COLLECTIVE BEHAVIOR IN SLAVE-MAKING ANTS: HOW ECOLOGY AND SOCIAL STRUCTURE SHAPE RAIDING STRATEGIES

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COLLECTIVE BEHAVIOR IN SLAVE-MAKING ANTS: HOW ECOLOGY AND SOCIAL STRUCTURE SHAPE RAIDING STRATEGIES

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In this dissertation, I studied the raiding behavior of a slave-making ant, *Temnothorax americanus*, a social parasite whose workforce consists of different ant species. ‘Slave’ workers are acquired in coordinated raids during which slave-maker workers attack a host colony and steal the immatures (brood). I focused on the mechanisms colonies use to reach collective decisions over where raid and on the attack once a raiding site was chosen. In Chapter One, I first established which nest characteristics make an optimal raiding choice by measuring the benefit of raids (stolen brood), and counterbalancing costs (mortality), at target colonies that differed in the number of brood, workers, or their ratios. These experiments showed that slave-makers are more successful when attacking colonies where brood outnumber workers and where there is an intermediate number of workers. In Chapter Two, I tested whether slave-maker colonies demonstrate a preference for such nests and characterize their decision-making strategy. Choice trials showed that slave-maker colonies exhibit no preference over host colony features. This result led to the question of why not be more selective? I then tested for ecological conditions that could favor their low acceptance threshold and show that slave-maker colonies encounter host colonies at a very low rate

relative to the time when brood is available to raid. Slave-maker colonies therefore ought to raid every nest they find rather than pay the opportunity cost of waiting to raid only the best host colonies. In Chapter Three, I investigated the mechanisms of attack and show that successful raiding parties effectively evacuate workers while guarding the door to keep the brood inside. I also tested how conflict, especially prominent in *T. americanus* colonies, affects this collective behavior. I found that workers from colonies with higher levels of worker reproduction initiate attacks alone rather than as a group. In Chapter Four, I explored how cooperation and collective behaviors co-evolve by modeling these interactions analytically. I adapted mathematical models of cooperative binding from biochemistry to characterize collective behaviors that arise in a non-linear fashion.

BIOGRAPHICAL SKETCH

Julie S. Miller grew up in Los Angeles, California, but always found great comfort in leaving the urban sprawl to spend time at the beach or up in the Sierras Nevada Mountains. The first sign that Julie needed to be outdoors was an early obsession with living under the sea as a mermaid so that she could befriend the creatures down there. Years later, she fantasized about exploring the jungle in search of the world's largest leaves or scaling high peaks in the Himalayas as a Buddhist monk. In the years that followed, Julie sought more reasonable life goals. At Brandeis University, she pursued her interest in neuroscience and genetics, and was romanticized by the idea of making scientific discoveries. However, as the unapologetic daughter of hippy parents, Julie also spent her time involved in activism and social justice work, especially with the Brandeis Labor Coalition advocating for workers' rights. These experiences fueled a fascination with how communities organize and cooperate while others perpetuate inequality. With her undergraduate career wrapping up, Julie decided to take a field biology course just for fun. In this fateful course, she realized she could enjoy both the outdoors and science at the same time, and was instantly hooked. That course, under the guidance of Dan Perlman, set her on the trajectory that led to this dissertation. A class field trip to the Ant Room at Harvard's Museum of Comparative Zoology led to an internship, where the sage Stefan Cover opened her eyes to the world of ant biology. With ants, she found the intersection of her otherwise opposing set of passions: ant seemed to epitomize the harmonious and cooperative society beyond the reach of most human societies. But they also satisfied her growing interest in field biology and entomology by providing a biologically rich tapestry of lifestyles and behaviors to study. After learning as much about ants and animal behavior as she could as a volunteer at the South West Research Station and the LA Museum of Natural History, and as a Master's student with Andy Zink at San Francisco State University, she entered the Ph.D. program in Neurobiology and Behavior.

To my parents,

Evi Jacoby-Miller and Bill Miller

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

A GOLDILOCKS STRATEGY LEADS TO OPTIMAL RAIDING OUTCOMES IN SLAVE-MAKING ANTS

Introduction

The study of host-parasite interactions has predominantly focused on the effects of parasites on their hosts, however there is relatively less known about how the host environment effects the fitness of the parasite (Schmid-Hempel 2011). In order to fully understand the co-evolution of hosts and their parasites, it is important to understand the basis of parasite host-choice, i.e. which features of a host are optimal from the perspective of the parasite.

When host density is sufficient for host-choice, some researchers have hypothesized that parasites ought to prefer hosts in the best condition, from which a maximum quantity or value of resources can be exploited (Valera et al. 2004). Alternatively, others have hypothesized that parasites ought to prefer hosts that can least effectively resist attack, such as hosts that are immuno-compromised (e.g. the tasty chick hypothesis; Christie et al. 1998). There is mixed support for either of these hypotheses, and it is more likely that parasites optimize host-selection though a compromise between the above two factors to optimize the net profitability of a host (Théron et al. 1998; Cervo & Turillazi), as is predicted by optimal foraging theory (Stephens & Krebs 1986).

These hypotheses have been investigated almost exclusively in ectoparasites that infect as a single autonomous organism, like flies (Valera et al. 2004), fleas (Krasnov et al 2005; Tschirren et al. 2007) or trematodes (Théron et al. 1998). However, parasites sometimes consist of multiple individuals, who attack as a collective, as is the case with social parasites, like slave-making ants (Buschinger 2009), or pathogens, like protozoa or bacteria (Schmid-Hempel 2011). When a parasite attacks as a group, the dynamics of attack or infection are likely to differ from those between a lone parasite and its host. For instance, groups may be more robust to host defenses than lone individuals, since injury of one individual may not be fatal to the success of the attack. If this were the case, groups may be less sensitive to host defenses than parasites consisting of a single individual and predicts that parasite groups ought to weigh the value of host resources more heavily than its defensibility. On the other hand, evidence from epidemiology, in which parasites or pathogens attack as a group, suggests that group size is of utmost importance. Pathogens that adopt a "frontal attack" strategy (Merrell & Falkow 2004) on their hosts typically withhold attack until their numbers reach a threshold, termed an "infectious dose" (Schmid-Hempel & Frank 2007). Quorum sensing bacteria are one example of this strategy, only inducing attack once they have reached a critical mass (Gama et al 2012; Miller & Bassler 2001). Similarly, attack is group-size dependent in the facultative slave-making ant *Myrmecocytus mimicus* (Hölldobler 1976). Colonies perform ritualized displays to advertise fighting power, and will only raid after determining that the size of its rival is less numerous. These attack strategies suggest that host defensibility, or more specifically, relative fighting power, should be weighed more heavily in decisions over host choice. By this logic, there is no single best host choice for the parasite, but rather the optimal choice depends more strongly on the parasite's condition or group size.

Here, I investigate host-choice in a parasite that attacks collectively by measuring the relative importance of several key host traits on parasite success. I use the obligate slave-making ant, *Temnothorax* (=*Protomagnathus*) *americanus*, which collectively attacks other ant colonies in coordinated raids (Wesson 1939). I measure both the benefits and costs to slave-maker colonies as a function of each of the following host features: the host's (1) resource value, (2) defensibility, (3) the ratio between the latter two, and (4) the host's group size relative to the parasite's. While slave-makers depend on chemical manipulation of their hosts in order to increase their chances of success (Regnier & Wilson 1971; Brandt et al 2006), it is especially unclear how relative fighting power influences raiding success in slave-making ants, and thus what constitutes an optimal host choice.

