation in movement within and among clones, and whether this variation differed between contact with a non-clonemate or a clonemate. Polyps from clones were chosen randomly from within each type for each clone (warrior scouts, warriors, interior and free edge). Runs were done concurrently for types and neighboring clones to control for any effects of time since collection.

Polyps were pinned to a movable piece of cork and given a minimum of 16 hours to attach. For the majority of this time, pinning constituted a single pin through the oral opening and pedal disc to reduce and standardize the damage to each polyp. After polyps attached, the pin was removed and the cork piece and its attached anemone were moved to the middle of a larger plastic container in which the movable cork piece was surrounded with affixed cork. The sea water was removed to simulate a low tide. During low tide, polyps were kept under uniform light at room temperature (20-21° C, except for a single run where low tide was at 25° C). After 2.5 hours, artificial sea water (15-17° C) was allowed to slowly fill the container for 45 minutes. The entering water was delivered from a container above the experimental containers that had been drilled with equally sized holes to control the amount of water entering each experimental
container. As it has previously been shown that *A. elegantissima* moves up an oxygen gradient (Fredericks 1976) these controls ensured each experimental container experienced the same water input at the same rate and therefore controlled for oxygen addition and mixing within the container. To control for the impact of where the water was entering the container, containers were randomly divided so that half had water enter from slightly below the focal anemone and the others from slightly above the focal anemone.

Ten minutes after the end of the low tide, and while the container was being filled with water, the focal polyps were stroked three times on one side (both column and tentacles were stroked) with either a clonemate or non-clonemate to simulate an interaction with one of those two agents without any agonistic behavior. This process was repeated 15 minutes later. The treatment each polyp underwent initially was selected randomly such that in every set of polyps tested concurrently, there were equal numbers of control and experimental treatments. After water flow into experimental containers ceased, containers were kept under uniform light in a water bath to keep water temperature constant between 15 and 17° C. The following day, at approximately 22.75 and 23.5 hours after the start of the low tide, photographs were taken to discern if and where the focal polyp moved.

After the photographs were taken polyps were re-set with pins by removing them from the cork and then re-pinning them as at the start of the experiment. The following day the experiment was repeated using the same polyps under the opposite treatment (i.e. polyps previously in contact with clonemates were exposed to non-clonemates and vice versa). Therefore, each polyp experienced both treatments, stimulation with a clonemate and a non-clonemate, in a randomized order.
3.2.4. Test of attack strength against a proxy clone

In our third experiment we tested the prediction that attack strength (the average reaction of the standard opponent clone to the focal clone) varied between clones by testing all clones against the same standard opponent clone. Warriors from each of the six focal clones were pinned to movable cork pieces placed in small plastic containers. Due to the small sizes of clones 1 and 2 from southern California, it was difficult to ensure that polyps would be able to be placed close enough to ensure sufficient interaction to generate an attack. Therefore, we preferentially selected polyps that attacked in experiment 1 or had a large number of acrorhagi to maximize the probability we would initiate an attack. As polyps from clones A-D were larger, we selected warrior polyps by the order which we massed them, and therefore quasi-randomly.

After 24 hours, the pins were extracted and water was removed to simulate a low tide. At the start of the low tide, the focal polyps were gently touched by, and put adjacent to, a polyp from the standard opponent clone such that the two polyps (focal and standard) were side-by-side during the low tide. Low tides lasted between 2 and 2.5 hr.; after which, sea water was added to the container and an aerator was placed in the container for 30 minutes to mimic an incoming tide. After the aerator was removed the container was closely monitored for 1.5-1.75 hours for attacks by the focal clone. If a focal polyp was attacked by a control polyp, the focal polyp was removed from the experiment for that day. If a focal polyp attacked a control polyp, we allowed the attack to occur until it appeared the focal polyp would no longer continue its agonistic behavior (acrorhagi no longer inflated) or we had reached the end of the observation time. These latter cases were rare and we were able to allow the focal polyp to finish any attack it initiated, hence the variation in observation time. Control polyps that were attacked were moved to their own container either at the end of the agonistic interaction or the
end of the observation period and photographed to note their location and physical state following the encounter. The following day these polyps were again photographed to evaluate movement and physical condition. We used these photographs to measure the distance, if any, that the control polyps moved in response to being attacked.

3.2.5. Data analysis

For each experiment data were collated and analyzed using IBM SPSS Statistics 19 (SPSS Inc. Chicago, Illinois) and R version 2.13.0. Our analysis of dyadic agonistic polyp encounters was run in SPSS Statistics 19, including T-tests and ordinal probit generalized linear models. As we were specifically interested in the differences in distances moved by the standard opponent clone to each focal clone, we ran T-tests comparing the average distances moved by the foreign clone in our attack strength experiment using this program.

For the effects of clonemate or non-clonemate contact we took our movement data and transformed it into three different datasets to ensure we had not biased ourselves toward movement when measuring how far polyps had traveled. To bias ourselves against movement, we first treated movement as a binary response using two different distance cut-offs. In our first case, a polyp was considered to have moved only if our measured movement was ≥ 0.44 cm (approximately one half the diameter of an average polyp). In our second case, we made the cut-off ≥ 0.24 cm. Lastly, we considered the actual distance measured, a continuous variable, as the true movement. The actual distances moved did not fit a normal distribution, but did fit a Poisson. We therefore used a Poisson function to fit these data. We built generalized linear mixed models (GLMMs) using the lme4 package in R (R Development Core Team 2005) (Pinheiro & Bates 2000) with a binary response for the first two datasets and a Poisson function for the
third dataset given the larger number of cases in which polyps did not move. For each GLMM we started with mass, number of acrorhagi, the treatment (clonemate or non-clonemate stimulation), polyp type, clone, and polyp type by clone interaction as fixed factors in our full model. Because each polyp was measured twice (once for each treatment) we had polyp identity as a random effect in the model. For factors in each model that were found to be not significant in the ANOVA associated with the model (fixed effects) or in a likelihood ratio test (random effects) we ran all possible model combinations with these factors. We report all models that are equivalent by AIC criterion ($\leq 2 \Delta AIC_c$). All comparisons between proportions of polyps that moved were completed using two sample $Z$ tests (Devore 2000). To test for behavioral consistency, we tested whether or not clone identity was a significant factor, as a random effect, in our GLMMs for clone movement across treatments. If clone was significant across treatments it would be indicative that there are consistent differences between clones across contexts and support the existence of behavioral consistency.

3.3. Results

3.3.1. General polyp data for mass and acrorhagi counts

ANOVAs for acrorhagi counts and the mass of polyps for each clone showed that for all clones, there was a significant difference among types for both variables (all $P \leq 0.01$) except for the mass of types for clone B (Fig. 3.1). As in previous work (Ayre & Grosberg 2005) we find that clones demonstrate variable degrees of division of labor as evidenced by the differing absolute values of mass and acrorhagi among all clones (Fig. 3.1). Cursory comparison of acrorhagi number in Fig. 3.1 shows that neighboring clones are similarly armed with acrorhagi when
compared to the other clones in the analysis (i.e. clone 1 warrior scouts are more similar to those of clone 2 than those of clone A) (Ferrell 2005). Neighbors do show significant differences between each other for each of these phenotypic characters, depending on the comparison (data not shown), but these differences are minor compared to the much larger differences among non-neighbors (Fig. 3.1). Although our interior type polyps constituted both reproductive and undifferentiated polyps, we did not see significant variation within this type as compared to the other, more finely distinguished types of polyps.

### 3.3.2. Individual competitive ability between neighboring clones

We found strong differences in individual polyp fighting ability between neighboring clones C and D (Table 3.1). In all pairings, clone D won more contests than clone C (although in no case was the difference significant) regardless of whether draws, in which one of the clones held an edge, were included as wins. If all contests were pooled, clone D won significantly more contests than C (binomial test, \( P < 0.01 \)); however, attacks were infrequent between these two clones (Clone D: 3 of 32 polyps attacked; clone C: 2 of 32 polyps attacked) (two sample Z test, NS). In 2 of the 3 cases in which clone C was attacked it moved, compared to 8 of the 29 remaining polyps tested moving at least a half centimeter (two sample Z test, NS). Clone D did not move in either of the two cases it was attacked, and only once in the remaining cases (two sample Z test, NS). Comparing the rates of movement between C and D in this experiment, it is apparent that the two responded differently to the presence of one another when not attacked. Clone C moved significantly more than clone D (two sample Z test, \( P < 0.01 \)), but not when attacked (two sample Z test, NS), although it is likely that the small number of attacks made it difficult to detect differences in this case.
Neighboring clones 1 and 2 also showed differences in competitive ability, although they were not as strong as those between C and D. Clone 2 won more contests than clone 1 for all treatments if edges were not included although none of these differences were significant. These trends held when edges were included as wins except for R₁ versus W₂ where the two clones were tied (Table 3.1). This trend favoring clone 2 occurred despite clone 1 initiating three times more attacks than did clone 2 (six vs. two attacks). If treatments were combined, the difference in wins was still not significant, regardless of whether draws with edges were included (binomial test, NS). For combined treatment, there was no difference in the total number of attacks between the two clones (Table 3.1). Clone 1 moved in 12 of the 26 cases (46%) it was not attacked, and 5 of the 6 cases (83%) in which it was attacked (two sample Z test, NS). Clone 2 moved in only 4 of 26 cases (15%) when it was not attacked, and 3 of 6 cases (50%) when it was (two sample Z test, \( P = 0.065 \)). Comparing the rates of movement between the two clones when neither was attacked we found that clone 1 moved significantly more often (two sample Z test, \( P < 0.02 \)) but this was not the case when comparing their rates when attacked (two sample Z test, NS).

Similar to the other two sets of neighbors, clones A and B initiated attacks at similar rates to one another (two sample Z test, NS). However, clone A won more contests in all treatments (except in W versus W when draws with edge were counted as wins, in which case both clones tied with 3 wins a piece). Similar to clones 1 and 2, this occurred despite clone A initiating fewer attacks. None of the individual differences in wins for the treatments proved significant (\( P < 0.05 \)) but when treatments were pooled, the differences were significant when draws with an edge were not included as wins (binomial test, \( P < 0.02 \)) and indicative of a trend if they were (\( P = 0.09 \)). Movement by Clone A did not differ significantly between when not
attacked (36% of polyps moved) compared to attacked (50%). This contrasts with clone B, which moved much more frequently (two sample Z test, \( P = 0.02 \)) when attacked (6 of 15, 40%) than when not attacked (1 of 16, 6%). When not attacked, the two clones moved at different rates compared to one another, but this was not the case when they were attacked (two sample Z test, \( P = 0.03 \) and NS respectively).

Although polyps that were attacked in the first run of competitive fighting moved further on average than those that were not attacked, only one of these differences was significant, and only barely so (for clone A when attacked by clone B, T-test for unequal variance, \( P = 0.044 \), all other \( P > 0.1 \)). This was largely due to the small number of attacks observed in each series. In each pair, there was an approximate two-fold difference in average distance moved between clones when attacked by the neighbor (clones A-B: 0.44 cm – 0.87 cm, clones C-D: 2.01 cm – 0.00 cm, clone 1-2: 1.45 cm – 0.80 cm) but none of these differences between clones were significant (e.g. the distance moved by A when attacked by B compared to the distance moved by B when attacked by A)(Pooled data for each clone; T-tests for unequal variances, \( P > 0.1 \)).

3.3.3. Results of repeated interactions

The partners of focal polyps for each of the two runs that we performed to test for the effects of repeated interactions did not show significant differences in either mass or acrorhagi (paired T-tests, all \( P > 0.1 \)). Table 3.2 shows the results of these contests for the two pairing types. Comparing the total number of resolved contests (for W-W and R-R pairings pooled together) between the two runs (see Table 3.2) we found a significant difference in the number of contests with clear winners (not including edges as a win) for the pairing of clones 1 and 2 (6
of 16 compared to 12 of 16, Z test, \( P = 0.0325 \). There was no difference in the number of resolved contests for the other two clone pairings. We ranked contests results from 1 to 5, with 1 and 5 representing clear wins by one of the two clones, 3 was a draw, and ranks 2 and 4 were used for contests that were ruled a draw with an edge given. Using Mann-Whitney U tests we compared the rank distribution between the first and repeated runs. There was no significant difference between runs for any clone pairing for W-W contests. For R-R contests, there was a significant difference in the distribution of contest outcomes for clones C versus D between the two runs (Mann-Whitney U test, \( P = 0.034 \)), but not for any of the other two clone pairings.

When we pooled W-W and R-R runs to increase sample size, and compared the first and second runs we found a difference among the distribution of contest outcomes for the C-D clone pairing (Mann-Whitney U test, \( P = 0.047 \)), with contests moving toward more egalitarian outcomes. We did not find any differences for the other two pairs between the two runs.

There was little evidence to suggest that polyps that were involved in both runs changed their attack behavior between the two runs. For clones 1 and 2 in the first run two polyps for clone 1 attacked, and only one for clone 2. These numbers reversed in the second run. For clones A and B only a single polyp attacked in the first run and that same polyp was again the only polyp, of those that were used in both runs, to attack in the second run. Clone B saw one polyp attack in both contests along with two others in the first run, and one other in the second run. This same trend held for movement by the polyps that took part in both interactions. We found no significant differences in the distances moved between runs within each clone (Paired T-tests). It is interesting to note though, that the changes in means occurred in opposite direction for clones within the A-B and 1-2 pairings.
We ran generalized linear models (ordinal probit) with winner of the second contest as the response for each set of pairings (using all second runs, so each pairing has one polyp that had been in an earlier interaction; balanced between clones) to determine if any of the following variables affected the outcome: the winner of the contests in the first run (previous winner), whether or not either clone attacked, whether it was a W-W pairing or a R-R pairing, and which clone had been previously used in the first run. In all cases the best fit model contained only an intercept. All other models had AIC values greater (by > 2) to the point that they are considered different by AIC criterion. Comparing the results of the first and second interactions for polyps that occurred in both interactions it appears that, for most of our tested clones, dominance by one clone over the other is less strong in the second interaction compared to the first (Tables 3.1, 3.2). This trend held for clones C, 1 and 2. Clones B and D did not see strong directionality in terms of wins for either interaction, and clone A regressed in ability with repeated interactions.

3.3.4. Results of touch by a clonemate or non-clonemate

For each pair of clones, the results of the GLMMs for all three response types (using movement above 0.24 cm as a binary variable, above 0.44 cm as a binary variable, and the actual measured movement fitted with a Poisson distribution) showed similar results. These results held whether we included all clone types in the analysis (full dataset) or only those warrior castes that would be expected to interact at the border (i.e. warrior-scouts and warriors)(warrior caste dataset). For space considerations, we present only the data for the 0.24 cm cut-off here for both datasets (Figs. 3.2). Results for the other two cut-offs and both datasets are presented in Appendix A.
There was little evidence for a difference in clone movement between clones C and D (Fig. 3.2). The best models fit to the 0.24 cm binary response variable for both the warrior caste and full datasets had mass as the only significant factor in the model (Tables 3.3, 3.4). Mass also appeared in the best models, with a slightly positive estimate, when 0.44 cm binary and the real distance response variables were used in the full dataset analyses, although it was not a significant factor in the model for any of these (Appendix A Table A.1). The best models for these response variables in the warrior caste dataset analyses had the treatment, (whether the focal polyp was touched by a clonemate or non-clonemate) appear as a factor in the best models, although again it was not significant (Appendix A Table A.1).

In contrast, clone identity was a significant factor in all models for all analyses comparing movement in clones A and B (Fig. 3.2; Tables 3.3 and 3.4; Appendix A Tables A.1 and A.2). Mass also appeared in many of these models or models that would be considered equivalent using AIC criteria (ΔAIC_c < 2) but mass was never found to contribute significantly to the model (Tables 3.3 and 3.4). In the best models mass’ estimate within the model was slightly above zero, although in lesser models it was estimated as slightly negative.

Similarly, clone identity was a significant factor in a majority of the best models comparing clones 1 and 2 (Tables 3.3 and 3.4; Appendix A Tables A.1 and A.2). Only the models fit to the 0.44 cm cut-off for movement, for both analyses (all polyp types and warrior castes only) lacked clone as a significant factor in the best models, although it was present in either the second or third best models in both cases although not in a significant capacity. Mass also appeared in most of the top models, and was a significant factor in analyses of all polyp types. However, unlike in the other two pairs, mass was fitted with a strongly negative estimate within the best models. In the GLMMs fitted for when there was no movement cut-off for the both
datasets, the number of acrorhagi was a significant factor in the best model whereas mass appears in the best model for the other datasets. This is likely because mass and acrorhagi are highly correlated and how the variance within the model is split between them depends on the model type.

3.3.5. Results of attack strength tests against a proxy clone

The small number of attacks initiated in the standardization experiment meant there were minimal results for this experiment (Table 3.4; range of n: 0 – 6). A T-test comparing the distance moved by the standard opponent clone was only possible for clones A and B (not significant) because of the lack of attacks for clones 2 and D. Each clone in a neighboring pair was given similar opportunity to attack (the same for each clone in a pair except for clones C and D where clone D was given a greater number of opportunities to attack).