As obligate social parasites, *T. americanus* depends on host workers for all necessary colony tasks, including foraging, brood care, and other nest maintenance (Alloway 1983). The more host-workers a colony has, the more individuals available to perform these necessary tasks, and as expected. Thus, the best measure of a colony's payoff in a raiding bout is the total amount of worker brood the slave-maker colony acquires, but I confirm this here. If resource value is the most important factor in host-choice, then slave-makers will always do best to target nests containing the largest quantities of brood. This hypothesis is supported by a prior study on *T. americanus,* which finds that slave-makers prefer the largest hosts, as determined through behavioral choice-tests (Pohl & Foitzik 2011). These findings are inconclusive, since their results could be explained by a biased discovery rate at larger nests rather than an innate preference.

Pohl & Foitzik's (2011) study are surprising because slave-makers are also predicted to consider the defensiblity of a host nest, or the number of workers defending the nest under attack. During raids, slave-makers release a chemical called a propaganda substance to interrupt host defenses (Regnier & Wilson 1971; Brandt et al 2006), but host workers still fight back by immobilizing, biting and stinging individual slave-maker workers (personal observation), sometimes leading to death. Slave-maker mortality has costs for future raiding opportunities, as

T. americanus conducts multiple raids per season (6 on average, Foitzik & Herbers 2001). In addition to posing a mortality risk, host workers act to protect their brood by carrying it away from the nest to hide in the leaf-litter, effectively reducing the quantity of brood available to the slave-makers (Kleeburg et al. 2014). If host defensiblity is the most important factor in host-choice, then slave-makers will acquire the most brood and suffer the fewest mortalities at target nests with the fewest workers.

The optimal host choice will therefore be one that maximizes brood acquisition while minimizing slave-maker mortality, or more specifically, which minimizes the worker-to-brood ratio. However, the range of possible ratios may be constrained by the biology of the host. In most host organisms, resource value and defensibility are inextricably linked, such that hosts in good condition tend to mount strong immune defenses (Sheldon & Verhulst 1996). Likewise, host nests containing high numbers of brood are also likely to contain a high number of workers, and vice versa (Hölldobler & Wilson 1990). To test the assumption that there are natural constraints on worker-to-brood ratios, I censused naturally occurring host nests and determined the degree of correlation between the two. I then investigated the optimal compromise between brood number and defensibility by examining the effects of worker-to-brood ratios and absolute colony size on raiding success.

The optimal host size (i.e. worker number) may be affected by a more complex set of factors, especially with constraints on the worker-to-brood ratio. Preferring hosts with few workers may make it easier to steal the brood, but such colonies are likely to have few brood in the first place. Conversely, hosts with large quantities of brood may also be guarded by more workers. It is not straightforward what balance of workers and brood will lead to the best outcomes for slave-makers.

Furthermore, optimal host size may also depend on the size of the slave-maker colony itself. *T. americanus* colonies are always smaller than the hosts they raid, so there will always be a colony size asymmetry, but the extent of this asymmetry can vary substantially. Even though they are outnumbered, the numerical advantage given to slave-makers for deploying propaganda substances or having larger bodies is unquantified in any slave-making species. To investigate the consequences of such battles on optimal host size, I analyzed the effect of relative fighting power (i.e number of workers between parasite and host) on raiding outcomes. If group attack strategies are more resilient to host defenses than solo strategies, then slave-maker colonies may be less vulnerable to larger host colonies (Oster & Wilson 1978). This hypothesis predicts a one-size fits all strategy of optimal host choice. Depending on the potency of the parasite's manipulative strategies or combat techniques, group attacks may alternatively be sensitive to group size asymmetries, affecting brood stolen and/or mortality. Such size-dependent dynamics have precedent in other social insects in which there is combat over territory or resources (Plowes & Adams 2005; McGlynn 2000). If relative colony size does have consequences for raiding success, then optimal host size would scale with slave-maker size. This latter hypothesis predicts that the greater the colony size asymmetry, the lower the raiding success and the higher the mortality.

I test the above hypotheses for which colony attributes make an optimal raiding site for the slave-maker *T. americanus* using experimental raids in the lab. I offer slave-maker colonies target nests that varied in their number of workers, brood and the ratio between the two, and measured the raiding outcomes of those trials in terms of their benefits (number of brood stolen) and their costs (mortality).

Methods

Colony Collection & Census

Temnothorax americanus uses three host species: *T. longispinosus*, *T. ambiguus*, and *T. curvispinosus*. The populations measured in this study consisted almost exclusively of *T. longispinosus* hosts, and all experimental host colonies were *T. longispinosus*. *T. americanus* and *T. longispinosus* colonies were collected at the Huyck Preserve (42° 30' 53.7876", -74° 8' 27.7836"), Cornell Plantations Land (42° 27' 55.2738", -76° 26' 34.299") and at Bear Mountain State Park (41° 18' 33.9768", -74° 0' 14.6808") between May and July, 2013 – 2016. Colonies were transported back to Liddell Labs in Ithaca, NY in Ziploc bags and stored in a refrigerator (4C) for up to three weeks before being censused for counts of the following: queens, workers, mature sexuals, larvae, and pupae (worker and sexual). In the trials below, mean slave-maker (*T. americanus*) colony size was 6.31±0.39 slave-maker workers (min: 2, max: 17), not including the queen.

Colony Maintenance & Preparation

Immediately following counts, a colony was placed inside a nest box containing an artificial nest, into which it moved. Artificial nests consisted of a balsa wood or plexiglass U-shape structure placed in between 2 microscope slides. Nest were then wrapped in foil to exclude light and housed in a plastic Ziploc box with a plaster floor, which was moistened twice per week to maintain humidity levels. Colonies were fed a teaspoon of Bhatkar diet twice per week and housed in a room at 26 C, with a 14L:10D daylight cycle.

Prior to raids, all slave-maker colonies experienced the same conditions to standardize any possible sense of urgency that might influence raiding behaviors. Two days prior to raiding, I removed all brood and some adult slaves from the colony so that there was a 1:1 ratio of slave-makers (including the queen) to slave ants. Slave-ants were relocated to a temporary nest without any slave-makers until after the raiding trial was complete. During the removals, each slave-maker worker was individually marked with RC Car Pactra Paint, with a dot on the abdomen, thorax and head. Ants were isolated in vials for at least four hours while paint dried before being reintroduced to the rest of the colony. Two days later, slave-maker colonies were used in raiding trials.

One to five days before a raid, *T. longispinosus* nests were prepared by randomly selecting a set number of workers and brood items from an existing lab colony. A mixture of larvae and pupae were selected for each trial, but the relative number of larvae and pupae were held constant between nests containing the same brood counts. When nests contained insufficient brood for the treatment, I added brood from other lab colonies, but always from colonies collected from the same population. These size-engineered colonies were then given a naturally emptied acorn or hickory nut to move in to, with a single entry hole that never exceeded 2 mm.

Raid Set-up

Raiding trials took place in glass arenas. On the morning of a raiding trial, a prepared slave-maker nest was positioned at one end of the arena and a target nest on the opposite end. Target nests were placed close to arena wall, but were not touching the wall to avoid unnatural bias in nest discovery from wall-following behavior. In all trials, the temperature was elevated to 30C the morning of the raid, and a plastic dish of food and a dish of wet cotton were placed in the center of the arena to prevent dehydration or starvation during the trials. The upper rims of the 13 cm high walls were painted with Vaseline to prevent ants from escaping.

The entrances of the target and the slave-maker nests were filmed using a webcam (Logitech C920 HD Pro), pointed through the transparent arena wall or positioned above the nest. If by 4pm, there were no slave-makers recruiting to or attacking the target nest, both colonies were removed from the glass arena and returned to their boxes until the next day. The colonies were given a maximum of five days to initiate a raid before the trial was aborted. If there was evidence of recruitment or a raid (i.e. the target nest had vacated, there was a raiding party in the arena, or slave-makers were entering or exiting the target nest), I left both colonies in the arena overnight and collected both colonies the next morning, so that colonies stayed in the arena for 24 (±4) hours. The raiding room was set to the same lighting regime as the room housing colonies, and I lowered the temperature back to 26C to coincide with darkness. After completion of a trial, I confirmed that a raid had been attempted or initiated by watching recordings of the two nests.

Experimental Raiding Trials

To test which aspects of target nests influence the number of brood stolen and slave-maker mortality rates, I conducted single-choice raiding trials using target nests that differed in their worker and brood combinations. Despite some variation in the size of the raiding arenas between trials, arena size did not have a significant effect on raiding success (45 cm versus 100 cm arenas for nests containing 25:25 workers : brood ($t = -0.3257$, $df = 16.032$, $p = 0.749$).

The morning after each raid, I retrieved both nests and collected any remaining ants from within the arena, i.e. outside of either nest, and counted the contents of both nests on that day. To determine the number of brood stolen by slave-makers, I counted the total number of brood in possession by slave-makers after the raid, as all slave-makers started each raid with zero brood. Occasionally, slave-maker colonies moved in to the host colony’s nest, taking control of the brood within, and I included these instances into counts of brood acquired from raids.

Benefits

(a) Worker-to-Brood Ratios

To test the effect of worker-to-brood ratios on brood stolen, I conducted two sets of trials with nests of variable ratios. I fist compared trials with targets containing 25:25 or 50:25 workers : brood, which kept brood number constant while varying the ratios (1:1 vs 2:1). Twelve *T. americanus* colonies were used in 19 trials, and colonies were never used more than once for the same treatment. I also compared trials with 20:20 or 20:60 workers : brood, which kept worker number constant while comparing a different set of ratios (1:1 vs 1:3). Thirteen *T. americanus* colonies were used in 19 trials. The above trials were conducted in rectangular glass arenas measuring 45cm x 25cm. For the latter trials, host colonies were housed in the plexiglass nests described above.

(b) Worker Number

To test the effect of absolute worker number on the proportion of brood stolen, I conducted three sets of trials with targets that differed in their number of workers (25, 50 & 100 workers), but which held the worker-to-brood ratio constant at 1:1 workers : brood. Fourteen *T. americanus* were used in 15 raiding trials. In these trials, the slave-maker and its target were placed 100 cm apart by connecting two glass arenas, described above, with a paper bridge 10 cm in length, and colonies were housed in balsa wood nests described above.

(c) Brood Number

To test the effect of absolute brood number on the total number of brood stolen, I used the same trials as above (B), but analyzed the total number of brood rather than the proportion.

(d) Relative Colony Size

To investigate the effect of the relative size of slave-maker and target colonies on the proportion of brood stolen, I pooled all raiding trials from above and included additional trials with target nests containing 50:30 and 30:10 workers : brood, totaling 57 raiding trials with 38 different *T.*

americanus colonies. The latter set of trials took place in square glass arenas measuring 75 cm x 75 cm.

Costs

Mortality was determined by taking the difference between a colony's living slave-maker workers on the day of the raid and the day after the raid. To determine which of the above factors influence slave-maker mortality, I pooled mortality data from all raiding trials described above, totaling 67 trials using 44 *T. americanus* colonies. More raids are included in this analysis than above due to missing data on brood stolen.

Analyses

All analyses were performed in R version 3.1.2. To determine the naturally occurring worker-to-brood ratios and absolute colony sizes if the target species, I measured the mean and standard error number of workers and brood from all *T. longispinosus* nests collected between 2014 and 2016. I compared the number of adult workers to the number of worker brood (larvae and pupae) with a linear regression, using the "lm" function in the "stats" R package. To confirm that colonies with more host-workers tend to produce a higher number of sexual offspring, I fit the number of reproductives per slave-maker colony (male and female alates) to the number of host workers using a linear regression.

I compared the number of brood stolen from nests containing a 25:25 worker-to-brood ratio with nests containing a 50:25 worker-to-brood ratio using a one-sided Wilcoxon signed rank test. I corrected for 3 ties in the data using the "wilcox.exact" function in the "exactRankTests" R package. I also compared the proportion of brood stolen from nets containing 20:20 and 20:60 worker-to-brood ratios using a one-sided Wilcox test.

For the following analyses, I used Generalized Linear Models (GLM) using the “glm” function in the “lme4” package version 1.1-12 (Bates et al. 2016). I performed multi-model averaging using the “dredge” and “model.avg” functions from the “MuMIn” package version 1.15.6 (Bartoń 2016) using model selection criteria of AICc <4 (Bolker et al. 2009; Burnham et al. 2011). I report the results of the conditional models. Additionally, I report standardized model estimates (β), derived by multiplying each coefficient by the ratio of the standard deviations of the predictor variable and response variable (beta = TRUE in the “model.avg” function). The specific global models and model distributions are described for each case below.

Using trials with target nests containing a 1:1 ratio of workers-to-brood (25:25, 50:50, and 100:100 workers:brood), I tested for the effect of absolute colony size on raiding success by running a Generalized Linear Model (GLM). To account for the different number of brood available in each nest, the response variable was modeled as a proportion, consisting of two vectors: number of brood successfully stolen and number of brood remaining. Due to the binary structure of the response variable, the GLM used a binominal distribution and logit link function (Zuur et al. 2009). The global model contained the following predictor variables: the number of host workers in the target nest, the number slave-maker workers and their interaction term (Table 1.1(A)). The data were overdispersed, so models were ranked using QAICc (Burnham & Anderson 2002).

To test the hypothesis that more brood will be stolen from nests containing an absolute greater number of brood, I ran a GLM and multi-model averaging using the same 1:1 worker-to-brood ratio trials as above, but with the absolute number of brood stolen as the response variable and with a Negative Binomial distribution. The global model included: the number of available brood in the target nest, the number slave-maker workers, and their interaction term (Table

1.1(B)). I did not include the number of workers in the target nest because it is perfectly collinear with brood number (1:1 worker-to-brood ratio).