3.4. Discussion

3.4.1. General conclusions

In our series of experiments we found that at the level of the individual polyp, clones generally have clear and consistent differences in their fighting ability, as previously argued by Ayre & Grosberg (1996, 2005). However, the degree of domination appears to decrease if interactions are repeated, suggesting both that clones are more equivalent in terms of competitive ability at this level, as Ferrell (2005) had hypothesized, and that habituation occurs between neighbors even when contact is agonistic (attack based) rather than merely tentacle-to-tentacle as had been demonstrated by Ayre & Grosberg (1995).
We hypothesized that one mechanism underlying the stability of clonal boundaries in the face of a discrepancy in polyp fighting ability was that clones that lost most dyadic polyp encounters would have stronger attack strengths. This hypothesis did not seem to be supported by clone pairs 1-2 or A-B. In the former, attack strengths appeared to favor clone 2 which also dominated in the agonistic contests between polyps of the two clones. For the A-B clone pairing, we saw nearly equivalent attack strengths for the two clones. The clone pairing C-D did not give clear results, but did seem to be in agreement with the results from the other two. The response of clone C to attacks from clone D was larger than the inverse interaction, suggesting the attack strength of D is greater than that of C, and D is also the stronger clone in the agonistic encounters. We find these results surprising since we hypothesized that clones may be able to counter increased losses at the polyp level if their attacks are able to push the dominant clone further back. We do not see this, although it does seem that clone C is more aggressive when faced with a new competitor than clone D (Table 3.5). This is likely because attack strength is intimately coupled to the outcomes of agonistic contests, and therefore cannot be separated as we initially hypothesized.

Rate of movement is another possible way by which clones may mitigate differences in agonistic ability in dyadic polyp encounters. In all but the C-D pairing we found differences between neighbors (Table 3.6). These results held both on the global scale of the clone (when all polyp types were included) and locally amongst polyp types that would encounter one another (warrior types). These strong differences in movement among clones may be a mechanism by which a clone deficient in fighting ability may maintain a stable boundary with its neighbor. We can imagine a scenario where a clone, via a higher intrinsic movement rate, can move polyps to the boundary before the dominant neighbor clone can move forward and take the place of
polyps it has defeated. Given our results here, we hypothesize this is likely case for the interaction between clones in pairs 1-2 and A-B (Table 3.6). If this is the case, our findings are consistent with those of Depickère et al (2008) who found that individual differences in movement of the ant species *Crematogaster scutellaris* created differences in aggregation time, a group level process. However, it is beyond the scope of these experiments to explain how this mechanism would work, but future modeling should be able to test the validity of this hypothesis.

3.4.2. General polyp data for mass and acrorhagi counts

Our results for the relative mass of polyps for each type within each clone and for counts of acrorhagi (Fig. 3.1) varied from clone-to-clone. These results match the findings of previous work (Ayre & Grosberg 2005; Ferrell 2005) that suggests the degree of division of labor within each clone is variable, and that neighboring clones that share an interclonal boundary appear to be well matched in terms of number of acrorhagi. The former point is demonstrated by the relative low degree of differentiation by clone B, specifically in the lack of difference in mass among types. Clones 1 and 2 also demonstrate relatively small range in acrorhagial counts. These clones contrast strongly with clones A and D, which show high degrees of differentiation between interior polyps and polyps at interclonal boundaries with regards to both the number of acrorhagi and mass of these types.

3.4.3. Individual competitive ability between neighboring clones

Similar to Ayre & Grosberg (1996), we found that in neighboring clones sharing an interclonal boundary, one clone will often win the majority of dyadic contests. In our three
pairings, we found that clones A, D, and 2 were dominant in their interactions (with clones B, C, and 1, respectively). Although sample sizes for each pairing type were not sufficiently large to generate statistically significant differences in competitive ability, by pooling these classes together, we found that the differences in contests won were significant for the C-D and A-B clone pairings. Clones 1 and 2 did not appear to be as competitively different as those in the other two pairings. This latter result supports the hypothesis put forward by Ferrell (2005) that clones that share an interclonal boundary are more competitively equivalent than clones that are randomly selected from the intertidal. Although our experiment gives no insight into the degree of competitive equivalence exhibited between randomly selected clones that is necessary as a baseline to formally test the hypothesis it does provide a degree of support for it. The lack of a strong dominance by clone 2 over clone 1 suggests the two clones are evenly matched, and possibly using similar behavioral strategies whereas for the other two pairings it is likely that the less competitive clone is somehow compensating for its relative weakness in this dyadic fighting through other means.

We found interesting results with regards to movement by clones in this series of experiments. Comparing the rates of movement for polyps that were not attacked to the rates of movement for polyps that had been attacked we found that although rates were higher following an attack for all clones except clone D, the increase was only significant for clone B, although clone 2 showed a clear trend ($P = 0.065$). The low number of polyps that were attacked prevented many of these comparisons from being significant, but overall these data strongly suggest an increase in movement following an attack. Interestingly, we did not find any significant differences between neighboring clones in the distance moved when attacked, although means were quite different in some cases. There are many possible explanations for
this. Some of these may be specific to the interaction: neighboring clones may be habituated to one another’s attacks; attacks are somehow comparable in strength between neighbors. The former of these hypotheses would be in agreement with the habituation of neighboring clones to one another found by Ayre & Grosberg (1995) where aggression between clones decreased after approximately five days of tentacle-tentacle stimulation between the two clones. Other hypotheses for why neighboring clones do not differ in the distance moved following an attack may be clone specific: polyps may simply move until they are out of contact with the attacking polyp; polyps may encounter physical constraints when moving beyond certain distances. Our experiments here do not provide us with the data to prefer one of these hypotheses over the others.

3.4.4. Results of repeated interactions

We found some evidence that repeated interactions lead to less clear conflict resolution between neighboring clones. For the neighboring pair of clones 1 and 2 there were a significantly fewer number of contests resolved in the second run compared to the first run. This trend held for clones C and D as well (8 of 16 in the 1st run, 4 of 16 in the 2nd run) but for the A-B clone pairing there was almost no change in the number of resolved contests, and they in fact slightly increased (7 of 16 in the 1st run, 9 of 16 in the 2nd run). These results provide further evidence of habituation between neighboring clones and that clones are changing their behavior and thereby the interaction as the time of the interaction is extended. This is similar to the “dear-enemy” hypothesis where neighbors reduce the intensity of their responses to one another (Fisher 1954; see reviews by Ydenberg 1988; Temeles 1994). However, it appears in this system that polyps may be able to de-habituate to one another, since attacks are consistently
observed across the boundary (Ayre and Grosberg 2005). This observation separates the habituation seen in this species from the “dear-enemy” interactions observed in other taxa.

Additionally, our results suggest polyps are not changing their attack strategies over the repeated interactions. Among those polyps that participated in both interactions, within each clone there was, at most, a single polyp difference in the number of polyps that attacked between runs.

In addition to not changing their attack strategies, polyps did not appear to alter the distances they moved in response to the interaction. Neighbors appeared to have inverse responses, in terms of movement, to a repeated interaction. While differences were not significant, it did occur in two of our three clone pairs. This may be indicative of different clone level strategies. Some clones may initially react very quickly to a new encounter before being habituated, whereas others may react slowly and ramp up their response as interactions increase. These two types of strategies, when paired, may lead to the clone level equivalence that we see at interclonal boundaries in the field.

The lack of a significant change in both movement and attack strategy by polyps in the repeated interaction belied the final results of the paired encounters, where outcomes were more equivalent than their first interaction (Tables 3.1 and 3.2 for comparison). It would appear then that clones reduce their response to a neighboring clone’s presence and attacks over repeated interactions. The increased equivalence would lead to clone level equivalence and this effect would likely only increase if we allowed for repeated interactions between the same two polyps. Because of limitations on the number of polyps we had available, and to control for multiple variables, only a single polyp was allowed into a repeated interaction. Given the responses we saw in the cases we were able to test, it is likely that repeated interactions
between the same two polyps would lead to an increase in the number of draws between the two. While there are good reasons for believing this to be the case, extrapolation from these results should be made cautiously because we forced the second interaction in our experimental design. Although Ayre & Grosberg (1995) found habituation over a daily contact regime this situation may be avoided in the natural habitat when contests that have clear winners. If losers of contests flee at the end of these losses there would likely not be repeated interactions. This would be especially true if the fleeing clone has low rates of movement when not attacked – thereby reducing the probability a polyp that had fled the interclonal boundary would quickly return to it. Polyps that lost, and possibly even those that won, would likely have time to de-habituate before encountering the neighboring clone again, especially if they had lost and retreated back to within their own clone as has been previously reported (Ayre & Grosberg 2005).

3.4.5. Results of touch by a clonemate or non-clonemate

The results of touch stimulation by a neighboring clone on focal polyps revealed a variety of insights into clone behavior. Surprisingly, we did not find any difference between treatments for any of the clones: simple contact failed to elicit a measurable response. Given these results and those of our individual competitive ability experiments, it would appear that movement rates are stimulated by attacks much more strongly than they are by contact, especially as neighboring clones habituate to one another.

Polyp type never appeared in the best GLMMs we tested to explain movement. This suggests that there is no strong difference in movement rates or distances among types within a clone. We were surprised by this, as bar graphs (not shown here) of movement rates appeared
to show such differences. Post-hoc comparisons of warrior-scouts, and warrior-scouts combined with warriors with interior polyps found no differences in movement rates for any of the clones, suggesting that the observed differences, given the sample sizes, are not sufficient to be significant. Additionally, the relative relationship among types differed among clones. In clones 1 and 2, warrior types moved more often than interior polyps, whereas in clones A and C the inverse was true. For clones B and D the two types moved either similarly or inverted their relationship depending on treatment. These differences do not seem to vary in any predictable way with fighting ability, and thereby may be far less significant in the interactions between neighbors than the general differences in movement between neighboring clones.

The strongest difference we found in this experiment was the difference between clones for neighbor pairings A-B and 1-2. For all but one of the best models fitted to the data for these pairs, clone was a significant factor (Tables 3 and 4; see Appendix A). This held for our analyses of our two datasets, the one with all polyp types included and the dataset containing only warrior types. From this, we conclude that it is highly probable that neighboring clonal mats use different behavioral or movement strategies. In addition to the strong persistence of a clone effect in our analyses, our general observations of these clones while in the lab with respect to how often they would move out of their containers strongly suggested differences among clones (Data not recorded). In combination, we conclude that such differences also persist in the field, although they may be tempered somewhat by habituation. Our observations of a different set of clones that had been housed in pairs within containers and allowed to interact (Chapter 4) suggests strongly that although some habituation may occur, these strong clone differences in movement persist.
Because the differences between each pair of clones (excluding clone pairing C-D) were consistent across both treatments (clonemate and non-clonemate contact) it suggests that these clones exhibit some elements of behavioral syndromes (see reviews: Sih et al. 2004; Bell 2007, shown in Cnidarians by Briffa & Greenaway 2011). Similarly, because we did not find any differences in attack rates in our experiments on repeated agonistic encounters between first and second runs, it is possible that clones are also consistent in attack behavior as well. The consistency of these behaviors is evidence of a clone personality within this species. Given our data here we are unable to discern the origin of these personalities. Whether they are determined genetically, or are adapted in part as a response to the social environment (the neighboring clone they are in contact with) they develop in.

3.4.6. Results of attack strength tests against a proxy clone

Although the paucity of attacks during our experiments reduces the power with which we can infer relative fighting ability, it would appear that the relative strength of the clones when compared to a standard are: 1 < 2; A = B; and likely C < D (Tables 3.5, 3.6). Although we had no cases in which D attacked the standard opponent clone, when comparing their responses to one another’s attacks in the agonistic encounter experiment we found C reacted more strongly to D’s attacks than vice-versa. Against the standard opponent clone the results are indicative of increased aggression by clone C compared to D when faced with a novel clone. Despite only a single attack by clone 2, the response by the polyp from the standard opponent clone was relatively strong (0.38 cm) whereas the standard opponent clone polyps did not respond at all to any of clone 1’s attacks. We therefore conclude that the attack strength of 2 is
greater than that of 1. The response of the novel clone to attack by clones A and B is similar and we therefore conclude that their attack strengths are approximately equal.

The results of the standardization experiment generally agree with those of the single encounter dyad fighting experiments (Table 3.1) in term of fighting ability. Nonetheless, each pair presented intriguing differences when all fighting experiments are considered. For example, clones A and B moved at similar rates in their neighbor pairing encounters, but on average clone B moved nearly twice as far when attacked than did clone A. This would suggest that A may have a slightly stronger attack, which is supported by the foreign clone moving slightly farther when attacked by clone A compared to clone B. These same trends held for clones 1 and 2, with clone 2 inducing a slightly stronger response in clone 1 and the foreign clone than what clone 1 could induce in the foreign clone and clone 2. Of note in all of these pairings is that attacks by either of the clones in a neighboring pair gave no strong evidence of being significantly different than one another. Although this may be due to the small sample sizes, these results suggest that clones, at least in terms of their reactions to one another’s attacks are evenly matched when compared to a randomly selected clone. This conclusion, at least for attack strength, is in line with the results of Ferrell (2005) when he suggested that clones that share an interclonal boundary are more competitively equivalent than would be expected by chance. Clones that have similar movement reactions to one another when attacked may be more likely to form an interclonal boundary than those that have disparate reactions to one another in this regard.

3.4.7. Final conclusions

Our work strongly suggests clone-specific behavioral strategies by the sea anemone *A. elegantissima*. These are tempered by habituation, leading to stability of interclonal boundaries
despite disparate polyp fighting ability. In agreement with our results, other data we have collected (unpublished data) indicate that attack and movement rates are clone specific and maintained over extended periods of interaction between clones. The existence of behavioral consistency and behavioral personalities within Anthozoa and Cnidaria has recently been demonstrated by Briffa & Greenaway (2011) in *Actinia equina* in regards to startle response. Future experiments should focus on examining the degree of DoL within each clone relative to the behavioral tendencies it exhibits relative to its neighbor. Additionally, larger scale studies that could identify relative ratios of different behavioral types in the intertidal would shed light on whether or not certain strategies are generally favored in this environment, and possibly for other systems where groups encounter one another.
Table 3.1. Results of polyp-on-polyp agonistic encounters between warrior (W) and interior (R) types from neighboring clones. Thickness of arrows indicate relative number of wins, numbers in the lower right corners of intersecting arrows indicate the number of unresolved contests. Numbers at the ends of arrows indicate obvious wins by that clone. Vertical arrows are wins by the first clone listed, horizontal arrows by the second clone listed. Numbers between these edges and the corner are contests in which one clone held an edge, but had not yet won the encounter. See text for further details.

<table>
<thead>
<tr>
<th>Clones</th>
<th>W versus W</th>
<th>W versus R</th>
<th>R versus W</th>
<th>R versus R</th>
<th>Total wins (if edges are included)</th>
<th>Dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A versus B</td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td>14 (16) 3 (7)</td>
<td>A &gt; B</td>
</tr>
<tr>
<td>C versus D</td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td>1 (1) 11 (18)</td>
<td>C &lt;&lt; D</td>
</tr>
<tr>
<td>1 versus 2</td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td><img src="image" alt="Arrow Diagram" /></td>
<td>6 (9) 14 (16)</td>
<td>1 &lt; 2</td>
</tr>
</tbody>
</table>
Table 3.2. Results of polyp-on-polyp agonistic encounters between warrior (W) and interior (R) types from neighboring clones for the second interaction of a repeated interaction. One polyp in each encounter had been in a prior contest, the other was new to the interaction, matched design for each set of neighbors. Notation is the same as in Table 3.1.

<table>
<thead>
<tr>
<th>Clones</th>
<th>W versus W</th>
<th>R versus R</th>
<th>Total wins (if edges are included)</th>
<th>Dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A versus B</td>
<td>0</td>
<td>3</td>
<td>3 (5) 6 (9)</td>
<td>A &lt; B</td>
</tr>
<tr>
<td>C versus D</td>
<td>0</td>
<td>1</td>
<td>1 (2) 3 (6)</td>
<td>D ~&gt; C</td>
</tr>
<tr>
<td>1 versus 2</td>
<td>3</td>
<td>1</td>
<td>4 (4) 2 (4)</td>
<td>1 ~ 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clones</th>
<th>W versus W</th>
<th>R versus R</th>
<th>Total wins (if edges are included)</th>
<th>Dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A versus B</td>
<td>0</td>
<td>3</td>
<td>3 (5) 6 (9)</td>
<td>A &lt; B</td>
</tr>
<tr>
<td>C versus D</td>
<td>0</td>
<td>1</td>
<td>1 (2) 3 (6)</td>
<td>D ~&gt; C</td>
</tr>
<tr>
<td>1 versus 2</td>
<td>3</td>
<td>1</td>
<td>4 (4) 2 (4)</td>
<td>1 ~ 2</td>
</tr>
</tbody>
</table>
Table 3.3. Best fit GLMMs to movement data from our experiment on the effects of clonemate and non-clonemate contact using AIC criteria. Only models equivalent by AIC criteria ($\leq 2 \Delta \text{AIC}_c$) are shown. Models presented are for all the analyses that utilized a 0.24 cm cut-off for movement, with all polyp types present, for each pair of neighbors. Mass is the mass of polyps, Clone is the effect of clone identity, Acro is the number of acrorhagi, Type is the polyp type, and Treatment in whether polyps were exposed to clonemates or non-clonemates. All models presented have polyp identity as a random effect. * = $P < 0.05$, model ANOVA, ** = $P < 0.01$. Models were built in R using the lme4 package.

<table>
<thead>
<tr>
<th>Clone Pairing</th>
<th>AIC</th>
<th>BIC</th>
<th>Models for 0.24 cm Dataset</th>
</tr>
</thead>
<tbody>
<tr>
<td>A vs. B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Clone** + Mass</td>
</tr>
<tr>
<td>1</td>
<td>4.6</td>
<td>5.2</td>
<td>Clone** + Mass + Acro</td>
</tr>
<tr>
<td>1.7</td>
<td>5.2</td>
<td>5.2</td>
<td>Clone** + Mass + Type</td>
</tr>
<tr>
<td>1.9</td>
<td>5.4</td>
<td>5.4</td>
<td>Clone** + Mass + Treatment</td>
</tr>
<tr>
<td>C vs. D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.2</td>
<td>0.2</td>
<td>Mass</td>
</tr>
<tr>
<td>1.1</td>
<td>4.6</td>
<td>5.2</td>
<td>Mass + Type</td>
</tr>
<tr>
<td>1.2</td>
<td>4.8</td>
<td>5.2</td>
<td>Mass + Treatment</td>
</tr>
<tr>
<td>1.6</td>
<td>5.2</td>
<td>5.2</td>
<td>Clone + Mass</td>
</tr>
<tr>
<td>1.7</td>
<td>5.2</td>
<td>5.2</td>
<td>Mass + Acro</td>
</tr>
<tr>
<td>1 vs. 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Clone** + Mass*</td>
</tr>
<tr>
<td>0.5</td>
<td>4.1</td>
<td>4.1</td>
<td>Clone** + Mass* + Treatment</td>
</tr>
<tr>
<td>1.3</td>
<td>4.8</td>
<td>4.8</td>
<td>Clone** + Mass** + Type</td>
</tr>
<tr>
<td>1.3</td>
<td>4.8</td>
<td>4.8</td>
<td>Clone** + Mass** + Type</td>
</tr>
<tr>
<td>1.9</td>
<td>5.4</td>
<td>5.4</td>
<td>Clone** + Mass** + Acro</td>
</tr>
</tbody>
</table>
Table 3.4. Best fit GLMMs to movement data from our experiment on the effects of clonemate and non-clonemate contact for border types (warrior/scouts and warriors) only. Abbreviation and notations are the same as in Table 3.3.

<table>
<thead>
<tr>
<th>Clone Pairing</th>
<th>AIC</th>
<th>BIC</th>
<th>Models for 0.24 cm Movement Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>A vs B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td></td>
<td>Clone** + Mass</td>
</tr>
<tr>
<td>1.1</td>
<td>4.9</td>
<td></td>
<td>Clone** + Mass + Acro</td>
</tr>
<tr>
<td>1.4</td>
<td>5.3</td>
<td></td>
<td>Clone** + Mass + Treatment</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td></td>
<td>Clone**</td>
</tr>
<tr>
<td>2</td>
<td>5.9</td>
<td></td>
<td>Clone** + Mass + Type</td>
</tr>
<tr>
<td>C vs D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.7</td>
<td></td>
<td>Mass</td>
</tr>
<tr>
<td>0</td>
<td>3.3</td>
<td></td>
<td>Mass + Type</td>
</tr>
<tr>
<td>1.2</td>
<td>4.5</td>
<td></td>
<td>Clone + Mass</td>
</tr>
<tr>
<td>1.3</td>
<td>2.1</td>
<td></td>
<td>Type</td>
</tr>
<tr>
<td>1.6</td>
<td>5</td>
<td></td>
<td>Mass + Acro</td>
</tr>
<tr>
<td>1.9</td>
<td>0</td>
<td></td>
<td>&lt;No Fixed Effects&gt;</td>
</tr>
<tr>
<td>1.9</td>
<td>7.9</td>
<td></td>
<td>Clone + Mass + Type</td>
</tr>
<tr>
<td>2</td>
<td>5.3</td>
<td></td>
<td>Mass + Treatment</td>
</tr>
<tr>
<td>1 vs 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.8</td>
<td></td>
<td>Clone* + Mass</td>
</tr>
<tr>
<td>1.1</td>
<td>0</td>
<td></td>
<td>Clone*</td>
</tr>
<tr>
<td>1.2</td>
<td>5.9</td>
<td></td>
<td>Clone* + Mass + Treatment</td>
</tr>
<tr>
<td>1.4</td>
<td>6.1</td>
<td></td>
<td>Clone* + Mass + Type</td>
</tr>
<tr>
<td>2</td>
<td>6.7</td>
<td></td>
<td>Clone* + Mass + Acro</td>
</tr>
</tbody>
</table>
Table 3.5. Results of the attack strength experiment. Data reported is the distance the foreign clone (clone F) moved in response to attacks from the clone listed.

<table>
<thead>
<tr>
<th>Attacking Clone</th>
<th>Standard clone's response to attack (cm moved)</th>
<th>n</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>clone A avg</td>
<td>0.20</td>
<td>4</td>
<td>A = B</td>
</tr>
<tr>
<td>clone B avg</td>
<td>0.15</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>clone C avg</td>
<td>1.08</td>
<td>6</td>
<td>unknown; C more aggressive</td>
</tr>
<tr>
<td>clone D avg</td>
<td>NA</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>clone 1 avg</td>
<td>0.00</td>
<td>5</td>
<td>1 ~&lt; 2</td>
</tr>
<tr>
<td>clone 2 avg</td>
<td>0.38</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.6. A summary of results from our three experiments. Fighting ability refers to polyp-on-polyp first encounters only. The results for this column are not as strong, or even reverse upon repeated interactions. See Tables 3.1 and 3.2. Movement refers to rates of movement by each clone. * against clone F D did not attack, thus against a proxy clone we are unable to compare its attack strength to that of C. See text for further discussion.

<table>
<thead>
<tr>
<th>Fighting ability</th>
<th>Movement</th>
<th>Attack Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>A &gt; B</td>
<td>A &lt; B</td>
<td>A = B</td>
</tr>
<tr>
<td>C &lt;&lt;&lt; D</td>
<td>C ~ D</td>
<td>unclear*</td>
</tr>
<tr>
<td>1 &lt; 2</td>
<td>1 &gt; 2</td>
<td>1 ~&lt; 2</td>
</tr>
</tbody>
</table>
Figure 3.1. Bar charts of the mean number of acrorhagi (+S.E.) for each polyp type within each clone, and line graphs of the mean mass (+S.E.) for each type within each clone. Neighboring clones are positioned adjacent to one another. Significant differences were found among types for each clone except mass for clone B (ANOVAs for each clone, see text for more details).
Figure 3.2. Results of the stimulation of polyps from each clone be either a clonemate (Self) or non-clonemate neighbor (Nonself). Shown is the proportion of movement (using 0.24 cm as the cut point) by polyps from each clone for each condition. We pooled data for all polyp types as type was not a factor in the best fit GLMM models to the data (Table 3.3).
CHAPTER 4

BEHAVIORAL SYNDROMES IN A GROUPING INTERTIDAL INVERTEBRATE: ATTACK AND

MOVEMENT CONSISTENCY IN THE SEA ANEMONE ANTHOPLEURA ELEGANTISSIMA

4.1. Introduction

Clones of the intertidal sea anemone Anthopleura elegantissima compete with both inter- and intraspecifics for intertidal space (Francis 1976; Ayre and Grosberg 2005). In the intraspecific case, this can lead to the formation of an interclonal boundary between competing clones (Ayre and Grosberg 2005; Ferrell 2005). Agonistic interactions that occur across these boundaries have been well characterized (Ayre and Grosberg 2005; Ferrell 2005; Chapter 3) at the level of the individual polyp but not at the level of the clone. Currently, there is data to suggest that polyps of a given clone use different behavioral strategies than those of a neighboring competitor clone (Chapter 3). These results suggest that these different strategies may mitigate individual clonal differences in polyp fighting ability (Ayre and Grosberg 1996; Chapter 3). Despite this research on polyp behavior there have been few studies focusing on polyp behavior when they are in groups (but see Francis 1973a; Ayre and Grosberg 2005). This gap in the research has limited our knowledge of the group level interactions in this system.

A better understanding of this species’ group behaviors has been limited for two reasons. First, no research has been conducted on the effects of multiple polyps interacting with one another to determine if there are non-linear effects that may trump the polyp level differences in fighting ability between neighboring clones (Ayre and Grosberg 1995, 1996;
Additionally, investigations of how consistent clones are in their behavioral patterns have been limited to single polyp interactions (Ayre and Grosberg 1995; Chapter 3) not interactions among groups of polyps. There is certainly good reason for this to be the case as previous work has shown evidence for behavioral personalities among polyps in both this species (Chapter 3) and another species of sea anemone, *Actinia equina* (Briffa and Greenaway 2011). Both *Ac. equina* and *A. elegantissima* are intertidal species and the presence of personalities in the former in conjunction with our suggestive evidence (Chapter 3) implies that *A. elegantissima* is likely to exhibit personalities in multi-polyp interactions as well.

The dearth of studies on group interactions in this species has prevented a full understanding of the presence, formation, and maintenance of interclonal boundaries between neighboring clones. How common these boundaries may be, and how easy they are to form is unknown. However, previous work (Ferrell 2005; Chapter 3) strongly suggests that their formation is rare. Ferrell (2005) hypothesized that boundaries only occur between relatively evenly matched clones and therefore should be infrequent. This implies that when clonal mats encounter one another one of the clones should typically dominate and eliminate the weaker clone. As dominant clones establish themselves in the intertidal and increase in size they will become more efficient at eliminating competing clones that attempt to establish. This is especially true for those dominant clones that grow to encompass entire boulders (Francis 1973; Ayre and Grosberg 2005). This species establishes via planula that settle on intertidal rock faces. Planula that settle near large, established clones will be attacked by multiple polyps and immediately eliminated. This will help prevent the formation of an interclonal boundary.

Here we test two hypotheses. First, we test Ferrell’s (2005) assertion that the establishment of interclonal boundaries is rare. To address this hypothesis we built artificial
interclonal boundaries in the laboratory similar to Francis (1973a). We paired clones randomly

\( n = 14 \) clones; 7 pairs) except, as a control, a pair of clones that had shared an interclonal
boundary in the field (artificial boundary experiment). Second, we test the hypothesis that A.

*elegantissima* exhibits behavioral personalities in multi-polyp settings. We compare the
movement response of a focal clone when alone to when a second clone is present (artificial
tide pool). We did so by placing groups of clonemates either alone or paired with non-
clonemates in a container. We then measured the inter-polyp distance for each clone daily as a
proxy for movement and clone spread over several days. We predicted that clones would vary in
how much they increased their average distance among polyps and that this distance would be
lower in the paired vs. unpaired treatment.

In both the interclonal boundary and tide pool experiments there was a strong effect of
clone identity on our models of behavior. This supports our hypothesis that individual clones
demonstrate differing behavioral syndromes in respect to attack and movement behaviors. This
agrees with the different strategies that appear to be employed by neighboring clones (Chapter
3). In our experiment on interclonal boundaries we found a strong individual polyp effect with
respect to both movement and attack rates, suggesting that individual polyps within each clone
of A. *elegantissima* demonstrate elements of personality, again in line with previous work
(Chapter 3). Maintenance of an interclonal boundary only occurred in the control case
suggesting that only certain strategies may be paired such that their competitive abilities, at the
clone level, are equivalent. This supports Ferrell’s (2005) hypothesis that the formation of such
boundaries is a rare occurrence between evenly matched clones.

4.2. Materials and methods
4.2.1. Animal collection and care

Polyps were collected on San Juan Island, WA (at Eagle Cove and Cattle Pt.) in spring 2008 (series 1 Artificial boundaries experiment (AB), Artificial tide pools experiment), and 2010 (series 2 AB) and from Bodega Bay, CA in fall of 2011 (series 3 AB). All polyps were transported back to either Friday Harbor Labs (series 1 and 2) or The Ohio State University (series 3) in plastic bags partially filled with sea water. All polyps were maintained in plastic containers in sea tables within the lab when not in use. During the course of each experiment polyps were not fed.

4.2.2. Artificial boundaries between clone pairs

Experimental setup

Pairs of clones were pinned to cork board attached to the bottom of a plastic container using hook and loop fasteners. Clones were initially separated by a plastic barrier while they attached. The animals were given a 2 h period without water (low tide) and 2 h of fresh running sea water (incoming tide) daily.

Series 1: Six pairs of clones (12 clones total) interacted for >20 days. These clone pairings had not been neighbors in the field and were collected from two distinct sites and then paired randomly. On two occasions per container we switched the position of polyps of the two clones to facilitate interactions and to simulate incursions by each clone (as observed by Ayre & Grosberg 2005). The first of these alterations occurred within the first two days of observation: we randomly chose between one large and up to three smaller polyps from each clone to move.
If possible, we swapped the position of these polyps between the two clones. If this was not possible, we placed each polyp in a position such that it was in contact with the opposing clone. We repeated this alteration between five and six days later (with the exception of clone pairing 6 vs. 8 which occurred at eight days). Polyps that left the container, when identifiable, were returned to the center of the container. Containers were observed closely for the 2 h following a low tide during which any attacks were recorded.

Series 2 and series 3: In each of these series we observed a single clone pairing extensively. Series 2 involved two clones that were not neighbors in the field. These clones were collected from separate localities (as in Series 1) and then paired in the laboratory. Series 3 was a single pairing of clones that were neighbors in the field and acts as our control. These clones were collected simultaneously from the field and then re-paired in the laboratory. All polyps in both series were massed before the start of the experiment using the same methods as in Chapter 2. Clones were allowed to interact over the course of 20 (series 2) and 22 (series 3) days without interference. Photographs were taken every 3 to 10 minutes following the simulated incoming tide for ~ 6 h. We did have a three day exception to this pattern during series 3 when our main camera broke. During this period the experiment was closely observed for ~ 2.5 h following the low tide to maximize the probability we would view any attacks. Photographs of these attacks and general position of polyps were taken with a back-up camera. Overnight photographs were taken hourly for series 2, but seeing that they added little to our analysis of series 2 they were not taken during series 3.

Data collection
For all three series we recorded polyp movements, distances moved, attacks, and which polyps were in contact with one another from photographs taken during the course of the experiment. We used a single day as our time scale for recording these variables. Photographs were analyzed in Adobe Photoshop® or GIMP v 2.6.11. In all cases distinct clones could be identified, however, in some rare cases, specific polyps could not. In these cases we used the polyps nearest to the previous position of the unknown polyp that were of the same clone and size, to assign polyp identity. We viewed this as a conservative under-estimate of polyp movement. In some cases polyps crawled out of the container. In these cases we used the most likely location the polyp would have left the container as its location. We then measured to that location for the distance moved for that movement bout, and as the distance between polyps. We view this as a conservative underestimate of the distance between polyps.

In order to determine the maintenance of clonal boundaries, we took photographs of containers on either the first day of the observation period (series 2 and 3) or immediately following the first and second polyp alterations (series 1). Using these photographs we defined the location of the interclonal boundary between the two clones by drawing a line over the photograph that appeared to most closely follow the midpoint of the distances between the two clones. We then drew this same line for the next day in the observation series where there was either another polyp alteration (series 1) or the experiment was terminated. This method gave us a pair of photographs for each series 2 and series 3 data. Because made alterations to the boundary in Series 1 we only look between these alterations and therefore have two pairs of photographs for these data. For each photograph we measured, using image software (GIMP v 2.6.11), the distances between the eight polyps for each clone that were furthest away from the side of the container on which that clone had originally been placed (e.g. if the clone was
originally stationed on the left side of the container, we measured to the interclonal boundary line for the eight polyps that were most to the right). Polyps that were beyond the boundary in the direction opposite the side they were placed were assigned negative distances. Polyps that left the container between the time the two photographs were taken were not used in the second photograph analysis. We again use this method as a conservative underestimate of the distances between polyps of a clone and the interclonal boundary.

**Data analysis**

All data were analyzed using generalized linear mixed models built in R using the lme4 package (R Development Core Team 2005) (Pinheiro and Bates 2000; Bolker et al 2009). We tested the effects of the following variables on the binary response variables of polyp attacks and polyp movements. Full models for all series included the following: clone pairing, whether it had been attacked, whether it was in contact with a non-clonemate, whether it was in contact with a clonemate, and which day it was. We also included polyp identity and clone identity as random effects in models tested. For series 2 and 3, we also knew polyp mass and the division of labor (DoL) class of the polyp (Ayre and Grosberg 2005), and both of these were added to the full models. We used Akaike information criteria (ΔAIC) to rank models and Wald Z tests for each model’s fixed effects and their contribution to each model (Pinheiro and Bates 2000; Bolker et al 2009). We used likelihood ratio tests to determine the effect of clone identity on the model (Pinheiro and Bates 2000; Bolker et al 2009).

For our series 1 data, we ran models in two ways: with moved polyps removed from the dataset for the day they were altered, and those same polyps not removed from the dataset. Since we did not alter polyps for series 2 or 3, we did not alter the data in this way.
We used a general linear mixed model run in SPSS v. 19.0 to test for the effects of time on the distance of polyps to the boundary. Inputs into the model included time (either time one or two) and set (for series 1 data) as fixed effects and clone as a random effect. We used a paired T-test to determine if variances for these distance data, among all series, had increased between time points.

### 4.2.3. Group behavior in artificial tide pools

**Experimental set-up**

Groups of five to six clonemates (variable due to limited polyp availability) were placed on corkboard that measured 10 cm by 12 cm inside a plastic container of dimensions 24 x 14 x 8 cm. On the bottom of the container there was also ~ 45 cm² of Velcro hooks (Fig. 4.1). Each clone had two replicates: one in which it was the only clone in the container and another in which it was paired with a second clone from the other locale (Eagle Cove paired with Cattle Pt.) (Clone pairs: 10 & 12, 11 & 13, 14 & 17, 15 & 16) (Fig 4.1). We chose polyps for each treatment randomly. We removed pins and water to simulate a low tide for ~3 h. We then ran fresh sea water into the container daily for between 4 and 9 h. We photographed each container daily to document polyp movement (Fig. 4.1b). We simulated a single low tide weekly to replicate the effect of a tide pool that was rarely emptied. This limited the desiccation stress clones experienced. After completion of a run, we performed a second run with polyps that were previously alone being paired and vice versa. Exceptions to this include clones 15 and 16 for their second run, unpaired (1 new polyp for clone 15 was used and 3 new polyps for clone 16 were used), and the second run for the paired case, which used polyps from the two clones that
had been used in a different experiment beforehand. Clones 10-13 were run for 18-23 days while the clones 14-17 were run for 7-14 days due to time constraints.

We used inter-polyp distance as a proxy for polyp movement although situations exist where after some initial spread movement directed toward clonemates would reduce inter-clonemate distance. We found this to be an unlikely scenario given the lack of desiccation stress and competition in the single clone treatment. In some cases polyps crawled out of the container. In these cases we used the most likely place the polyp would have left the container (straight line from previous known location to where it was outside the container, marking the point where the line crossed the container as the final location of the polyp) and measured to that location from that point on. We viewed this as a conservative underestimate of the distance between polyps.