To test the hypothesis that relative size of slave-maker and target colonies will affect raiding success (i.e. proportion of brood stolen), I ran a GLM that included data from all raiding trials. The global model included the following predictor variables: number of workers in the target nest, number of slave-maker workers, their interaction term, number of brood, and the interaction between number of brood and workers in the host nest (Table 1.2). I partitioned the analysis with a hurdle model because the data were overdispersed and zero inflated (20.7% of trials had zero success). First, I ran a binomial model of whether brood were captured using the entire data-set. Then, using only non-zero data, I ran a model of the proportion of brood stolen using the two-vector response variable (brood stolen, brood remaining) with a binomial distribution and logit link function. The non-zero model of proportions was still overdispersed (448 residual deviance on 56 residual df) after removing zeros, so I performed model averaging using QAICc.

To test for the effects of target nest characteristics on slave-maker mortality, I modeled mortality rates both as a proportion and as count data using a GLM. The global model included the following as predictors: number of slave-maker workers, number of host colony workers, their interaction term, the number of brood in the host nest and the interaction between number of host workers and their brood. Mortality data were overdispersed and zero-inflated (50.7% of trails contained zero deaths), so I modeled mortality using a hurdle mode. First, I ran a binomial model of whether mortality occurred. Then, I modeled mortality rates for non-zero data in two ways, first as a proportion and second as count data of absolute number of deaths. For the proportion model, the response variable consisted of two vectors, the number of deaths and the number of survivors, following a binomial distribution and logit link function. For the count model, I used a Poisson distribution.

Results

Natural Colonies

For the 244 *T. longispinosus* nests censused, the mean worker-to-brood ratio was 1.34±0.19 (SE). The mean nest contained 36.1±2.2 workers and the mean number of worker brood was 50.8±2.7 (larvae + worker pupae). Brood number was significantly positively related to worker number ($p<0.0001$, $R^2=0.15$, $F_{(1,242)} = 44.09$; Fig. 1.1). Slave-maker reproductive success was positively related to the number of host workers in that colony ($p<0.0001$, $R^2=0.18$, $F_{(1,233)} = 51.98$; Fig. 1.2).

Benefits

(a) Worker-to-Brood Ratios

As predicted, more brood is stolen from nests with proportionally fewer workers than brood, i.e. with a smaller worker-to-brood ratio. When target nests were matched for number of brood, marginally more brood was stolen from nests containing 25 workers than nests containing 50 ($p = 0.0468$, $W = 23.5$, $n=19$ raids; Fig. 1.3A). As expected, proportionally more brood was stolen from nests containing 60 brood than 20 brood, even though they were matched for worker number ($p = 0.0014$, $W=8$, $n=19$ raids; Fig 1.3B).

(b) Worker Number

The absolute number of workers in the target nest had a negative effect on the outcome of raids. This result arises from raids on nests where the worker-to-brood ratio was kept constant at 1:1 (Fig 1.4) and when the data from all raids were pooled (see *Relative Colony Size*; Table 1.2).

The proportion of brood stolen at nests with a 1:1 ratio, but that varied in their absolute numbers, was significantly negatively related to the number of workers guarding the nest ($p < 0.0001$, standardized estimate = -0.044, n= 15 raids; Table 1.1A), and had a stronger influence than the size of the slave-maker colony (Worker importance = 1, Slave-maker worker importance = 0.32). The size of the attacking slave-maker colony had a small, but positive effect on the proportion of stolen brood ($p < 0.001$, estimate = 0.021). QAICs were calculated using the overdispersion parameter c-hat =13.89. Relative colony size did not explain the variation in the data and was not included in final models. In summary, increasing the absolute number of defending workers at the target nest resulted in proportionally fewer stolen brood for a slave-maker colony.

(c) Brood Number

Despite the negative effect of worker number on the proportion of brood stolen, I predicted that the *absolute number* of brood stolen in these same trials (with 1:1 worker-to-brood ratios) would be greater in nests containing more brood; however, this prediction was not satisfied. None of the factors were strong predictors of the number of brood stolen in these trials. The null model had the lowest AIC score, which did not include any of the predictors. In addition, the results of model-averaging showed that the number of brood in the target nest did not have a significant effect on the absolute brood stolen ($p = 0.127$; Table 1.1B), and its effect was in the opposite direction than predicted (standardized estimate = -0.07). The effect of the slave-maker colony size was marginally significant ($p = 0.045$) and had a weak positive effect on the number of brood stolen (Table 1.1B). Model selection eliminated any models containing the interaction term.

(d) Relative Colony size

When all raiding trials are pooled, the relative colony size (Table 1.2), between a slave-maker colony and a target colony, is not an important determinant of raiding success. For the first part of the hurdle model, i.e. the binary response, model-averaging resulted in a model that included all factors, with slave-maker colony size as the most important factor (relative importance = 0.95). For comparison, the relative importance of relative colony size (i.e. the interaction between slave-maker and target colony sizes) was 0.18 (Table 1.2A). However, none of the factors had significant p-values (Table 1.2A).

The results of the second part of the hurdle model, i.e. non-zero data, also fail to support the importance of relative colony size in raiding success (Table 1.2B). Relative colony size (i.e. the interaction between slave-maker and target colony sizes) was eliminated in model selection. The final model-averaged results indicate that number of workers in the host nest was the most important factor, with a small negative effect ($p < 0.001$, estimate = -0.030, relative importance = 1.00; Table 1.2B). QAICc values used in model selection were calculated with the overdispersion parameter c-hat = 6.757.

Costs

None of the host nest characteristics were strong predictors of slave-maker mortality, however slave-maker colony size had a weak effect (Table 1.3). In all models of mortality, high AIC values eliminated the interaction term between target and slave-maker colony sizes before model-averaging due to AICc criteria. In the binary part of the hurdle model, none of the factors were significant predictors of mortality, but of those in the final model, slave-maker worker number had the highest relative importance (0.84) and a positive effect ($\beta = 1.161$; Table 1.3A). A similar pattern results from the proportion mortality model, in which slave-maker worker number also has the highest relative importance (1.00), but a weak negative effect ($\beta = -0.161$;

Table 1.3B). The count model poorly explained the variation in mortality, as the best model (i.e. the model with the lowest AICc score) was the null model, containing none of the predictor variables.

Discussion

T. americanus colonies experience differential benefits based on the characteristics of the colony they raid. The most influential factor in the success of raids was the absolute number of host workers defending the target nest (Tables 1.1 & 1.2). The more host workers defending a nest, the fewer brood a slave-maker colony was able to steal (Fig 1.3A & 1.4). This is consistent with the prediction that better defended hosts will lead to lower pay-offs for the parasite.

One the other hand, the number of brood in the nest was not universally a strong predictor of brood stolen. The number of brood in the nest was a strong positive predictor of stolen brood at nests with small to moderate numbers of workers (20 workers; Fig 1.3B). However, when brood varied along with worker number (in 1:1 worker-to-brood nest trials), the effect of worker number at high levels (i.e. 100 workers) made any benefit of high brood numbers obsolete because those brood were inaccessible (Fig 1.4).

As expected, minimizing worker-to-brood ratios had a positive effect on the number and proportion of brood stolen (Fig 1.3). The natural variation in these ratios from census data reveal that slave-makers could benefit by discriminating hosts based on worker-to-brood ratios, however the margin of benefit may be quite small. There is a strong correlation between the number of workers and brood in natural host nests (mean ratio = 1.34±0.19; Fig 1.1), confirming that there is a biological constraint on the range of possible worker-to-brood ratios. Hence, nests with extreme ratio values are unlikely to be found (i.e. few workers with many brood and vice versa). The importance of this constraint is that it places upper bounds on what the optimal host size can be. Because brood number will likely track worker number, maximizing brood is limited by a slave-maker colony’s capacity control the host workers in that same nest. Given these results, slave-maker colonies will do best to avoid the best defended nests, even though they are likely to contain more brood. This constraint also suggests that slave-makers ought to avoid very small targets because those nests are likely to contain very few brood. Thus, moderately sized hosts appear to maximize profitability.