Data analysis

For Part 2, we used a fourth root transformation of clonal spread data so that residuals were normally distributed (Kolmogorov-Smirnov test, $N = 3433, P = 0.733$). We then used this transformed data set for our GLM and GLMMs. Our inputs into the model included the clone identity as a random effect, the day as to when the measurements took place, whether it was a paired or unpaired replicate, and whether it was the first or second run were all modeled as fixed effects. As above, we ordered the various models by their $\Delta AIC_c$.

4.3. Results

4.3.1. Series specific behavior of clones in artificial boundaries
Series 1:

We found no qualitative differences between the best models for series 1 data for our altered dataset, where we removed polyps from the dataset if they had been altered on that day, and unaltered datasets. For simplicity we present only the modified dataset here. In our best GLMMs for both attacks made and movement bouts as response variables (Table 4.1), clone identity contributed significantly to the models (Likelihood Ratio (LR) test, all $P < 0.05$, Table 4.1). Similarly, the focal polyp having been attacked also contributed significantly to models for either response variable as a fixed effect. It was modeled with a positive estimate for both response variables (Wald Z test, all $P < 0.001$). Which clone pairing it was (Pairing), occurred in many of the best models describing attacks and one of the best models for movement, although it did not contribute significantly to either. Models for the response variables varied: being in contact with the enemy clone was found to contribute significantly and positively to the GLMMs with attack behavior as the response variable. It did not contribute significantly when movement was the response variable. The day of the experiment contributed significantly to our best models for movement (Wald Z tests, all $P < 0.001$) although it was given only a very slight positive estimate in these models.

Series 2:

Our best GLMMs (Table 4.2) describing the attacks performed by polyps consistently contained whether or not the focal polyp was in contact with an enemy polyp and polyp mass as significant factors ($P < 0.01$). Time, in the form of the day of experiment, also appeared in many of the best models. Wald Z tests revealed that the $P$-value associated with this fixed effect varied about 0.05, in some models being slightly below this threshold, and in others slightly
above. In all models containing both polyp and clone as random effects, polyp identity had a very strong effect on the model (Likelihood ratio (LR) test, $P < 0.01$), whereas clone identity did not.

Our models for movement of polyps in this series also found that polyp identity contributed significantly (LR tests, $P < 0.001$) as did clone identity (LR tests, $P < 0.001$). The fixed effects of day and contact with an enemy clone were both highly significant in all models (Wald Z tests, $P < 0.001$) whereas the fixed effects of the focal polyp being attacked, was in contact with a clonemate, and the DoL class it was labeled as all also contributed significantly to the best models, although $P$-values were not as strong (Wald Z tests, $P < 0.05$).

Series 3:

There were minimal attacks ($n = 7$) and movement bouts ($n = 35$) in this artificial boundary experiment. For attacks as the response variable, no fixed factors contributed significantly to any of our models (Table 4.3). Similarly, neither polyp nor clone identity contributed significantly as random effects to the model. Fixed effects in our best models included the focal polyp having been in contact with and attacked by the enemy clone. These results are similar to those of our best GLMMs for movement data in this series.

Polyp identity was a significant factor in all GLMMs for polyp movement (LR tests, all $P < 0.01$) but clone identity was still not found to be significant (Table 4.3). The fixed factor that occurred most frequently in the best models (being in contact with an enemy polyp) was significant in all of the best models (Wald Z tests, all $P < 0.05$). Mass also occurred as a covariate in some of the best models, but was not significant; the same was true for day of the experiment and contact with self (Table 4.3).
4.3.2. General results for stability of artificial boundaries

In no case for series 1 or 2 did a boundary appear to form between the pair of clones that remained present for any extended length of time. The boundary that was initially created by us was not maintained. In contrast with our observations of the first two series the boundary that was put into place was maintained in the third series.

Quantitatively, we found that this observation was less supported by our observed data. Our GLM had only clone and set as contributing significantly to the model (F-test, $P < 0.05$). Neither time nor any of the interaction terms were significant.

In order to explain the discrepancy between our qualitative and quantitative results, we analyzed the change in variance in our distance to boundary measurements between the two time periods at which we measured. Combining data for all series we found that variance increased between the first and second time periods (Paired T-test, $P = 0.008$).

4.3.3. Group behavior in artificial tide pools

The inter-clonemate distance (spread) was clone dependent, with clones 10, 11, 16 and 17 having a greater average distance between constituent polyps than other clones (Fig. 4.2). Our best GLMM model had both Day and Run as the only fixed effects although neither contributed significantly (Wald Z test, $P > 0.05$). Clone identity was also present in this model as a random effect. Current software limitations made it untenable for us to test the effects of clone on the model since it was the only random effect present. To avoid this problem we concern ourselves only with the specific clones used in this study and model Clone as a fixed effect. Doing so, we found that it, along with the day of the experiment, the run, whether it was
a paired run, and the interaction between being paired and the run were all strong factors in the model (Wald Z test, all $P < 0.001$). These data are supported by Fig. 4.2 and Table 4.4. For unpaired treatments, a difference typically emerged between runs, with run 2 (using polyps that had been paired with another clone in run 1) seeing clones spread more than those unpaired in run 1, although $P$ values were not always low (Fig. 4.2; Table 4.4). We saw a similar increase in spread for paired treatments in run 2 (Fig. 4.2). Differences in paired and unpaired treatments occurred for clones 11, 12 and 14 in run 1 (Table 4.4). In all but one of these comparisons, the unpaired treatment had greater spread than the paired treatment. Such differences disappeared almost entirely in the second run (Table 4.4).

4.4. Discussion

4.4.1. General conclusions

Across both of our experiments, artificial boundaries and artificial tide pools, we found clone specific differences in movement, response to a competitor, and attack behaviors. Our data strongly suggest the presence of behavioral personalities at the group level, which is in accord with our previous work on this species using polyp level observations (Chapter 3). The presence of these personalities and differences suggest that such personalities likely have fitness consequences for competitive interactions with other clones and possibly other species in the intertidal.

4.4.2. Artificial boundaries: Personality in *A. elegantissima*
Previous work within Cnidaria by Briffa and Greenaway (2011) found that personality, or at least behavioral syndromes, exist within sea anemones. The results from our own experiments were suggestive that this was the case in *A. elegantissima* (Chapter 3) and we followed that up with a series of experiments described here to test this hypothesis. Our results were consistent with respect to movement and attack behaviors and as such we discuss them together with movement behaviors in the context of this experiment.

In all of our best GLMMs for all three series and both response variables (movement and attacks performed) polyp identity was present (Tables 4.1-4.3). In observations series 1 and 2 clone identity was also present in these models, with the exception of models for attacks in series 2 (Tables 4.1, 4.2). The strong presence of both polyp and clone identity in these models, with their presence being significant factors in most of these models, is strong evidence that clones, and even individual polyps within each clone, express their own behavioral syndromes. It is somewhat surprising that polyp identity is ubiquitous in our models whereas clone identity is less so, given that all polyps of a given clone will have the same (on the whole) genetic identity. This is likely a result of the specialization of polyps within a clone do different tasks (e.g. fighting or sexual reproduction) (Ayre and Grosberg 2005) although prior work did not show any consistent patterns between clones with regards to DoL caste and these behaviors (Chapter 3).

This assertion, that DoL within the clone may be responsible for intra-clone behavioral differences, is supported here by the presence of polyp class in models for series 2 movement data although it does not appear in the best models for either attacks or any of the series 3 data. These particular data were not recorded for any of the series 1 data so that factor is not present in the full models for those datasets. Still, it would seem that the physiological changes that are associated with the differentiation into different classes, in addition to differential past
experience with an enemy clone (e.g. the habituation discussed by Ayre and Grosberg 1995 and Chapter 3) would lead to sufficient differences among polyps of a clone to have behavior be specific to each polyp.

More important for this particular species is that each clone appears to have its own behavioral set with regards to these two behaviors. Many explanations have been put forward to explain the existence of personality types. These include; that adaptive personalities can evolve when personalities play against one another preventing evolution to a single behavioral type (Smith and Blumstein 2008; Dall et al 2004) or when there may be a trade-off, such as between current and future reproductive success (Duckworth 2010; Wolf et al. 2007). These hypotheses may explain the existence of personalities in different clones of *A. elegantissima*. Clones that have low chances of future reproductive success, such as smaller clones that may be less likely to survive both stochastic events in the intertidal and agonistic interactions with either other clones or species, are likely to find an advantage in using a different behavioral strategy than larger clones. These smaller clones will have less tissue, at a clone level, devoted to gamete production than a clone covering the same amount of intertidal space that has larger reproductive polyps. We might then expect the former of these two to be bolder, e.g. show higher degrees of movement and aggression, than the latter that has a larger investment in gametes. Future studies in this group should look at examining this hypothesis more explicitly.

4.4.3. Artificial boundaries: Maintenance of interclonal boundaries

Qualitatively, in our three observational series we only saw the formation and stability of an interclonal boundary in our control run, where we paired two clones that shared an
interclonal boundary in the intertidal. Within this series there was reduced movement and attacks compared to our other series. We do not know why this was the case. Although there is evidence that clones will show some habituation to one another (Ayre and Grosberg 1995; Chapter 3) this habituation does not appear to last very long when contact ceases (~24-48 h Personal observation). The clones in this particular case had been separated for longer than this period before the start of the experiment. Our only explanation is that, by chance, neither of these two clones was inherently very aggressive although we cannot rule out prior habituation to one another.

Despite our qualitative observations, our quantitative measurements found no difference in the average distance of the eight polyps furthest from the side of the container they were placed in to the artificial boundary between our first and second time periods. This likely occurred for a variety of reasons. In many cases, there was one polyp per clone that was closer to the opposing clone than the other polyps per our alterations at the first time point. This greatly increased the variance at our initial time point making it less likely we would find a difference. Furthermore, clones that were likely to move toward the competitor polyp, if they were moving randomly, may also have been likely to move away. This seems to be supported by the increased variance at our second time period measurements compared to those at our first time period. This suggests that the boundary is more ragged than it was initially, and is in line with our qualitative observations. Furthermore, when polyps exited the container they were no longer available for this analysis. This meant that many of the polyps that had moved across the container into the midst of the opposing clone and out of the container were not used. By eliminating these polyps we underestimated the change in position of the boundary. Inclusion of
these polyps into our dataset would have likely reconciled any differences between our quantitative results and our qualitative observations.

Our observations are in contrast with what was found by Francis (1973), who found maintenance in the single clone pairing that she performed. This disparity could be for a variety of reasons, including the possibility that by chance the two clones Francis chose to use were competitively equivalent or nearly so. Furthermore, without quantitative measurements it is hard to determine exactly how ‘stable’ the boundary was in Francis’ case. With our increased sample size, we therefore give greater weight to our results here although we note that Francis’ (1973) results certainly demonstrates that, qualitatively, boundaries can form between clones that are not neighbors in the intertidal.

The lack of interclonal boundary formation and stability in our randomly paired clones suggests that the formation of these boundaries is a rare occurrence that must occur under a unique set of circumstances. This supports Ferrell’s (2005) hypothesis that pairs of clones that share an interclonal boundary are more evenly matched, in terms of competitive ability, than would be expected by chance. It also indicates that although different behavioral strategies can be used to negate polyp level differences in agonistic ability (Ayre and Grosberg 1996; Chapter 3) it would be expected that only rarely do different strategies match well enough at the clone level to be equivalent.

4.4.4. Artificial tide pools

We also saw large differences in implied movement, measured as distance between clonemates, between clones over an extended observation period (Fig. 4.2; GLMMs) not placed in direct competition with another clone. These data demonstrated not only differences among
clones, but an increase in spread throughout the experiment for run 2, which may simply be a result of polyps acclimating to the artificial surrounding. The apparent inhibition of movement by a secondary polyp in run 1 for clones 11, 12 and 14 is interesting but the results are not strong enough across all clones to conclude that this is an actual effect. Still, it is interesting to note that for most clones in run 1 the unpaired spread is greater than the paired spread (Fig. 4.2). This relationship disappears for run 2, perhaps because runs were done consecutively so polyps that were previously paired may have still been inhibited in their movement (Chapter 3).

We note that increased movement does not necessarily translate into increased inter-clonemate distance. However, for the unpaired case, we removed most desiccation stress, and there should be little benefit to clumping. Random walking by clones in this environment would naturally increase intra-clonemate distance, which it does. This is in agreement with Sebens (1982), who found clones are often discontinuous, especially in protected areas. Our simulated tide pool would be considered a well protected area, and as shown here clones quickly spread across the available substrate, presumably in a bid for space.

4.4.5. Future directions

Our results here demonstrate that clones and their constituent polyps exhibit behavioral syndromes with respect to attack and movement behavior. Furthermore, they show that when clones encounter one another it is unlikely that their behavioral types match in such a way that they are competitively equivalent at the clone level and that their responses to the presence of a competitor are both clone and possibly history (in terms of what they have encountered before) specific. However, at this point we do not have a good estimate of the breadth of behavioral syndromes that are exhibited within the population nor how the frequency of these
types may change over time (e.g. if there is frequency dependent selection on behavioral types). Additionally, although this species is confined to rocky intertidal, it has an extremely large range (Baja California to Alaska (Hand 1955)) over which it will encounter an environmental cline in addition to the variety of microhabitats that occur within the intertidal. Whether or not specific behavioral types perform better either at different latitudes or different microhabitats is a question that bears investigation. Additionally, our results here confirm the presence of behavioral syndromes in Cnidaria, organisms that employ only a simple nerve net for their nervous system. Further work on what is minimally required for the existence of behavioral syndromes should also be done as it has now been described in a variety of organisms that demonstrate relatively simple control systems for behavior (Davidson and Surette 2008; Briffa and Greenaway 2011).
Table 4.1. GLMMs for series 1 data for both attacks made and movement bouts completed with both treated as binary variables. We show all equivalent models with a ΔAICc ≤ 2 from the best model in both cases. * indicates a random effect, all other effects are fixed effects or covariates. 
P values were generated for fixed effects and covariates using Wald Z tests, and for random effects using LR tests. In all models polyp identity is present as a random effect although, for clarity, we do not show it below.

<table>
<thead>
<tr>
<th>Response Variable: Attack enemy clone (0/1)</th>
<th>ΔAICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model tested</td>
<td></td>
</tr>
<tr>
<td>Pairing + Was Attacked*** + Was in Contact with Enemy*** + CloneRE* + PolypRE***</td>
<td>0</td>
</tr>
<tr>
<td>Was Attacked*** + Was in Contact with Enemy*** + CloneRE* + PolypRE***</td>
<td>0.2</td>
</tr>
<tr>
<td>Pairing + Was Attacked*** + Was in Contact with Enemy*** + Was in Contact with Self + CloneRE* + PolypRE***</td>
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</tr>
<tr>
<td>Was Attacked*** + Was in Contact with Enemy*** + Was in Contact with Self + CloneRE* + PolypRE***</td>
<td>1.5</td>
</tr>
<tr>
<td>Pairing + Was Attacked*** + Was in Contact with Enemy*** + Day + CloneRE* + PolypRE***</td>
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<table>
<thead>
<tr>
<th>Response Variable: Moved (0/1)</th>
<th>ΔAICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model tested</td>
<td></td>
</tr>
<tr>
<td>Was Attacked** + Was in Contact with Self*** + Day*** + CloneRE*** + PolypRE***</td>
<td>0</td>
</tr>
<tr>
<td>Was Attacked** + Was in Contact with Self*** + Day*** + In Contact with Enemy + CloneRE*** + PolypRE***</td>
<td>0.1</td>
</tr>
<tr>
<td>Was Attacked** + Was in Contact with Self*** + Day*** + Pairing + CloneRE*** + PolypRE***</td>
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</tr>
<tr>
<td>Was Attacked** + Was in Contact with Self*** + Day*** + Pairing + In Contact with Enemy + CloneRE*** + PolypRE***</td>
<td>0.6</td>
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Table 4.2. GLMMs for series 2 data for both attacks made and movement, with both treated as binary variables. We show all models with a $\Delta AIC_c \leq 2$ from the best model in both cases. $\text{RE}$ indicates a random effect, all other effects are fixed effects or covariates. Abbreviations and notations are the same as in Table 1. $P$ values were generated for fixed effects and covariates using Wald Z tests, and for random effects (when the number of random effects was greater than 2) using LR tests.

<table>
<thead>
<tr>
<th>Response Variable: Attack enemy clone (0/1)</th>
<th>$\Delta AIC_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model tested</td>
<td>$\Delta AIC_c$</td>
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<tr>
<td>Day + Mass** + Was in Contact with Enemy*** + Poly$p_{RE}$</td>
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<tr>
<td>Mass** + Was in Contact with Enemy*** + Poly$p_{RE}$</td>
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<tr>
<td>Day + Mass** + In Contact with Enemy*** + In Contact with Self + Poly$p_{RE}$</td>
<td>1.1</td>
</tr>
<tr>
<td>Day + Mass** + In Contact with Enemy*** + Clone$<em>{RE}$ + Poly$p</em>{RE}$***</td>
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<table>
<thead>
<tr>
<th>Response Variable: Moved (0/1)</th>
<th>$\Delta AIC_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model tested</td>
<td>$\Delta AIC_c$</td>
</tr>
<tr>
<td>Day*** + Attacked Others* + In Contact with Enemy*** + In Contact with Self* + Class* + Was Attacked** + Clone$<em>{RE}$*** + Poly$p</em>{RE}$***</td>
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</tr>
<tr>
<td>Day*** + Attacked Others* + In Contact with Enemy*** + In Contact with Self* + Class* + Was Attacked* + Mass + Clone$<em>{RE}$*** + Poly$p</em>{RE}$***</td>
<td>2.0</td>
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</table>
Table 4.3. GLMMs for series 3 data for both attacks made and movement, with both treated as binary variables. We show all models with a $\Delta AIC_c \leq 2$ from the best model in both cases. Abbreviations and notations are the same as in Table 1. P values were generated for fixed effects and covariates using Wald Z tests, and for random effects (when the number of random effects was greater than 2) using LR tests.

**Response Variable: Attack enemy clone (0/1)**

<table>
<thead>
<tr>
<th>Model tested</th>
<th>$\Delta AIC_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Was Attacked + In Contact with Enemy + PolyPRE</td>
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</tr>
<tr>
<td>Was Attacked + In Contact with Enemy + CloneRE</td>
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</tr>
<tr>
<td>Day + Was Attacked + In Contact with Enemy + PolyPRE</td>
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</tr>
<tr>
<td>Was Attacked + In Contact with Enemy + CloneRE + PolyPRE</td>
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</tr>
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</table>

**Response Variable: Moved (0/1)**

<table>
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<tr>
<th>Model tested</th>
<th>$\Delta AIC_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Contact with Enemy* + PolyPRE</td>
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</tr>
<tr>
<td>Mass + In Contact with Enemy** + PolyPRE</td>
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</tr>
<tr>
<td>In Contact with Enemy* + CloneRE + PolyPRE**</td>
<td>2.0</td>
</tr>
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</table>
Table 4.4. Data showing the total number of comparisons of average spread made between the two unpaired runs for each clone and the number of those comparisons with Bonferroni adjusted $P$ values less than 0.05. Similar data for each clone within each run comparing spread in paired versus unpaired runs (Right side of table).

<table>
<thead>
<tr>
<th>Clone</th>
<th>Unpaired</th>
<th>Run 1</th>
<th>Run 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total comparisons</td>
<td>$P &lt; 0.05$</td>
<td>Total comparisons</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
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</tr>
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<td>12</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>0</td>
<td>6</td>
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<td>0</td>
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<tr>
<td>16</td>
<td>4</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>17</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>
Figure 4.1. Photographs of the setup and execution of the experiment for Part 2 for the paired case. In paired case polyps from one clone were placed on one side of the cork (boxed polyps in a.), polyps from the other clone on the other side (unboxed polyps in a.). Photographs were taken to document the movement of polyps (b.).
Figure 4.2. Average distance between polyps for each clone for each run and each treatment. Solid lines indicate run 1, dashed lines run 2. Filled points indicate unpaired treatments, unfilled points are paired treatments.
CHAPTER 5

AN AGENT BASED MODEL OF BEHAVIOR IN THE SEA ANEMONE ANTHOPLEURA ELEGANTISSIMA

5.1. Introduction

The sea anemone *Anthopleura elegantissima* inhabits the west coast of North America (Hand 1955). It reproduces both sexually and asexually, the latter by longitudinal fission that results in large mats of genetically identical sea anemones known as clones (Hand 1955; Francis 1973a, 1973b). When these mats encounter one another in the field they form an anemone-free interclonal boundary (Francis 1973a; Ayre and Grosberg 2005; Ferrell 2005). These boundaries have been observed to not change (e.g. they are stable) for upwards of four years (Francis 1973a) despite differential polyp ability in agonistic encounters (Ayre and Grosberg 1996; Chapter 3).

How clones maintain stable boundaries despite unequal polyp fighting ability has been the subject of multiple studies (Ferrell 2005; Chapters 3 and 4). Each of these studies concludes that the formation of boundaries is a rare occurrence. They suggest that usually when clones encounter one another one of the two clones should dominate and eliminate the weaker clone. These previous studies conclude that it is the rare case that two clones are competitively equivalent such that a boundary forms and exists for an extended period of time.

In the literature there have been two hypotheses put forth to account for the stability of interclonal boundaries. The first, indirectly proposed by Ayre and Grosberg (1995), is that neighboring clones habituate to one another. These authors found that, with extended contact,
polyps reduced the number of aggressive actions they made toward non-clonemates. This hypothesis is similar to the ‘dear-enemy’ hypothesis that has been proposed for the formation and stability of territory boundaries in other species (Fisher 1954; see reviews by Ydenberg 1988; Temeles 1994). A second explanation for competitive equivalence between neighboring clones was put forward by Ferrell (2005). The author asserts, somewhat contrary to the results of Ayre and Grosberg (1996), that clones that share an interclonal boundary are more competitively equivalent than they would be had they been selected by chance from the local population. Neighboring clones have similar numbers of acrorhagi, the nematocyst laden structures that anemones use to damage non-clonemates in agonistic encounters, suggesting that interclonal boundaries are largely a stand-off between two similarly competitive clones. Ferrell (2005) looked at multiple species and came to similar conclusions regarding three species of Hydractinia that form similar such boundaries.

Both Ferrell’s (2005) and Ayre and Grosberg’s (1996) conclusions were largely supported by my work in Chapters 3 and 4 of this dissertation. Neighboring clones were often more similarly armed with acrorhagi than if clones had been paired by chance. Additionally, these clones also tended to differ in polyp fighting ability with one clone dominating the dyadic polyp interactions between neighbors. In addition to finding support for these prior studies the work in Chapters 3 and 4 may help explain the stability of interclonal boundaries. First, clones demonstrated consistent differences in movement rates (Chapter 3 and 4) in a variety of settings in both single and multi-polyp settings. Second, repeated interactions between clones appear to lead to habituation and more equivalent results in binary polyp encounters (Chapter 3). This is in line with the results of the habituation Ayre and Grosberg (1995) found when they initiated clone contact, but not fighting, between two clones.

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With these data we can test the effects of these variables on the mobility of the interclonal boundary using an agent based model for behavior in *A. elegantissima*. Here we build such a model for two neighboring clones in Netlogo (Wilensky 1999). We address only one of the hypotheses put forward by our work in Chapters 3 and 4, that differences between clones in the probability that a given polyp will move can offset differences in individual polyp fighting ability. As a result, the boundary between two clones is maintained. Although we restrict our model to a 1-dimensional environment we believe this biases our model against finding situations where the boundary is immobile and therefore not necessarily an inappropriate simplification (discussed further in our Discussion section). We specifically test the effects of varying movement rates in two situations that polyps may find themselves in that we have tested experimentally (Chapter 3) on the maintenance of an interclonal boundary. We find that varying movement rates between clones can mitigate competitive differences such that the interclonal boundary is, on average, stationary. The validity of our model is supported by our parameterization to the clone pairs tested in Chapter 3. In two of the three pairs we test, the measured values for the traits of interest (Chapter 3) fall within the region where we expect an interclonal boundary to be maintained according to our model output.

5.2. Materials and methods

5.2.1 The agent-based model

We built our agent-based model using Netlogo (Wilensky 1999) version 5.0. For simplicity, we limited our model to a single one-dimensional (1-D) row. In this scenario the boundary between the two clones occurs only between two polyps (one per clone) since the
world is only one anemone wide (Fig 5.1). We utilize this simplification to a 1-D world under the assumption that expanding the model to a second dimension would add unnecessary complexity. In two dimensions, polyps can interact between rows, thereby greatly increasing the total possible number of interactions. The assumptions we would need to make on how agonistic interactions scale to this level would, initially, hinder our inquiry into the effects of the variables we are concerned with. We therefore restrict our model to a 1-D world as an initial step in examining the system. Additionally, for simplicity and clarity we do not model death or reproduction.

In our model, polyps undergo a series of behavioral decisions over the course of a series of time steps. These decisions are diagrammed in Fig. 5.1. We view each time step as analogous to an incoming tide bout because previous work (Ayre and Grosberg 2005) found that most movement and agonistic behaviors occurred on an incoming tide. At the start of each time step each polyp performs a search behavior of the surrounding area. Because polyps in the second and third row of a clone are still able to touch and attack non-clonemates (Ayre and Grosberg 2005; Personal observation) we use a search radius of three body diameters. This search behavior matches what was observed by Ayre and Grosberg (2005), where polyps were found to engage in this behavior after a low tide. During the search behavior polyps note if any non-clonemates are within the three polyp diameter search radius. Whether or not polyps encounter non-clonemates determines which of two decision pathways in the model they will follow.

If the focal polyp does not detect any non-clonemates (e.g. it is alone or surrounded by clonemates) the only decision it has to make is whether to move or not. It does so, in a random direction so long as there is an available patch, with probability $M_s$ (see Table 5.1 for a list and description of variables in the model). For all clones we restrict polyp movement to a maximum
of two patches away from its initial location. Although polyps can move relatively large distances between tidal bouts this is unusual (Chapter 3; Personal observation). Additionally, in previous experiments, we found no significant difference between clones in the distance they moved in this scenario (Chapter 3).

If a focal polyp does encounter non-clonemates it must decide whether to engage or not in an agonistic encounter. For simplicity we combine decisions for all polyps involved in this decision. We do this because in the experiments (Chapter 3) that we used to parameterize this model, we did not record the data on which of the polyps initiated the encounter. Therefore, we parameterized on total contests engaged using measured values from Chapter 3. So in cases where only a single polyp from each clone is deciding to engage with another the engagement probability is the probability the polyps engaged in agonistic contests in Chapter 3. In cases in which multiple polyps interact with one another, we assume that engagements between pairs of polyps occur independently of one another and therefore we determine the probability that our focal polyp is part of an interaction as one minus the probability that it is involved in no encounters:

$$1 - (1 - E)^N$$  \hspace{1cm} (5.1)

Where \(E\) is the probability that a pair will engage and \(N\) is the total number of non-clonemates that the focal polyp detects.

When polyps engage, they win contests with probability \(W\). We make the assumption, based on our observations of this species in the lab, that losses are more important than wins and therefore polyps will behave as winners only when they do not lose (this is only the case when multiple polyps are involved, not dyadic encounters). Therefore the probability that a focal polyp wins all encounters it engages in is:
$W^{N_E}$

(5.2)

Where $N_E$ is the ceiling of $N^*E$. This is the number of non-clonemates that will, on average, engage given the total number of non-clonemates in contact with the focal polyp.

If the focal polyp does not win all contests then it is marked as having a loss. We determine the average number of wins the focal polyp has as:

\[ W * N_E \]

(5.3)

We then choose this number of the surrounding non-clonemates at random and give each of them a loss. These polyps may receive additional wins or losses when we switch focal polyps in the model. Similarly, we determine the average number of victories that non-clonemates experience interacting with the focal polyp and distribute wins randomly among the set of nearby non-clonemates that did not have a loss.

After we have determined the wins and losses experienced by all simulated polyps, we then determine whether or not polyps will move. When polyps lose they move away from the attack with probability $L_{mb}$. If polyps win, they move toward the opponent with probability $W_{mf}$ and away from the opponent with probability $W_{mb}$ (Fig. 5.1). $L_{mb}$, $W_{mf}$, and $W_{mb}$ are parameterized using the averages of the neighboring clones using the data from the antagonistic interactions performed in Chapter 3. If polyps encounter non-clonemates, but do not engage with them, they move with clone-specific probability $N_b$. Of the times they move in this situation, they move toward the non-clonemate some percentage of the time, which we parameterize using the movement experiments performed in Chapter 3. To limit the number of variables, we use the average across both clones of the probability a polyp moves toward the opponent and away. We use the 0.24 cm binary response variable dataset to parameterize.
When polyps moved we allowed them to move over clonemates but not over non-clonemates. We make these assumptions for a few reasons. We make the first assumption because polyps have been observed to retreat toward the center of their own clone (Ayre and Grosberg 2005) and the soft bodies of these organisms allow them to easily slide by one another. We assume polyps cannot move over non-clonemates for two reasons. The first is that allowing non-clonemates to move past one another would disrupt the integrity of the clone given our 1-D model. In a true two dimensional model this would be less of an issue as an invading polyp would be clearly outnumbered by polyps of the invaded clone. The invading polyp would likely be killed through repeated attacks (as observed by Ayre and Grosberg 2005; Personal observation). As we do not model either multiple rows or death in our model, allowing non-clonemates to skip over one another would be less representative of the natural case. Second, it allows us to clearly demarcate the location of an interclonal boundary at all points in time of the simulation. If polyps were able to leapfrog non-clonemates, it would be unclear how an interclonal boundary could be defined given the 1-D nature of the model.

5.2.2 Simulation runs and analysis

We performed our simulations in a 33 by 1 arena using eight polyps per clone (Fig. 5.1). We positioned polyps such that they were regularly spaced. Each clone was confined to one side of the arena at setup. The two nearest polyps from each clone had only a single empty patch between them. We ran each individual simulation for 100 time steps at the end of which we determined the location of the interclonal boundary as the midpoint between the two nearest polyps of each clone. For each set of parameters we replicated the simulation 100 times. We compared the final interclonal boundary location to the initial location using a Student’s T-test.
for unequal variances \((df = 198)\). Specifically, we observed the effects of three parameters. The probability that one of the clones wins a contest, \(W\); the probability that polyps move when they encounter a non-clonemate but do not engage with them, \(N_b\); and the probability that the polyps move when there are no non-clonemates around, \(M_s\). We performed these experiments for three distinct cases. Each case was parameterized to match a pairing of clones, 1-2, A-B or C-D, that we investigated previously (Chapter 3).

5.3. Results

In all three pairs of neighboring clones that we modeled we found instances where there was no evidence to suggest the interclonal boundary moved over the course of the 100 simulations performed (Fig. 5.2, 5.3). This was the case for varying values of the win probability \((W)\) for both the probability a polyp moves in response to itself \((M_s)\) (Fig. 5.2) and the probability they move in response to contact by a non-clonemate \((N_b)\) (Fig. 5.3). Across all three pairs of clones, a consistent interclonal boundary occurred for a wider range of parameter values when varying \(N_b\) than for varying \(M_s\) at a given value of \(W\) where such outcomes were present. However, the appearance of a consistent boundary occurred for a wider range of \(W\) when varying \(M_s\) across all three pairs. In all cases the clone with the lower \(W\) value had higher \(M_s\) values than its opponent when the boundary remained stationary.

5.3.1 Clones 1 and 2

In this pairing of clones from Chapter 3 we found that clone 1 won 25% of finished contests among only warrior types and 36% of contests across all dyadic pairs tested. As shown
in Fig. 5.2 across this range of $W$ we found a wide swath of parameter values where the interclonal boundary was motionless. For this range of $W$ the majority of this swath occurred within the measured range of values of $M_i$, for each clone. This swath also clearly demarcated the border between parameter settings that led to expansion by clone 1 and contraction of clone 2 and vice versa.

This was not the case when we fixed $M_i$ and vary $N_b$ (Fig. 5.3). Here we only show the upper range of $W$ as we found lower values resulted in the interclonal boundary shifting in favor of expansion by clone 2 for all combinations of $N_b$. Unlike when we varied $M_i$, there was not a clear pattern. Parameter values resulting in no movement by the interclonal boundary appeared to occur randomly for any given setting of $W$. Additionally, the range of $W$ for which we found conditions such that the boundary did not move was very small. Although we limited ourselves to the upper limit of $W$ that we observed experimentally, we found a variety of parameter settings that fell within the range of $N_b$ measured for each clone that resulted in the interclonal boundary remaining motionless.

5.3.2 Clones A and B

Our results for this clone pairing mirrored our results for clones 1 and 2. Clone A was measured to win between 50% (warrior-warrior contests) and 70% (all contests) in Chapter 3. Varying $M_i$ across these values of $W$ we again found a large swath where the boundary was not found to move (Fig. 5.2). This remained relatively consistent across the range of $W$ tested. This swath was well within the values of $M_i$ we had previously found for these clones. When we varied $N_b$ we found similar results for the upper limits of $W$ that we tested, but dominance by clone B for lower values (Fig. 5.3). There did appear to be a clearer pattern for $N_b$ here than for
clones 1 and 2. Here it appeared that lower values of \( N_b \) relative to the opponent lead to increased competitive ability.

5.3.3 Clones C and D

In no case did our results suggest that the clones could maintain an interclonal boundary. In our experimental inquiries clone C won a maximum of only 5% of encounters with a clear victor. We purposely extended our range here to match the range of \( W \) we used for the other two clone pairings. When we varied \( M_s \) we found that settings where the boundary was immobile and within the range of \( M_s \) that we had measured only occurred for extremely high values of \( W \) (\( W = 0.25 \))(Fig. 5.2). And even for this value of \( W \) the region where this immobility would occur was very small. The results for when we varied \( N_b \) are similar (Fig. 5.3). For slightly more reasonable values of \( W \) our observed ranges of \( N_b \) included regions where the border was stationary.

5.4. Discussion

Our results demonstrated that members of neighboring clones that experience disadvantages in dyadic polyp encounters may mitigate these differences through the usage of different movement rates compared to their neighboring clone. This result is one explanation for how clones of \( A. \) elegantissima can be competitively equivalent despite differences in agonistic ability between polyps (Ayre and Grosberg 1996; Chapter 3). It is the first explanation for this equivalence that we are aware of that takes into account the observed differences in polyp fighting ability and incorporates these differences into the explanation for competitive equivalence. Ferrell (2005) argued that such competitive differences at the polyp level were
insignificant compared to the general equivalence of neighbors compared to clones chosen at random. However, he never actively measured individual agonistic ability and instead used a proxy for agonistic ability (acrorhagi counts). Not all values of $W$ we tested, especially those extremely different from 0.5, had regions where any pairing of $M$ or $N$ resulted in group equivalence (Fig. 5.3). This result supports Ferrell's (2005) hypothesis that clones must be at least somewhat equivalent at the polyp level for there to be group level equivalence.

In two of the three cases that we model, clone pairings 1-2 and A-B, our simulation results demonstrated occasions where the interclonal boundary remained stationary. These results occurred for parameter values of $N$, $M$, and polyp fighting ability within the measured ranges for these clones (Chapter 3)(Fig. 5.2, 5.3). However, for the clone pairing C-D this was not the case. The interclonal boundary remained stationary, on average, only for values of $M$ outside our observed range for these clones (Fig. 5.2)(Chapter 3).