Being a larger slave-maker colony does not grant easy access to the largest host nests, although it does ease the challenge slightly (Tables 1.1 & 1.2). However relative colony sizes between the host and parasite never played a significant role in raiding success in my trails. Differential success based on slave-maker colony size alone indicates that colonies could scale their preferences based on their own size, although the failure of relative colony size to influence success suggests that any preference adjustments would have minimal effects. Instead, the strong negative effect of host nest defensibility suggests that slave-makers ought to universally reject very large colonies, but does not conclusively suggest that all slave-maker colonies, regardless of size, should have the same host preferences.

It is unclear from this study whether the slight advantage given to larger slave-maker colonies is due to their enhanced success in combat and/or due to their advantage in retrieving brood. More detailed analysis of the behavioral interactions between slave-makers and their target colony could reveal how colony size confers this advantage. Depending on the details of combat, further investigation could reveal at which host colony sizes there are diminishing returns for invasion, and refine predictions about the optimal host size. Given that several slave-maker species have adapted chemical strategies to deal with more numerous workers at the site of attack (Lenoir et al. 2001), it is possible these chemical strategies dilute the added benefit of increased fighting power. Knowledge of the specific effects of propaganda pheromone on the dynamics of combat between differently sized colonies, as suggested by Franks & Partridge (1986), would clarify the effect of relative colony size on raiding success.

Contrary to expectations, host nests characteristics (i.e. worker number, brood number, etc.) did not predict rates of slave-maker mortality during raids. Patterns of mortality were instead weakly, but positively, associated with the size of the slave-maker colony itself. These results indicate that mortality rates have less to do with the features of the raided nest, and more to do with features intrinsic to the slave-maker colony. The prediction that large slave-maker colonies would experience lower mortality rates than smaller colonies was not satisfied, suggesting that larger colonies are not necessarily any better at protecting themselves from nest defenders than smaller ones. Because slave-maker colonies do not appear to pay a mortality penalty for raiding larger nests, colonies should be indifferent to host size as it pertains to risk.

These mortality findings also suggest that workers in larger colonies take more risks. Other studies on social insects show that older, and therefore larger, colonies expose themselves to greater risk. For example, younger harvester ant colonies will more readily cease foraging when patrol workers fail to return, whereas mature colonies are less likely to stop or reduce foraging rates under the same conditions (Gordon 1987). On the other hand, larger colonies may simply experience greater risk because more total slave-maker workers participate in raids than in small colonies.

These findings here are inconsistent with the conclusions reached by Pohl & Foitzik (2011), which found that *T. americanus* colonies prefer nests containing high worker numbers. As with other behavioral tests of host preference, Pohl & Foitzik (2011) could not robustly distinguish preference from discovery bias since slave-makers rarely sampled both options before making their choice. The next obvious step is to investigate whether slave-maker colonies behaviorally demonstrate a preference for the predicted nest characteristics.

Other studies investigating the optimal host choice for social parasites have only examined species which attack as a lone individual. A study of a social parasite on Allodapine bee colonies showed that the parasite always preferred the largest colonies (Smith & Schwarz 2008). This contrast may be due to the fact that the parasite peacefully co-exists with host and never initiates attack, suggesting defensibility is unimportant to the parasite; however, the findings of this study also could not distinguish detectability from preference. The only other study of social parasite host preference, on *Polistes sulcifer*, had results more consistent with the findings here (Cervo & Turillazzi 1996). The parasite preferred larger colonies, but had a stronger preference for nests with the highest density of pupae, regardless of nest size. Like *T. americanus*, the parasite attacks the host colony, but will usurp the nest for herself, suggesting that host defensibility is relevant in optimal host choice for this species, as well.

Although not considered in this study explicitly, distance is likely to weigh into the balance of costs and benefits of what constitutes an optimal raiding target. Although I found no effect of distance in this study, measures of costs and benefits of distance in the lab are unlikely to capture the true threats that more distant nests pose, other than energy expenditure. Experiments in rock ants demonstrate that distance is an important factor for colonies in selecting a new nest site, but that they weigh nest volume and integrity higher than distance (Franks et al. 2008). This may not be the case with slave-makers, since the colony must also transport brood between the site of attack and home in multiple trips, which is potentially more risky and energetically expensive than a single relocation event.

Another potential consideration for slave-makers is the aggression of the host colony, which has been shown to vary considerably both between and within populations (Jongpier et al. 2014; Kleeburg 2015; Pamminger et al. 2011). More aggressive colonies defend their nests more effectively and cause more harm to slave-maker workers. It is unclear at this point how a scout might evaluate the aggression level of a colony without being attacked herself, thereby preventing recruitment entirely. Nonetheless, it has the potential to be an important consideration for a slave-maker colony should they be capable of this assessment.

Even though aggression may be difficult to measure for a slave-maker worker, gathering information about worker number and brood number are also likely to be challenging. But given there are benefits to this knowledge prior to initiating an attack, it is plausible that slave-maker nervous systems have evolved sensitivity to various odors associated with workers and their brood. Work is currently underway to investigate the mechanisms by which slave-maker workers can detect these relevant nest features and whether they behaviorally respond to stimuli of different worker or brood numbers.

The determinants of optimal host choice in the slave-making ant are similar to those involved in other parasites. Attacking as a group had little to no effect on the outcomes of host selection, and are inconsistent with the patterns of infection shared by other parasites that attack as a group (i.e. pathogens, *Myrmecocystus* ants). Further knowledge of the specific attack strategies of *T. americanus* will help explain why parasite size has little influence over host choice. Finally, by establishing the features of an optimal raiding choice, future research on slave raiding behavior can look deeper into the mechanisms for how colonies reach decisions over where to raid, and whether the features of host value or defensibility are communicated or deliberated over as a colony.

Figure 1.1: The contents of natural *T. longispinosus* nests are plotted, with worker number on the x-axis and brood number on the y-axis. Brood counts consist of larvae and worker pupae, excluding reproductive pupae. Brood number is positively correlated with worker number, however most points fall above or below the line of regression (R^2 =0.15). The majority of nests contain fewer than 75 workers but can contain as many as 300.