It is unclear why our model fails to explain the maintenance of an interclonal boundary between clones C and D (but see further discussion below). It does seem that this pairing is anomalous compared to the other two. As noted in Chapter 2, this pairing does not follow the same patterns as clone pairings 1-2 and A-B. In those pairings the clones that dominated dyadic polyp on polyp interactions (clones 2 and A) consistently moved less than their neighbor. This was regardless of circumstance (clonemate or non-clonemate contact). For clones C and D, the latter not only dominated contests but had higher movement rates in the presence of a non-clonemate compared to clone C. In the presence of clonemates, C moved a greater proportion of the time than did D in the same environment.

We believe our model gives valid insight into clone behavior despite its simplicity. Despite restricting our model to a single line of interacting polyps and ignoring the effects of
repeated contact between clones our model showed agreement with observations for two of the three modeled clone pairings (1-2, A-B; Fig. 5.2, 5.3). We imagine that multiple rows of polyps that form full clonal mats will only heighten the maintenance of the boundary. As polyps move toward a competitor clone they will encounter an increasing level of resistance as polyps within the model are not limited to interacting only with other polyps in their row. Their search area is a circle surrounding their location allowing them to interact with many other polyps. This means that in a multiple row setting invading polyps will face resistance from not only the polyps in their row but from those in surrounding rows as well. Our restriction to a 1-D line of polyps thereby biases the model against finding clonal equivalence between neighbors.

Furthermore, in our model we do not consider habituation between neighboring clones. This habituation was found to occur with regard to initiation of agonistic behavior in response to touch by the tentacle of a non-clonemate by Ayre and Grosberg (1995). Additionally, we found differences in the outcome between first and second agonistic encounters in repeated agonistic interactions (Chapter 3). These behaviors should serve to limit interactions at interclonal boundaries and lead to greater competitive equivalence at the polyp level. Again, our simplification here biases our model against finding regions in parameter space where the interclonal boundary is stationary. Implementation of these variables should allow for the interclonal boundary to remain stationary for a greater range of parameter values than we found here.

Another simplifying assumption we make is that we do not consider differences in movement behavior between polyp types dependent on their type (see Table 1.1). Although we did not find that the task differentiation of polyps contributed significantly to differences in movement behavior across clones in previous work (Chapter 3) it may be that our sample size
was not sufficiently large to detect this effect. Since we tested the range of movement probabilities that we found in this prior work we find it unlikely that modeling clone behavior more finely would change our results here.

While our model worked well despite our simplifying assumptions, it did have its shortcomings as highlighted by the C-D clone pairing and the relatively narrow range of parameter space where clonal boundaries would appear to be maintained (Figs. 5.2, 5.3). Here we provide a series of hypotheses for why our model failed for clones C and D and give some discussion on the second point. First, our range of measured values for these clones may not be accurate, although this seems unlikely given the match between our results and Chapter 3 for the other clone pairings. Second, our simplified model did not account for the effects of multiple interactions or multiple rows of polyps. While we have strong reasons for tackling the system in a 1-D method initially, the effect of these variables is likely to be significant. As discussed, it is likely that these variables would promote group equivalence rather than oppose it. This would allow for a larger range of parameter values to lead to group level equivalence, thereby increasing the match between our model and our parameter estimates. Future modeling work should focus on exploring these hypotheses. Lastly, it is possible that the C-D boundary, as observed in the intertidal, is not stationary. Since we did not perform extended observations we cannot comment on whether this may be true. It is possible that the boundary is slowly moving in favor of clone D’s expansion. However, this explanation also seems unlikely as the boundary itself would be unlikely to form between the two clones initially if such dominance was present. If none of the above proposed hypotheses is correct and can account for group equivalence, it would appear that there is some aspect of this particular interaction that we are missing.
Our model is unique in that it offers an explanation, independent of the “dear-enemy” hypothesis (Fisher 1954; see reviews by Ydenberg 1988; Temeles 1994), for how two warring groups can come to a stalemate despite consistent supremacy of individual agents of one group over the other. This occurs through variation in movement behavior between the two groups in response to contact with their own group and the opponent. This supports the hypothesis we first proposed in Chapter 3 for group equivalence. Groups that are dominated at the agent level must move more often when in contact with self, and less often in response to contact with an opponent in order to overcome the advantage afforded to their opponent. Future work specific to this species should focus on incorporating the effects of multiple rows, and repeated interactions and should test the stability of the boundary in a more formal analysis. Additionally, given the generality of this model, it makes predictions that can be tested in other taxa and situations where groups demonstrate stalemates despite consistent competitive differences among constituent agents.
Table 5.1. Table showing the model variables and their definitions.

<table>
<thead>
<tr>
<th>Model variables</th>
<th>Description</th>
<th>Investigated?</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_s$</td>
<td>Probability a polyp moves when not in contact with a non-clonemate</td>
<td>Yes</td>
</tr>
<tr>
<td>$N_b$</td>
<td>Probability a focal polyp moves when in contact with a non-clonemate but there was no agonistic encounter. This is the also the sum of $N_{bb}$ and $N_{bf}$, the probabilities that a focal polyp moves away and toward, respectively, a non-clonemate in this scenario</td>
<td>Yes</td>
</tr>
<tr>
<td>$E$</td>
<td>The probability that a polyp from both neighbors will engage in an agonistic encounter when in contact with one another</td>
<td>No</td>
</tr>
<tr>
<td>$W$</td>
<td>Probability a polyp wins a dyadic polyp encounter against its neighbor</td>
<td>Yes</td>
</tr>
<tr>
<td>$L_{mb}$</td>
<td>Probability a losing polyp moves away from an opponent</td>
<td>No</td>
</tr>
<tr>
<td>$W_{mf}$</td>
<td>Probability a winning focal polyp moves toward an opponent</td>
<td>No</td>
</tr>
<tr>
<td>$W_{mb}$</td>
<td>Probability a winning focal polyp moves away from an opponent</td>
<td>No</td>
</tr>
</tbody>
</table>
Figure 5.1. Flow chart of the decision tree polyps face and a photograph of the initial starting position of polyps in simulations. A) Flow chart of the model decision tree for a single time step. B) Snapshot of the initial position of polyps at the start of the model in Netlogo. Arrows indicate the midpoint between the two clones. Polyps to the left of the arrows are of one polyp type, to the right the other polyp type.
Figure 5.2. (Following page) Results of parameter sweeps for varying $M_s$ with values of $N_b$ fixed to the values measured in Chapter 3. Clone pairings are listed at the top. The win probability ($W$) of the first clone in the pairing is in the upper right of the panel and increases as you move down each column. Dashed and dotted boxes indicate the range of values for measured in that clone experimentally. Interior dashed lines indicate the average of the trait across all polyp types. Squares indicate where there was no significant difference between the final position of the interclonal boundary from its initial position ($P > 0.05$). Diamonds and triangles indicate cases where the interclonal boundaries final position varied from its initial position ($P < 0.05$). Diamonds are cases where the first clone listed (1, A or C) moved forward and triangles are cases where the second clone (2, B or D) moved forward. Each point is the result of 100 simulations for those values of $M_s$. 
Figure 5.3 (Following page) Results of parameter sweeps for varying $N_b$ with values of $M_t$ fixed to the values measured in Chapter 3. Graphs and symbols are the same as in figure 5.2.
6.1. Introduction

Division of labor (DoL) generally refers to collectives in which individual agents act as specialists and perform specific tasks. The actions of specialists performing complimentary tasks contribute toward a common goal. DoL has been studied in a great variety of biological systems, including humans (Smith, 1763), wolves (Murie, 1944), green algae (Kuhn, 1971), organ systems (Buss, 1987), slime molds (Bonner, 1988), social insects (Beshers and Fewell, 2001) including honeybees (Johnson, 2003) and wasps (Naug and Gadagkar, 1999; Wenseleers et al., 2003), birds such as noisy miners (Arnold et al., 2005), sea anemones (Ayre and Grosberg, 2005), and bottlenose dolphins (Gazda et al., 2005). In the theoretical literature, DoL has been used to refer to systems with complete division of labor, where every individual is a specialist. Here we use “incomplete DoL” to refer to the coexistence of generalists and specialists at evolutionary equilibrium. Incomplete DoL presents a theoretical challenge. How can generalists who must pay an extra cost for their capacity to perform more than one kind of task persist in the presence of specialists who pay no such inefficiency penalty.
To explore conditions favoring such coexistence, we modify Wahl’s (2002a, b) landmark model. Inspired by coviruses that replicate only when complementary virions are present in a host cell, Wahl envisioned a system of specialists and generalists (and, in one version of the model, parasites). She considered well-mixed populations of individuals associating in randomly formed groups each generation, where reproduction depends on the agent’s group successfully completing two complementary tasks. By assumption, generalists flawlessly recognized specialist partners and always responded by performing the correct, complementary task. Generalists were assumed to pay either just the cost of the task performed (marginal cost) or the full combined cost of both tasks (fixed cost). Wahl concluded that fixed costs drive evolution toward complete DoL.

Here we explore conditions favoring incomplete DoL. We do so by studying the impact of relaxing two key assumptions. First, recognizing that all adaptive decision processes are intrinsically subject to error (Dukas, 1998), we relax the assumption that generalists have complete information and are perfectly rational (cf. Kokko, 2002; Hamilton, 2004). Second, while recognizing that generalists may bear some intrinsic cost for their capacity to perform more than one type of task, we relax the assumption that generalists must pay the full fixed cost (i.e., as if they performed both tasks) for their multitasking flexibility. By increasing biological realism in these two ways, we reveal plausible conditions under which incomplete DoL could emerge, where the types of co-existing agents outnumber the tasks that must be performed for the group to be successful (see analogous work on species coexistence e.g., Abrams 2006). We show that coexistence of generalists and specialists can occur even though generalists must pay a partial inefficiency penalty, and even though they may often perform the wrong task.
6.2. Model and results

The system we model resembles nest construction in *Metapolybia* wasps (Karsai and Wenzel, 2000). Successful construction depends on the completion of three main tasks: water collection, pulp collection, and building. It appears that some specialists collect water, other specialists build, and generalists can perform any of the three tasks. Other individuals appear to enter an idle state and so complete no task. For building to be successful, individuals must act in pairs to transfer either pulp or water to builders. Whom each wasp encounters appears to be random. Because knowledge in the colony is decentralized, generalists must flexibly decide which task to perform (e.g., to collect pulp or to build; Karsai and Wenzel, 2000). Feasibly, a generalist can make the wrong choice. By performing the non-optimal task, she can hurt the colony’s prospects. Using this system as inspiration, we extend Wahl’s (2002a, b) model.

Consider four types of agent: 1) specialists that perform task 1, 2) specialists that perform task 2, 3) generalists that can perform either task 1 or 2, and 4) parasites (considered later) that do not perform any task. Agents coalesce into groups composed of *n* individuals selected at random from the population. A group is successful, and agents therefore receive reproductive benefit *b*, provided at least one agent completes task 1 and at least one other agent completes task 2. Specialists always complete their task and pay the associated cost. Generalists pay that cost plus an extra cost, Δ, for their ability to perform either task. Parasites pay no cost.

Departing from Wahl (2002a, b), we assume: 1) each generalist can perform one task or the other, but not both, at any given time; and 2) generalists do not necessarily correctly identify other agents in the group and then perform the correct (complementary) task. In discrete time, the frequency *p* of agent type *i* in the next generation *t*+1 is given by:
\[ p_{i,t+1} = \frac{p_{i,t} \omega_{i,t}}{\sum_{i=1}^{t} p_{i,t} \omega_{i,t}}, \tag{6.1} \]

where \( \omega_{i,t} \) is the fitness of agent type \( i \) in generation \( t \) (Wahl, 2002a; Hofbauer and Sigmund, 2003). Equilibria occur when \( p_{i,t+1} = p_{i,t} \) for all agent types, which have identical fitnesses at these equilibrium frequencies.

### 6.2.1. Generalists pay extra costs and make mistakes (no parasites)

Here we explore how errors and extra costs impact generalist-specialist coexistence. (In Appendix B, we consider the specific case where generalists act without any knowledge about task types performed by other agents and pay no extra cost for their multitasking capability [i.e., \( \Delta = 0 \)].) We ask whether generalists can coexist with specialists when generalists must pay some extra cost for their flexibility and sometimes perform the wrong task. These conditions conspire to reduce generalist fitness, making coexistence less likely.

### 6.2.2. \( n=2 \)

We start with the case where \( n = 2 \). Fitness expressions are given in Table 6.1. The fitness for each specialist is the net benefit that accrues if successful (i.e., per agent reproductive benefit \( b \) from group success minus performance cost \( c_i \)) multiplied by the probability of encountering either a complementary specialist or a generalist that acts correctly by performing the complementary task. We assume reproductive value is zero for agents in groups that fail. We represent accuracy of information processing and decision making for generalists by parameter, \( \zeta \), which takes values ranging from 0 (generalist always acts incorrectly) to 1.
(generalist is perfect and the group invariably succeeds). A \( \zeta \)-value of 0.5 indicates generalists err half the time, on average. Wahl (2002a) discovered that in the special case where \( \zeta = 1 \) and \( \Delta = 0 \) (marginal costs), the system at equilibrium is composed strictly of generalists and the specialist performing the cheaper of the two tasks. Provided the benefit \( b \) exceeds the cost of either task, frequency of the specialist type present increases as the difference in costs between the two tasks increases. When the cost differential is equivalent to the reproductive benefit \( b \), this specialist type drives generalists to extinction. The population itself will then go extinct as it has lost the ability to complete the second task (Wahl 2002a). Here we do not focus on relative cost differences, but rather explore the effects of varying \( \zeta \) and \( \Delta \) per se.

The fitness for each generalist is the net benefit multiplied by probability of encounter summed across all types of encounter (Table 6.1). For encounters with specialists, the probability of meeting each type of specialist is multiplied by \( \zeta \) and by the net benefit (i.e., \( b - c_i - \Delta \), where \( \Delta \) is the extra cost generalists must pay). For encounters with generalists, we could model the interactions and payoffs in various ways. We consider two scenarios. First, we arbitrarily imagine that when generalists A and B encounter one another, both agents can discern which task the other agent plans to perform with probability \( \zeta \). We refer to this as method 1. In some interactions, generalist A might get it wrong and read that generalist B will perform task 1; if generalist B also gets it wrong, then both perform task 2 and the group fails. We assume, however, that if generalist B gets it correct, it counters generalist A’s mistake and the group succeeds. Under these suppositions, for a pair of generalists to succeed, only one of them needs to act correctly. This should happen with probability: \( 1 - (1 - \zeta)^2 \). If they both get it correct, the group obviously also succeeds. Second, we also consider the possibility that generalist-generalist pairs succeed in \( \zeta \) percent of interactions. We refer to this as method 2.
For \( n = 2 \) and method 1, we first solve analytically for internal stable equilibria (i.e., not on the edge of the simplex). The steady-state equations are (Hofbauer and Sigmund, 2003; Nowak, 2006):

\[ 0 = p_1 (\omega_i - \sum p_j \omega_j) \Rightarrow \omega_i = \omega_2 = \omega_g. \]

We begin by setting the fitnesses of type 1 and 2 specialists (from Table 6.1) equal, \( \omega_1 = \omega_2 \).

Then, solving for \( p_2 \) yields:

\[
 p_2 = \frac{(p_1 + \zeta p_g )d_2 - \zeta p_g d_1}{d_1}, \tag{6.2}
\]

where \( (b - c_i) = d_i \). We then set the fitnesses of type 1 specialists and generalists equal to each other, \( \omega_1 = \omega_g \). Substituting for \( p_2 \) and solving for \( p_1 \) gives:

\[
 p_1 = p_g \left[ \frac{\zeta^2 d_2 (d_1 - \Delta) / d_1 - \zeta^2 (d_1 - \Delta) + [1 - (1 - \zeta)^2] [(d_1 + d_2) / 2 - \Delta] - \zeta d_2}{d_2 - \zeta (d_2 - \Delta) - d_2 (d_1 - \Delta) \zeta / d_1} \right], \tag{6.3}
\]

\[
 = p_g \phi.
\]

Using the relationship \( p_1 + p_2 + p_g = 1 \), we can solve for \( p_g \) using equations 2 and 3:

\[
 p_g = \frac{1}{1 + \phi + ((\phi + \zeta)d_2 - \zeta d_1) / d_1}. \tag{6.4}
\]

Note that this equation does not involve any frequencies of the various types and therefore \( p_1 \) and \( p_2 \) are dependent on \( p_g \). We can therefore write \( p_2 = 1 - p_1 - p_g \) instead of (6.2). The equations do not constrain the frequencies to the interval \([0,1]\) and therefore find internal equilibria only.
These equilibria equations for method 2 remain the same except for the numerator of $\phi$. In the numerator, the $[1 - (1 - \zeta)^2]$ term is replaced by $\zeta$ for method 2. These areas or internal equilibria are indicated for both methods in Fig. 6.1 by the red overlays.

To solve for equilibria on the edge, we use the same method used above. That is, the $\omega$'s are set equal to each other and we then use the relationship that the two frequencies must sum to 1. For the case where generalists are absent:

$$\omega_1 = \omega_2$$

$$p_2d_1 = p_1d_2$$

$$p_2d_1 = (1 - p_2)d_2$$

$$p_2 = d_2/(d_1 + d_2)$$

For the parameter values of $d_1=0.8$ and $d_2=0.4$, this yields $p_2=1/3$ and $p_1=2/3$.