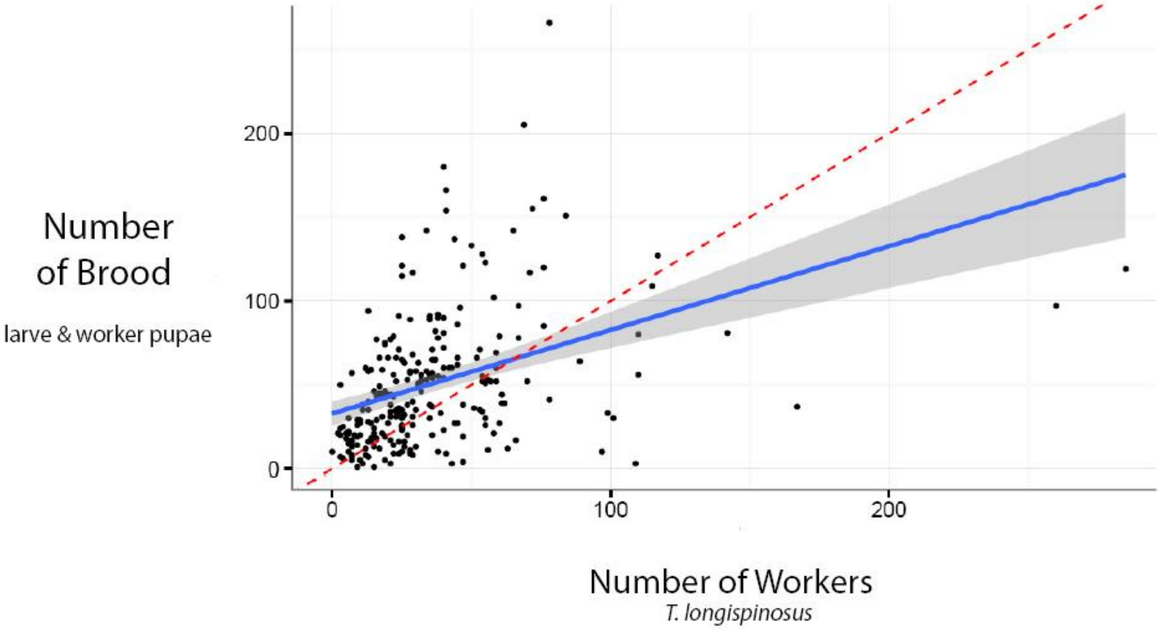

Figure 1.2: Slave-maker reproductive success in terms of male and female sexuals (alates) plotted is positively correlated with the number of host workers in the colony (p<0.0001, n=264 colonies).

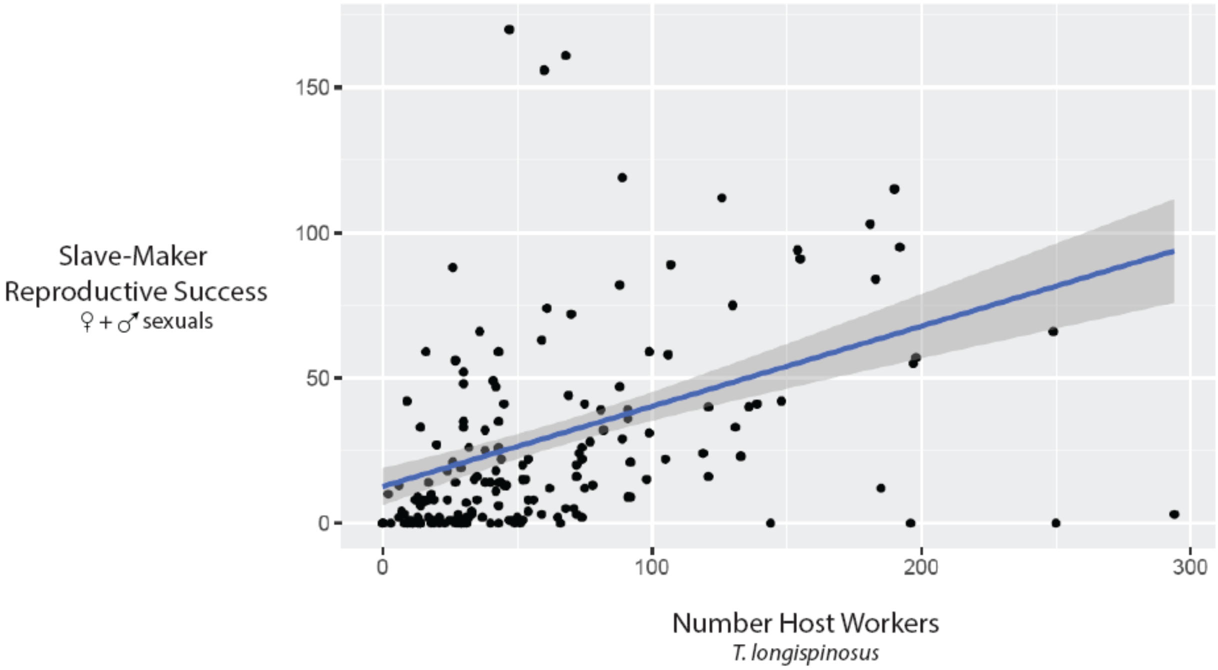

Figure 1.3: Slave-maker colonies obtain more brood when raiding nests with lower worker-to-brood ratios. Worker-to-brood ratios are indicated on the x-axis and underneath are the actual number of workers and brood used to create the corresponding ratios. Box-plots refer to the median and 25% and 75% quantiles. (A) Nests contained 25 workers, but differed in the number of defending workers, with either 25 or 50 workers per nest (25 workers, 1:1 ratio; p = 0.0468, n=19). (B) Nests contained 20 workers, but differed in the number of brood items, with either 20 or 60 brood per nest. A greater total number of brood is stolen from nests that contained more brood (60 brood, 1:3 ratio), even though they were matched for number of workers defending the nest (p = 0.0014, n=19 raids).

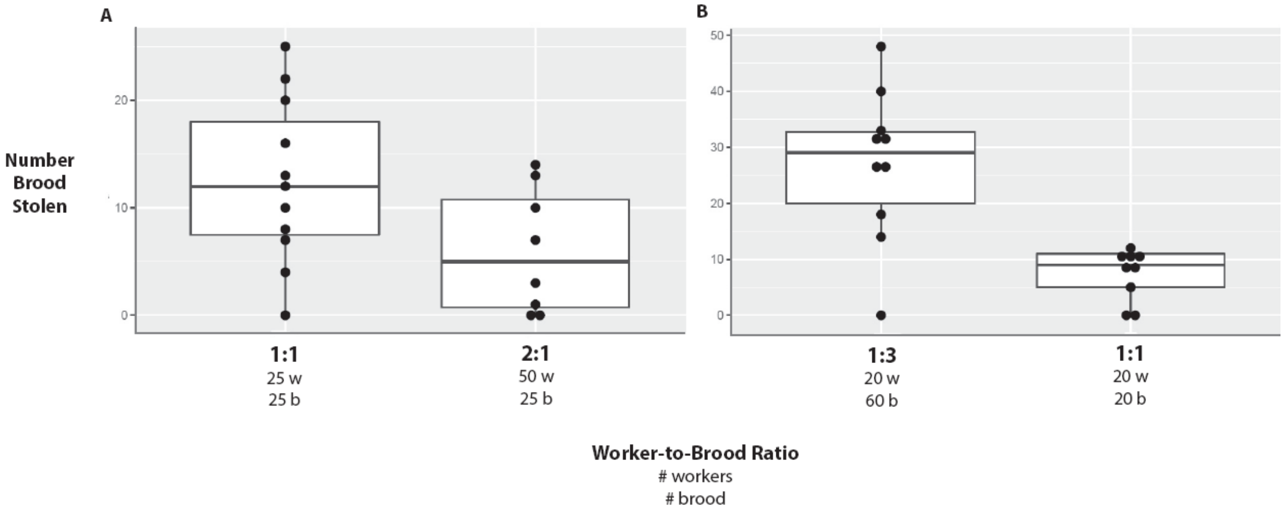

Figure 1.4: Absolute number of stolen brood is plotted for trials at nests containing 1-to-1 worker-to-brood ratios, but which differ in their size. Target nests contained either 25, 50 or 100 workers and brood items. Box-plots refer to the median and 25% and 75% quantiles. Although nests of 100 or 50 contained more total brood, raids at nests containing 25 workers and brood resulted in higher numbers of brood stolen (p <0.0001, n= 14 raids).