Similarly, when one of the specialists is missing (say specialist 2), we have the following expressions for method 1:

$$\omega_1 = \omega_g$$

$$p_g\zeta d_1 = p_1\zeta (d_2 - \Delta) + p_g\{1 - (1 - \zeta)^2\}(d_{avg} - \Delta)$$

$$p_g = (d_2 - \Delta)/(d_1 + (d_2 - \Delta) - \{1/\zeta\}^2\{1 - (1 - \zeta)^2\}(d_{avg} - \Delta),$$

where $d_{avg} = (d_1 + d_2)/2$

Again, for method 2, the $[1 - (1 - \zeta)^2]$ term is replaced by $\zeta$. For the case involving specialist 2 and the generalist, the resulting equations are the same, with $d_2$ replacing $d_1$.

The trivial cases occur at the vertices of the simplex where only one type remains. These vertices, of course, also act as equilibrium points, but only in the case where $p_g=1$ would the
groups succeed in completing the complimentary tasks. The other two cases would result in global extinction because groups would be able to complete just one of the two tasks and so would fail.

In addition to the above analysis, we ran a numerical simulation (Fig. 6.2). For each \( \zeta-\Delta \) pair, we ran the model 500 times with initial population frequencies chosen at random. We let the model run to equilibrium using the method 1 fitness expressions (Table 6.1) or the altered generalist fitness equation for method 2. We varied \( \zeta-\Delta \) by increments of 0.01. If a type’s equilibrium frequency was less than 0.00005, it was considered to be nonexistent. We used the following parameter values: \( b=1 \), \( c_1=0.2 \), and \( c_2=0.6 \). Given these values, we considered scenarios ranging from the fixed-cost case, \( (c_1+c_2)/2 \) (i.e., \( \Delta=0.4 \)), where generalists must pay costs as if performing both tasks to a synergistic case where generalists pay less than the ordinary cost of performing the task (\( \Delta=-0.1 \)). When \( \Delta=0 \), generalists pay for the completed task only, which corresponds to the marginal-cost case.

6.2.3. Results for \( n=2 \)

We found two regions of internal equilibria. One region occurred for large \( \zeta \) and intermediate \( \Delta \) denoted in Fig. 6.1A by the red overlay to the right hand side of the graph. We subjected these points to linear stability analysis and found all real parts of their eigenvalues to be negative, which indicates these points are locally stable and attracting. This was confirmed in simulation by attraction to these points within this region (black starred points in Fig. 6.1A). The second region of internal equilibria occurred for smaller values of \( \zeta \) and a range of \( \Delta \), \([-0.15, \sim 0.15]\]. Local stability analysis of the eigenvalues for these internal equilibria revealed these points were unstable. The system moves away from these points to one of two edge equilibria.
Frequently they move to strict (complete) DoL, but occasionally they move to the generalist-type 1 specialist equilibrium, which we refer to as “partial DoL.” This region is shown in Fig. 6.1 by the circles, indicative of multiple equilibria occurring in the numerical simulation.

The analytic solution and numerical simulation produced matching equilibrium points for stable and unstable internal equilibria. Likewise, they also produced matching edge equilibrium points for strict (complete) and partial DoL. Our analytic solutions and linear stability analyses were limited to this \( n=2 \) case. Analytic solutions for larger group sizes were not pursued, but we note that none of our numerical simulations, regardless of group size, produced any complex dynamics such as oscillations or chaotic solutions. Taken together, these observations suggest that our simulation results for \( n>2 \) provide meaningful, provisional insights.

A generalist-only equilibrium emerged for small \( \Delta \) combined with \( \xi \) around naivety (see Appendix B for a discussion on strict naivety at \( \Delta=0 \)), apparently reflecting the fact that pairs of generalists are likely to succeed (with >75% probability). The probability of success is twice as high for generalist-generalist pairs as for generalist-specialist pairs, giving generalists paying small extra costs an advantage over specialists.

The left side of the graph changes dramatically when generalist-generalist pairs are modeled using method 2 and succeed only \( \xi \) percent of the time (Fig. 6.1B). The region of generalist-only equilibria disappears, as does the region of unstable equilibria for lower levels of \( \xi \) above \( \Delta=0 \). The right hand side of the graph shows a region of stable internal equilibria occurring over a larger \( \xi-\Delta \) range, and a reduced region of partial DoL. In fact, it appears from this graph that coexistence of all three types is a phase transition between strict and partial DoL.

6.2.4. \( n>2 \)
Analytic solutions and fitness expressions are less straightforward for \( n > 2 \). As with the \( n = 2 \) case, the difficulty lies in defining what it means for a generalist to act correctly. Here we model generalists as being able to “see” only one other group member, selected at random. The generalist then performs the complementary task with probability \( \zeta \). If this generalist fails to act correctly, the group may still succeed through the actions of other members. The fitness for specialists is the probability that the rest of the group is not composed entirely of the same specialist type or generalists acting incorrectly multiplied by the appropriate net benefit.

Because generalists are able to see one other member of the group, it is possible for a group of generalists to see only each other (e.g., two generalists see each other). Because of this, these two generalists could get it wrong relative to one another, but in so doing they could perform the complementary task to that performed by a specialist in the group. That is, the group can succeed (e.g., the two generalists both play type 2 but there is a type 1 specialist also in the group). We kept this term for when \( n = 3 \) and found that it had no appreciable impact on the outcome. We therefore ignore its presence for larger group sizes. The fitnesses for the two specialist types are:

\[
\omega_1 = \frac{1 - (p_1 + (1 - \zeta) p_g)^{n-1}}{d_1} 
\]

and

\[
\omega_2 = \frac{1 - (p_2 + (1 - \zeta) p_g)^{n-1}}{d_2}. 
\]

(6.5)

(6.6)

Derivation of expressions for generalist fitness is more complicated, so we begin by defining the following abbreviations:

\[
p_1 + (1 - \zeta) p_g = p_1 p_g
\]

\[
p_2 + (1 - \zeta) p_g = p_2 p_g
\]

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\[(b - c_1 - \Delta) = \Delta_1\]
\[(b - c_2 - \Delta) = \Delta_2\]

Incorporating these definitions, the first component of generalist fitness is given by:

\[\omega_{G_1} = p_1\zeta\Delta_2 + p_2\zeta\Delta_1 + p_1(1 - \zeta)[1 - (p_1, p_2)_{g}^{-2}]\Delta_1 + p_2(1 - \zeta)[1 - (p_2, p_2)_{g}^{-2}]\Delta_2. \] (6.7)

Here a generalist can succeed if it sees a specialist and chooses to perform the complementary task and pays the cost of that task. This is encompassed in the first two terms. The last two terms represent cases in which the generalist sees a specialist and acts incorrectly but then receives the benefit because another individual in the group completes the correct task.

When a generalist sees another generalist, the second generalist can either see the first generalist with probability \(1/n\) (called \(\Psi\) here) or it can see a different individual in the group with probability \((n-1)/n\) (called \(\Omega\) here). The next component of generalist fitness represents the former of these two possibilities:

\[\omega_{G_2} = p_1[1 - (1 - \zeta)^2]\Psi \alpha + p_2(1 - \zeta)^2 \Psi \left\{ \frac{1}{2} \left[ 1 - (p_1, p_2)_{g}^{-2} \right] \Delta_1 + \frac{1}{2} \left[ 1 - (p_2, p_2)_{g}^{-2} \right] \Delta_2 \right\}. \] (6.8)

When two generalists both see each other, they succeed provided one of them acts correctly and they pay on average half the total cost of both tasks plus \(\Delta\) (called \(\alpha\) here). This situation is expressed in the first term of (6.8). If they see each other and both act incorrectly, we assume they both act as type 1 or as type 2 with equal probability. Yet, the group will still succeed if another agent, whether a specialist of the other type or a generalist acting correctly, performs the complementary task. This possibility is expressed by the second term.

When a generalist sees another generalist but this second individual sees a different member of the group, the first generalist may act correctly depending on what the second generalist sees:
\[ \omega_{G_j} = p_g \zeta \Omega \left[ \cdots + \left( \cdots \right) \right] + \left[ \cdots + \left( \cdots \right) \right] . \tag{6.9} \]

The \( p_g \alpha' \) expression is an approximation for group sizes larger than 3. This approximation is explained in Appendix C.

Finally, the first generalist may act incorrectly when the second generalist sees a different group member:

\[ \omega_{G_i} = p_g \left( 1 - \zeta \right) \Omega \left\{ \left[ \cdots \right] + \left[ \cdots \right] \right\} . \tag{6.10} \]

Expressions ending with \( \alpha' \) and \( \alpha'' \) are approximations. The sum of these components gives the fitness for generalists in large groups when paying extra cost \( \Delta \) and acting with probability \( \zeta \) for group success:

\[ \omega_G = \omega_{G_1} + \omega_{G_2} + \omega_{G_3} + \omega_{G_4} . \tag{6.11} \]

To simplify, we follow Wahl’s (2002a) example and do not scale the benefit \( b \) with task completion. That is, the benefit to individuals in the group is the same regardless of whether each complementary task is completed just once or more than once by the group. (A scaled version can be found in Appendix D.) As with the \( n=2 \) case, numerical solutions were obtained for \( \zeta-\Delta \) combinations, varying \( \zeta \) from 0.5 to 1 and \( \Delta \) from -0.15 to 0.4, both by increments of 0.01. If a type’s equilibrium frequency was less than 0.00005, it was considered to be nonexistent.

6.2.5. Results for \( n>2 \)

Specialist-only equilibria dominate for high levels of \( \Delta \) for all group sizes (Fig. 6.2). As group size \( n \) increases, this regime dominates the \( \zeta-\Delta \) parameter space. As this occurs, the regions of incomplete and partial DoL shrink. As \( n \) increases so too does the probability of group success.
Due to the lower cost of their task, type 1 agents dominate the population, which increases the likelihood that type 2 individuals will succeed and generalists with large \( \zeta \) will play the type 2 role. Provided generalists pay some additional cost, type 2 specialists become increasingly favored as \( n \) increases, shrinking the region of partial DoL. This corroborates Wahl’s (2002a) observation that increasing group size drives the evolution of complete DoL.

A region of incomplete DoL emerges for values of \( \zeta \) only moderately above the chance level of 0.5 combined with intermediate values of \( \Delta \). Under these conditions, neither generalists nor type 2 specialists are sufficiently advantaged to drive the other to extinction. In this region, the effects of \( \zeta \) and \( \Delta \) appear to balance each other: \( \Delta \) is not so high as to cost the generalist much in the presence of type 2 agents and \( \zeta \) is sufficiently large that generalists typically act correctly. Thus, generalists can coexist with both types of specialist.

For large \( n \), the numerical methods identified the region of coexistence for large \( \zeta \) as well as a region of multiple stable equilibria for smaller levels of \( \zeta \). By contrast with the \( n=2 \) case, this latter region probably reflects the existence of an unstable internal equilibrium per \( \zeta-\Delta \) pair. The system is then driven away from this point to one of the edges, creating the multiple stable equilibria.

When \( \zeta \) is large and \( \Delta \) is small, generalists drive to extinction the specialist doing the more expensive task (type 2). This occurs because generalists sometimes perform the cheaper task when paired with other generalists, which lowers their average payment. Meanwhile, the other specialist (type 1) enjoys favorable prospects for success because it is likely to be paired with a generalist that acts correctly.

Average group-level fitness (data not shown) was high in this region where generalists were accurate in their task choice (large \( \zeta \)) and paid only small extra costs (small \( \Delta \)) for their
flexibility. It was highest in the region favoring incomplete DoL, intermediate in other regions favoring persistence of generalists, and lowest in the region favoring complete DoL. In general, groups with generalists had relatively high fitness, reflecting the advantage of the generalist’s multitasking capability.

For larger \( \zeta \), the generalist-only equilibrium found in the \( n=2 \) case disappears. Type 1 specialists are able to invade in the larger-group setting because generalists perform the correct task sufficiently often.

6.2.6. Generalists pay extra costs and make mistakes, with parasites present

When parasites are present, they do little to change the fitness of the non-parasitic members of the population. When a generalist sees a parasite, we assume the generalist learns nothing and so randomly chooses to perform either task 1 or task 2. Due to this assumption, the probability \( (1-\zeta) \) does not apply. This effect is most clearly seen in the fitnesses of specialists:

\[
\omega_1 = \left[ 1 - \left( p_1 + (1-\zeta)p_g \Psi + (1-\zeta)p_g \Omega(1-p_p) + 0.5 p_g \Omega p_p + p_p \right)^{-1} \right] d_1. \tag{6.12}
\]

and

\[
\omega_2 = \left[ 1 - \left( p_2 + (1-\zeta)p_g \Psi + (1-\zeta)p_g \Omega(1-p_p) + 0.5 p_g \Omega p_p + p_p \right)^{-1} \right] d_2. \tag{6.13}
\]

Here, \( \Psi \) and \( \Omega \) represent probabilities that generalists perform the same task as the specialist of interest (see 6.2 for explanations of these terms). Here each specialist fails if the remaining members of the group act as that specialist type or as parasites.

Parasite fitness is the (gross) benefit multiplied by the probability that at least one agent performs task 1 and one performs task 2 within the group:
\[ \omega_p = b\left\{1 - e_1^n - e_2^n + p_n \right\} + p_g \left(1 - \zeta \right) \left(1 - p_p \right)^n + 0.5 p_g \Omega p_p + 0.5 p_g \Psi + p_p, \]

where

\[ e_i = p_i + p_g \Omega \left(1 - \zeta \right) \left(1 - p_p \right) + 0.5 p_g \Omega p_p + 0.5 p_g \Psi + p_p. \]

Fig. 6.3 shows the equilibrium regimes in the \( \zeta - \Delta \) space when parasites are present. We again explored the \( \zeta - \Delta \) equilibrium space with simulation, varying \( \zeta \) and \( \Delta \) by 0.01. In these graphs, the labeling scheme is the same as in Fig. 6.2, but parasites are present in all cases except where the point markers are red. We found patterns similar to those in parasite-free case. The state space occupied by a specialist-and-parasite-only equilibrium increases gradually with increasing group size.

6.2.7. \( n > 2 \) with parasites present

These results show that for the smallest conceivable group size where parasites could survive (i.e., \( n = 3 \)), there is a region of large \( \zeta \) and small \( \Delta \) where parasites are extinct at equilibrium. This region mainly consists of partial DoL. There is also a small region of incomplete DoL where parasites are nonexistent. As group size increases, this parasite-free region disappears. Also, in the synergistic case (i.e., \( \Delta < 0 \)), we find a region where parasites are excluded at equilibrium for either a generalist-type 1 equilibrium or a generalist-only equilibrium for intermediate \( \zeta \). This region is suppressed as group size increases and as \( \zeta \) decreases. The failure of parasites when \( n = 3 \) apparently reflects the high probability of group success when a generalist sees a specialist. Moreover, because we assume parasites give the generalist no information, when a generalist sees a parasite it chooses randomly, and in a group
of three agents it is possible that the third agent is either another parasite or a complementary specialist. If a type 1 specialist were to replace the parasite, the group would have a higher probability of success. It follows that parasites should be vulnerable to extinction. For the region lacking parasites at intermediate $\zeta$, the cause again appears to be the high probability of success for generalist-generalist pairings (see 6.1.3).

When $n = 3$, all four types are present at equilibrium in the far right region of the graph for a moderate range of $\Delta$ and large $\zeta$. However, this space disappears when $n = 5$ and the coexistence of all four types no longer occurs on the $\zeta$-$\Delta$ scale investigated.

Fig. 6.4 shows how the frequency of parasites at equilibrium for the maximum $\zeta$ and $\Delta$ values (1.0 and 0.38, respectively) changes with group size. The figure shows that parasite frequency climbs quickly initially and then, by $n = 10$, begins to level off (see similar observation in Wahl 2002a).

6.3. Discussion

We explored conditions favoring incomplete division of labor (DoL) – the stable coexistence of generalists and specialists. In doing so, we uncovered conditions in which three or even four types of agents could coexist (two kinds of specialist, a generalist, and even a parasite) despite the fact that only two types of tasks must be performed. (Our work superficially resembles models of coexistence of three consumer species exploiting two resources [Wilson and Yoshimura, 1994; Abrams, 2006].) In particular, we identified conditions favoring persistence of generalists in the presence of two types of specialists, even though generalists were assumed to pay extra costs for their multitasking flexibility and even though they often chose to perform the wrong task (Fig. 6.2). Thus, the inefficiency and error
proneness of multitasking agents (generalists) is not sufficient to drive evolution to complete DoL (specialists only).

These findings prompt the conjecture that inefficient, error-prone multitaskers may be a fundamental feature of many biological systems characterized by division of labor. Their rampant error-making may be inconsequential to group success, especially if information is incomplete, as in wasps (Forsyth, 1978; Jeanne, 1986; Karsai and Wenzel, 2000), stingless bees (Hofstede and Sommeijer, 2006), and possibly sea anemones (Ayre and Grosberg, 2005). Under some conditions, perfectly naïve multitaskers, with their flexibility in task choice, could exclude more efficient specialists (Fig. 6.1A). At the level of the group or collective, it may be adaptive to contain such seemingly disadvantageous agents. Multitaskers can dramatically elevate group-level fitness, and our results suggest that small groups lacking generalists are especially prone to extinction via the Allee effect (Stephens and Sutherland, 1999).

Empirical work, possibly in one or more of the systems mentioned above, is needed to evaluate evidence of such multitasking in nature. Where such evidence is found, performance costs and decision-making accuracy of generalists should be quantified. These data could be used to parameterize models tailored to these specific systems. Such work could help clarify conditions favoring the persistence of inefficient, error-prone generalists in systems with DoL.