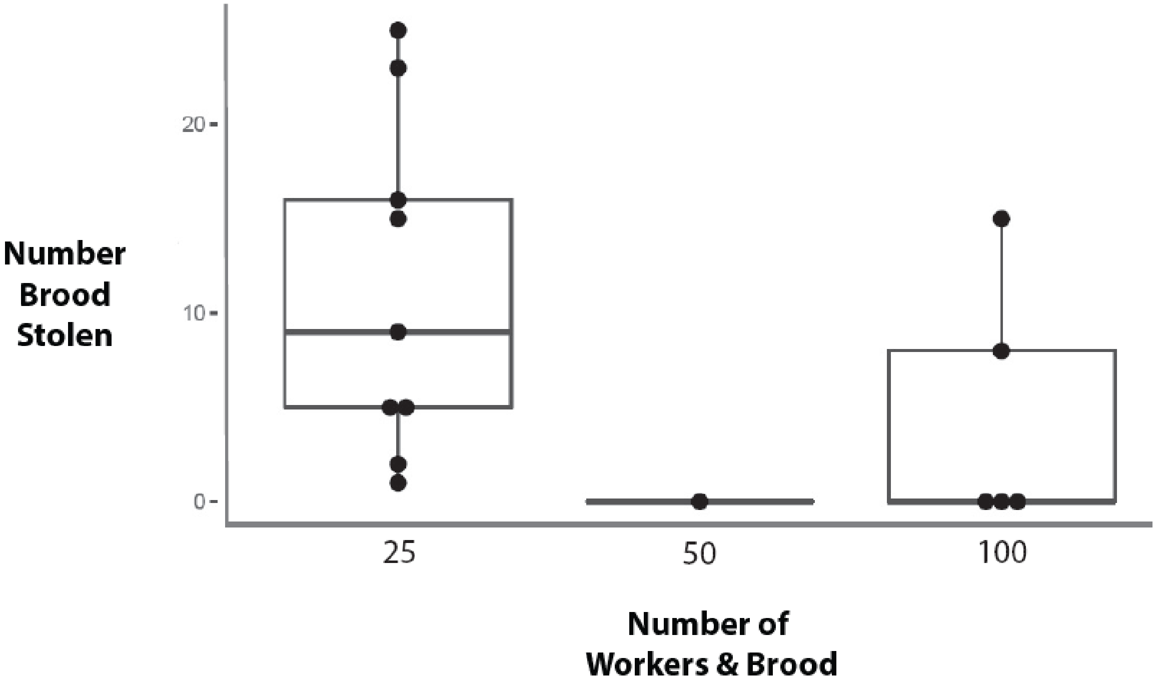

Table 1.1: Factors affecting brood stolen as (A) a proportion and (B) as an absolute number, from nests of varying sizes with 1:1 worker-to-brood ratios. Summary of results from model averaging of each global model. N = 14 trials

Parameters		**Standardized Estimates (±SE)**	**95% Confidence Interval (2.5,97.5)**	***Z***	***P*-value**	**Relative Importance**
Importance of Worker Number? **A) Proportion of Brood Stolen** GLOBAL MODEL: Proportion Brood Stolen ~ Host Workers + Slave-Maker Workers + Host Workers x Slave-Maker Workers						
Intercept		0	0	N/A	N/A	N/A
Host Workers		-0.044 (±0.005)	-0.055, -0.034	8.287	< 0.001	1.00
Slave-Maker Workers		0.021 (±0.004)	0.012, 0.029	4.801	< 0.001	0.32
Importance of Brood Number? **B) Absolute Number of Brood Stolen** GLOBAL MODEL: Number Brood Stolen ~ Host Brood + Slave-Maker Workers + Host Brood x Slave-Maker Workers						
Intercept		0	0	N/A	N/A	N/A
Slave-Maker Workers		0.084 (±0.038)	0.002, 0.167	2.006	0.045	0.47
Host Brood		- 0.070 (±0.042)	- 0.161, 0.020	1.527	0.127	0.41

Table 1.2: Factors influencing brood stolen for all trials. Due to zero-inflation, data were analyzed in a hurdle Model: (A) First, with brood capture as a binary response variable (Yes/No), and (B) then, as a continuous variable, using only non-zero data. N = 57 trials.

Parameters		**Standardized Estimates (±SE)**	**95% Confidence Interval (2.5,97.5)**	***Z***	***P* - value**	**Relative Importance**
Hurdle Model: Binary Response GLOBAL MODEL: Capture (Yes/No) ~ Slave-Maker Workers + Host Workers + Host Brood + Slave-Maker Workers x Host Workers + Host Workers x Host Brood						
Intercept		0	0	N/A	N/A	N/A
Slave-Maker Workers		3.343 (± 2.427)	-1.501, 8.187	1.353	0.176	0.95
Host Workers		- 2.470 (± 1.891)	-6.242, 1.302	1.283	0.199	0.80
Host Brood		-1.699 (± 1.607)	-4.900, 1.503	1.04	0.298	0.46
S-M x H Workers		1.932 (± 4.526)	-7.112, 10.978	0.419	0.675	0.18
Host W x Brood		3.209 (± 4.687)	-6.160, 12.578	0.671	0.502	0.06
Hurdle Model: Continuous, Non-Zero Data Global Model: Proportion Brood Stolen ~ Slave-Maker Workers + Host Workers + Host Brood + Slave-Maker Workers x Host Workers + Host Workers x Host Brood						
Intercept		0	0	N/A	N/A	N/A
Host Workers		-0.030 (± 0.003)	-0.036, -0.023	9.005	<0.001	1.00
Slave-Maker Workers		0.005 (± 0.003)	-0.002, 0.012	1.47	0.141	0.22
Host Brood		-0.004 (± 0.003)	-0.011, 0.003	1.153	0.249	0.20

Table 1.3: What contributes to Slave-Maker mortality? Due to zero-inflation, data were analyzed in a hurdle Model: (A) First, with mortality as a binary response variable (Yes/No), and then, only using only non-zero data, (B) with mortality as a proportion of slave-maker colony size and (C) with mortality as a total count per trial. N = 67 trials.