Our analysis also revealed conditions that could lead to multiple equilibria (Fig. 6.2). Alternate evolutionary trajectories could converge on complete DoL, partial DoL, or no DoL at all. The emergence of these multiple equilibria may help explain why some phylogenetic groups contain taxa with DoL (complete or incomplete) and other taxa without DoL. Such phylogenetic groups include slime molds (Bonner, 1988), green algae (Kirk, 1999), and sister groups of sea anemones (Ayre and Grosberg, 1996, 2005; McFadden et al., 1997; Pearse and Francis, 2000).
To conclude, our work was inspired by Wahl (2002a, b), who found that the inefficiency of generalists tended to drive evolution toward complete division of labor, with only specialists remaining at equilibrium. Here, by relaxing two key assumptions, we have explored plausible conditions favoring the coexistence of generalists and specialists. We have shown that extra performance costs and less-than-perfect accuracy in decision-making by generalists do not necessarily drive evolution to complete DoL. Stated another way, generalists can coexist with specialists provided the combined effect of the inefficiency and error proneness of generalists falls below some threshold. Generalists can persist in the presence of efficient specialists and even parasites, even if generalist make decisions in a naïve way. Future work should attempt a more comprehensive analysis of theoretical conditions favoring stable coexistence of generalists and specialists (for some additional causal factors see Bonner, 1988; Buss, 1987; Michod et al., 2003; Michod and Roze, 2001). Future efforts should also attempt to model the evolution of incomplete DoL in real systems ranging from sea anemones to human social organizations.
Table 6.1. Fitness expressions for specialists and generalists, where $n = 2$.

<table>
<thead>
<tr>
<th>Type of agent</th>
<th>Specialist, Type 1</th>
<th>Specialist, Type 2</th>
<th>Generalist, Type $g$</th>
<th>Average fitness ($\omega$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specialist, Type 1</td>
<td>0</td>
<td>$b-c_1$</td>
<td>$\zeta(b-c_1)$</td>
<td>$(p_2+p_g\zeta)(b-c_1)$</td>
</tr>
<tr>
<td>Specialist, Type 2</td>
<td>$b-c_2$</td>
<td>0</td>
<td>$\zeta(b-c_2)$</td>
<td>$(p_1+p_g\zeta)(b-c_2)$</td>
</tr>
<tr>
<td>Generalist, Type $g$</td>
<td>$\zeta(b-c_2-\Delta)$</td>
<td>$\zeta(b-c_1-\Delta)$</td>
<td>$(1-(1-\zeta)^2)(b-(c_1+c_2)/2-\Delta)$</td>
<td>$p_1\zeta(b-c_2-\Delta)+p_2\zeta(b-c_1-\Delta)+p_g(1-(1-\zeta)^2)(b-(c_1+c_2)/2-\Delta)$</td>
</tr>
</tbody>
</table>
Figure 6.1. (Following page) Equilibria arising for various combinations of decision-making accuracy ($\zeta$) and extra costs ($\Delta$) paid by generalists, in groups of 2. Panel A is for method 1, where generalist-generalist pairs succeed with frequency $\left\{1 - (1 - \zeta)^2\right\}$. Panel B is for method 2, where generalist-generalist pairs succeed with frequency $\zeta$. In both graphs, for each $\zeta$-$\Delta$ pair, an initial frequency distribution was chosen at random and the system was allowed to evolve until it reached an equilibrium frequency distribution, or until the run timed out after 10,000 iterations. Each $\zeta$-$\Delta$ pair was simulated 500 times. Areas overlain with red indicate areas containing internal equilibria found analytically. Plus signs = specialists only, stars = all three types present, squares = generalists and type 1 specialists, x’s = generalists only, circles = multiple stable equilibria, and diamonds = run timed out. Parameter values: $b = 1$, $c_1 = 0.2$, and $c_2 = 0.6$. 
Figure 6.2. The equivalent graphs to Fig. 6.1A for groups sizes 3 (Panel A), 5 (Panel B), and 10 (Panel C). Parameter values and symbols meanings remain the same as in Fig. 6.2.
Figure 6.3. (Following page) Equivalent graph to Fig. 6.2, but with parasites included in the simulation. Group sizes of 3 (Panel A), 5 (Panel B), and 10 (Panel C). Parasites present at equilibrium except where indicated by red markers. The other agent types present are indicated by the same marker points as Fig. 6.1. Parameter values: $b = 1$, $c_1 = 0.2$, and $c_2 = 0.6$. 
Figure 6.4. Maximum parasite frequency at equilibrium, over the entire ζ-Δ state space, plotted against group size. Parameter values: $b = 1$, $c_1 = 0.2$, and $c_2 = 0.6$. 
Appendix A

Here we present the data for the effects of contact with a clonemate or non-clonemate for the unadjusted movement data, and movement data where we used 0.44 cm as a cut-off for movement. Data presented here are for the full dataset (all polyp types)(Table A.1) and the warrior-caste dataset (Table A.2).
Table A.1. (Following page) Best fit GLMMs to movement data from our experiment on the effects of clonemate and non-clonemate contact using AIC criteria. Only models equivalent by AIC criteria (≤ 2 ΔAIC_0) shown. Models presented are for all two analyses (0.44 cm cut-off for movement, No cut-off for movement) with all polyp types present, for each pair of neighbors. Mass is the mass of polyps, Clone is the effect of clone identity, Acro is the number of acrorhagi, and Type is the polyp type. All models presented have polyp identity as a random effect. * = $P < 0.05$, model ANOVA, ** = $P < 0.01$. Models built in R using the lme4 package.
<table>
<thead>
<tr>
<th>Clone Pairing</th>
<th>AIC</th>
<th>BIC</th>
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Table A.2. (Following page) Best fit GLMMs to movement data from our experiment on the effects of clonemate and non-clonemate contact for border types (warrior-scouts and warriors) only, for the 0.44 cm and no cut-off analyses. Abbreviation and notations are the same as in Table A.1.
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Appendix B

Naïve generalists pay no extra costs; no parasites

Under the assumption of naïveté, we assume each generalist randomly decides which of the two tasks to perform. For groups of two \((n = 2)\), the fitness equations for the three types are given in Table B.1.

As generalists choose either task with equal probability, they can be thought of as having the fitness of either specialist half the time. To solve for equilibrium frequencies, we note the following to be true:

\[
\omega_g = 0.5 \omega_1 + 0.5 \omega_2.
\]

If we then set \(\omega_1 = \omega_2\) and substitute into the above, we find \(\omega_g = \omega_1\) whenever \(\omega_1 = \omega_2\). We therefore set the fitness expressions for the two specialist types equal to each other, substitute for \(p_g\) using \(p_g = 1 - p_1 - p_2\), and solve for the equilibrium frequency of type 2 specialists:

\[
p_2 = p_1 + \frac{(c_2 - c_1)/2}{-b + (c_1 + c_2)/2}.
\]

This equation defines the line of neutrally stable equilibrium points plotted on the simplex in Figure B.1. Thus, generalists paying marginal costs can coexist with specialists, even if they make completely uninformed (random) choices about which task to perform.

Figure B.2 shows the similar effects of increasing group size and widening the difference in costs of performing task 1 versus 2. In both instances, the stable population frequency shifts to a greater percentage of specialists performing the cheaper task (task 1 in this case).
If naïve generalists are forced to pay an additional cost, they quickly go to extinction (data not shown) and the system consists solely of specialists in a strict (complete) DoL.
Table B.1. Fitness equations for types when generalists are naïve.

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<th>Type of agent</th>
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<td>$(p_2 + 0.5p_g) d_1$</td>
</tr>
<tr>
<td>Specialist, Type 2</td>
<td>$(p_1 + 0.5p_g) d_2$</td>
</tr>
<tr>
<td>Generalist, Type $g$</td>
<td>$0.5[(p_2 + 0.5p_g) d_1] + 0.5[(p_1 + 0.5p_g) d_2]$</td>
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</table>
Figure B.1. Simplex for $n = 2$, where generalists have no knowledge (i.e., perform correct task with 50% probability) and pay only marginal costs (i.e., only of the one task performed). Vertical line indicates neutrally stable equilibria. Parameter values: $b = 1$, $c_1 = 0.2$, and $c_2 = 0.6$. 
Figure B.2. Simplexes showing effects of group size and differential costs of two tasks. Parameter values for column on left: $b = 1$, $c_1 = 0.2$, and $c_2 = 0.6$; for column on right, $c_1$ was changed to 0.5. Descending rows have increasing group size from 2 to 5 to 10. Lines indicate neutrally stable equilibria.
Appendix C

Explanation of the approximation \( p_g \alpha' \):

The \( p_g \alpha' \) expression is an approximation for group sizes larger than 3, where \( \alpha' \) term expands to:

\[
\left( \zeta \Omega \left[ (1 - \zeta) p_2 A_2 + (1 - \zeta) p_1 A_1 + p_g \alpha' \right] + (1 - \zeta) \Omega \left[ (1 - \zeta) p_2 A_1 + (1 - \zeta) p_1 A_2 + p_g \alpha' \right] \right) + \Psi' \alpha
\]

This expansion would be repeated for each additional group member beyond the fourth. That is, for a group of 4, the above expansion would be sufficient. But for a group size of 5, this expansion would be substituted and a similar expansion would be done to the \( \alpha' \) terms in this insertion. The top line in this expression represents the case where the third generalist in the chain of generalists sees someone other than the first two generalists. Because the first two generalists are now linked, \( \Omega \) and \( \Psi \) have become \( \Omega' \) and \( \Psi' \), respectively, where \( \Omega' = (n-2)/n \) and \( \Psi' = 2/n \). These terms change in a similar fashion with each expansion. The first term in braces on the top line represents the case where the second generalist in the chain acts correctly and hence \( \zeta \) is written out front, whereas the second term containing braces represents the case where the second generalist acts incorrectly. The middle line, where the third generalist sees either of the first two, is this probability multiplied by the average fitness for generalists interacting with each other. Here the first generalist is already implicitly assumed to be acting
correctly (as indicated by the first $\zeta$ in (5a)), meaning the group succeeds no matter what the other two generalists do.

The second approximation, $\alpha''$, is slightly different. Where this term appears the first two generalists in the chain have acted incorrectly, so either the third generalist needs to be correct (hence the last $\zeta$ term in $\omega_{G_4}$) or another group member needs to act as the opposite type (see the final set of terms multiplied by $(1-\zeta)$ in equation (10)). While this is correct for $n = 3$ because the $(1-\zeta)$ term disappears, for larger $n$ we need to define what the third generalist “sees.” Thus $\alpha''$ expands to:

$$\Omega [p_1\Delta_2 + p_2\Delta_1 + p_3\alpha'] + \Psi'\alpha.$$

This is true when $\alpha''$ is multiplied by the last $\zeta$ in equation (10), but this expansion does not hold when $\alpha''$ is multiplied by $(1-\zeta)$. This is beyond our scope here. We assume that when the first three generalists are wrong and at least one of the other types is present, the generalist receives the benefit half the time, on average.
Appendix D: extensions of Chapter 6

Naïveté, large groups and differential costs of tasks

With parasites

Imagine a parasitic agent (Wahl, 2002a) that does not complete any task but accrues a benefit if the group succeeds. These agents act as defectors in the population. When parasites are present, the fitness expression for each type in a group of size \( n \), where generalists choose tasks naively (i.e., randomly), is shown in Table D.1. These fitnesses were calculated as in the main body of the paper. The population should be in equilibrium when the fitness for any type equals that of any other type. Under the condition that \( \omega_1 = \omega_2 \), these two fitnesses are also equal to \( \omega_g \). This implies that there are multiple equilibria for the system, and that they fall along a line. The constraints on the equilibrium frequencies can be obtained from the authors upon request.

Less-than-perfect knowledge and costs

Scaling of \( b \)

We briefly explored the effects of scaling \( b \) for larger group sizes with the number of times the pair of tasks were completed by a group in a time step. For simplicity, we chose a linear scaling where we simply multiplied the reproductive benefit, \( b \), by the number of times the group completed both tasks. This system is analytically very laborious, if not intractable, and we chose to model it using an agent-based model of our system. Simulating 5000 individuals
acting over 1000 time steps where each time step was treated as a generation in which 10 agents coalesced to form a group, we found that the system continuously stabilized. However, for any given $\zeta$-$\Delta$ pair, the system found different stable equilibria. These points were always internal, where all three types coexisted. This is not surprising. If it pays the group and the individual to perform both tasks more often, the advantage type 1 specialists had in doing the cheaper task is reduced and fitnesses of the other two types increased. Given the stochastic nature of the system and the large group size, these conditions should favor the coexistence of all types. We speculate that these preliminary results may demonstrate how large social groups, such as ant colonies, can maintain so many different types of generalists and specialists.

With parasites

When parasites are present, they do little to change the fitnesses of the nonparasitic members of the population. When a generalist “sees” a parasite, we assume the generalist lacks knowledge and so randomly chooses to perform either task 1 or task 2. Because a generalist can never act incorrectly when it sees a parasite, the probability $(1-\zeta)$ does not really apply. This effect is most clearly seen in the fitnesses of the specialists. Here each specialist fails if the rest of the group acts as that specialist type and/or is a parasite.

$$
\omega_1 = 1 - \left( p_1 + (1-\zeta) p_g \Psi + (1-\zeta) p_g \Omega(1-p_p) + 0.5 p_g \Omega p_p + p_p \right)^{n-1} \left( b - c_1 \right) \quad (D.1)
$$

$$
\omega_2 = 1 - \left( p_2 + (1-\zeta) p_g \Psi + (1-\zeta) p_g \Omega(1-p_p) + 0.5 p_g \Omega p_p + p_p \right)^{n-1} \left( b - c_2 \right) \quad (D.2)
$$
The terms raised to $n-1$ power in the above equations are built by adding the probabilities that each additional group member is the same specialist type or a generalist that: sees the specialist in question and performs the wrong task, sees a different group member that is not a parasite and performs the wrong task, or sees a different group member that is a parasite and does the same task as the specialist or a parasite. We call these terms being raised to $n-1$ power $\gamma_1$ and $\gamma_2$. Again, we ignore the possibility that generalists could see one another and perform the same task, which happens to complement the specialist in question. As above, this does not appear to change the results. The equations can now be written as:

$$\omega_1 = [1 - \gamma_1^{n-1}] (b - c_1)$$  \hspace{1cm} (D.3)$$

$$\omega_2 = [1 - \gamma_2^{n-1}] (b - c_2)$$  \hspace{1cm} (D.4)$$

Following the approach above for the case without parasites, we now re-write the equations for the generalist with parasites corresponding to equations (7) through (10) in the main text together with the additional terms $\omega\_G_5$ and $\omega\_G_6$ for when generalists see a parasite and when the generalist they see in turn sees a parasite. As in the case without parasites, after this point in the chain of decision-making we make some small approximations.

$$\omega_{G_5} = p_1 \zeta \Delta_2 + p_2 \zeta \Delta_1 + p_1 (1 - \zeta) [1 - \gamma_1^{n-2}] \Delta_1 + p_2 (1 - \zeta) [1 - \gamma_2^{n-2}] \Delta_2$$  \hspace{1cm} (D.5)$$

$$\omega_{G_6} = p_g [1 - (1 - \zeta)^2] \Psi \alpha + p_g (1 - \zeta)^2 \Psi \left[ \frac{1}{2} [1 - \gamma_1^{n-2}] \Delta_1 + \frac{1}{2} [1 - \gamma_2^{n-2}] \Delta_2 \right]$$  \hspace{1cm} (D.6)$$

$$\omega_{G_7} = p_g \zeta \Omega [\zeta p_1 (1 - \zeta) p_2 + 0.5 p_p \Delta_1 + [\zeta p_2 + (1 - \zeta) p_1 + 0.5 p_p] \Delta_2 + p_g \alpha]$$  \hspace{1cm} (D.7)$$
The terms $\alpha'$ and $\alpha''$ are approximations along the lines of those explained in the preceding section, except now parasites must be accounted for in the expansions. For $\alpha'$ we take the first term from the expansion in the previous section and show it expanded for this case:

$$\omega_{G_i} = 0.5 p_p (1 - \gamma_1^{n-2}) \Delta_1 + 0.5 p_p (1 - \gamma_2^{n-2}) \Delta_2$$  \hspace{1cm} \text{(D.9)}$$

$$\omega_{G_s} = (1 - \zeta) 0.5 p_p \Omega p_p \left[ (1 - \gamma_1^{n-3}) \Delta_1 + (1 - \gamma_2^{n-3}) \Delta_2 \right] + \zeta 0.5 p_p \Omega p_p \left[ \Delta_1 + \Delta_2 \right]$$  \hspace{1cm} \text{(D.10)}$$

$$\omega_G = \omega_{G_i} + \omega_{G_s} + \omega_{G_1} + \omega_{G_2} + \omega_{G_s} + \omega_{G_1}$$  \hspace{1cm} \text{(D.11)}$$

The terms $\alpha'$ and $\alpha''$ follow in similar fashion.

Parasite fitness, as shown in the main body of the paper is the benefit multiplied by the probability that at least one agent performs task 1 and one performs task 2 within the group:

$$\omega_p = b \left[ 1 - \varepsilon_1^{n-1} - \varepsilon_2^{n-1} + p_p^{a-1} + \left( p_g \Omega (1 - \zeta) (1 - p_p)^{a-1} \right) \right], \text{ where}$$
\[ \varepsilon_i = \rho_i + p_g \Omega(1 - \zeta)(1 - p_p) + 0.5 p_{-g} \Omega p_p + 0.5 p_{-g} \Psi + p_p. \]
Table D.1. The average fitness value for each type of agent in a group of size $n$, assuming marginal costs and naïve (i.e., random) decision-making. Fitness is the probability of group success multiplied by the type-specific net benefit, $b-c_i$, where $b$ is the benefit obtained if the group completes both tasks, $c_i$ the cost for doing task $i$, and $p_i$ the frequency of type $i$, where $i$ is 1, 2, and $g$ for generalist or $p$ for parasite.

<table>
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<th>Average fitness ($F_i$)</th>
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<tr>
<td><strong>Type $g$</strong></td>
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<tr>
<td><strong>Type $p$</strong></td>
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