Parameters	**Standardized Estimates (±SE)**	**95% Confidence Interval (2.5,97.5)**	***Z***	***P* - value**	**Relative Importance**
A)Hurdle Model: Binary Response GLOBAL MODEL: Death (Yes/No) ~ Slave-Maker Workers + Host Workers + Host Brood + Slave-Maker Workers x Host Workers + Host Workers x Host Brood					
Intercept	0	0	N/A	N/A	N/A
Slave-Maker Workers	1.161 (± 0.669)	-0.176,2.500	1.702	0.089	0.84
Host Workers	1.107 (± 0.740)	-0.370,2.584	1.469	0.142	0.70
Host Brood	0.095 (± 0.700)	-1.300,1.486	0.134	0.894	0.20
S-M x H Workers	-0.390 (± 1.593)	-3.573,2.794	0.240	0.810	0.12
B) Hurdle Model: Non-Zero Data as Proportion Global Model: Proportion Mortality ~ Slave-Maker Workers + Host Workers + Host Brood + Slave-Maker Workers x Host Workers + Host Workers x Host Brood					
Intercept	0	0	N/A	N/A	N/A
Slave-Maker Workers	**-0.161 (± 0.062)**	**-0.288,-0.034**	**2.490**	**0.013**	**1.00**
Host Workers	0.088 (± 0.079)	-0.073,0.249	1.075	0.282	0.61
Host Brood	0.080 (± 0.067)	-0.056,0.215	1.157	0.247	0.37
S-M x H Workers	0.072 (± 0.109)	-0.150,0.295	0.641	0.521	0.13
C) Hurdle Model: Non-Zero Data as Count Global Model: Number of Deaths ~ Slave-Maker Workers + Host Workers + Host Brood + Slave-Maker Workers x Host Workers + Host Workers x Host Brood					
Intercept	0	0	N/A	N/A	N/A
Host Workers	0.148 (± 0.180)	-0.218, 0.514	0.793	0.482	0.41
Slave-Maker Workers	0.136 (± 0.162)	-0.195, 0.466	0.804	0.421	0.39
Host Brood	0.156 (± 0.162)	-0.174, 0.485	0.927	0.354	0.34
S-M x H Workers	0.242 (± 0.265)	-0.300, 0.783	0.876	0.381	0.04

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CHAPTER 2

COLLECTIVE DECISION-MAKING IN SLAVE-MAKING ANTS IS OPTIMIZED FOR HOST COLONY QUANTITY, NOT QUALITY

Abstract

While the mechanisms underlying collective decision-making are under intensive study, very little is still known about how collective decision-making evolves. Convergent evolution in the mechanisms of collective decision-making across taxa and levels of biological organization suggest that common principles guide optimal decision strategies. To investigate the role of ecology in shaping mechanisms of collective decision-making, I study collective decision-making the slave-making ant *Temnothorax americanus* as a contrast to existing model systems. *T. americanus* colonies make collective decisions over where to conduct raids, in which they attack heterospecific ant colonies to steal their brood. I begin by describing a colony's sampling tactics and preference function using choice experiments, which show that colonies use a sequential-choice strategy with low acceptance thresholds for host colony size. I then investigate what suite of ecological conditions have favored the evolution of this decision strategy using theory developed for mate choice. I test whether the conditions known to favor sequential-choice are met by *T. americanus* by making behavioral observations of raids and by measuring demographics of colonies in the field. The data presented here show that raiding is constrained by the timing of host brood development and low encounter rates of host colonies. Variation in host nest quality, however, is unlikely to favor low acceptance thresholds of slave-maker colonies. The implications of these results are that slave-maker colonies are maximizing their

payoff by raiding as many nests as possible, and not by selectively exploiting the few most profitable ones.

Introduction

Collective behavior presents a puzzle when adaptive patterns arise from local interactions that are not dictated by any single leader or blueprint (Camazine et al. 2001). An analogous puzzle exists at the ultimate level, where it is not obvious how selection operates on local interactions to generate behaviors that are adaptive (i.e. fitness enhancing) at the group level (Sumpter 2010). Even though a mechanistic understanding of collective behavior deepens our evolutionary understanding (Boomsma & Franks 2006), we also need to be informed about the ecological context of collective behavior if we want to understand how it has been shaped by selection (Couzin & Krause 2003, Gordon 2014). Relevant aspects of ecological context may include environmental factors, the nature of the group’s problem (e.g finding food, nest-site, defense) and the relative weighting of certain trade-offs (e.g speed-accuracy, quality-quantity, etc.). By linking ecological context to specific mechanisms of collective behavior, we can begin to build a framework for predicting when certain mechanisms will evolve, potentially exposing principles governing collective organization.

There is already evidence that convergent mechanisms of collective behavior have evolved in response to similar problems (Visscher 2007, Marshall et al. 2009, Jeanson & Deneubourg 2009, Reid et al. 2015). The most well-studied example of convergence occurs in the problem of nest-site selection, in which both colonies of honeybees and ants reach collective decisions over new nest sites using convergent processes (e.g. independent assessment of options, positive feedback via recruitment, cross inhibition and quorum sensing; reviewed in Visscher 2007). Even outside of the context of nest-site selection, comparable processes take place between neurons in the mammalian brain during sensory processing (Shadlen & Newsome 2001) and in groups of microbes during the production of virulence factors (Ross-Gillespie & Kümmerli 2014). The similarities in how these disparate systems make decisions are of interest because they suggest that selection has repeatedly arrived at the same solution to the problem of collective decision-making. To what extent do these similarities represent optimal solutions that generalize beyond the details unique to each system's ecology and basic biology?

One shared feature among these different systems is their use of the best-of-n decision strategy (Visscher 2007, Seeley & Buhrman 2001, Shadlen & Newsome 2001), which involves sampling the environment and selecting the best option among all sampled possibilities. While certain ecological conditions are known to favor the best-of-n decision strategy over others, such as the sequential choice strategy (Janetos 1980, Real 1990), it remains unclear to what extent the specific details of each system's ecology and basic biology will fine tune the mechanisms of decision-making within a given decision-making strategy. For example, the relative importance of speed over accuracy (Chittka et al. 2009) in a given problem will certainly shape the mechanisms that promote or hinder speed in any decision-making strategy.

The question of how ecology shapes mechanisms of collective decision-making remains relatively unexplored because work in the field of animal behavior has focused predominantly on nest-site selection in social insects (Visscher 2007), a problem that is fundamentally similar across the systems studied so far. To investigate the role of specific ecological factors in shaping mechanisms of collective decision-making, I investigate collective decision-making in a novel context by studying a slave-making ant. Slave-making ant colonies make collective decisions over where to conduct a raid, during which the slave-maker colony attacks a host colony to steal the young (i.e brood; Hölldobler & Wilson 1990, Buschinger 2009). As with nest–site selection, brood raiding requires that a colony select a specific location in the local environment and physically go to there as a group. However, brood raiding may differ in that the trade-offs among variables, such as time, energy expenditure, accuracy and risk, may be weighed differently given that raiding is a more short-term decision and includes the risks of combat (Franks & Partridge 1993; Kleeburg et al. 2014). Taking advantage of the similarities and differences from nest-site selection, I investigated the mechanisms of raid-site selection to create an informative comparison.

I focus this study on *Temnothorax americanus*, an obligate slave-making ant native to the temperate forests of eastern North America (Wesson 1939, Alloway 1979, Alloway & Del Rio Pesado 1983). As a first step, I investigated the decision-making strategy (i.e. best-of-n versus sequential choice) used by slave-making ant colonies over where to initiate a raid. Since decision-making strategies are defined by both sampling tactics and preference functions (Widemo & Saether 1999), I examined both in *T. americanus* colonies using choice experiments. If colonies sample multiple different options before initiating a raid at the most preferred option, even if the best option is found first, then they satisfy the predictions of the best-of-n strategy. If, however, colonies choose the first option they encounter that meets a quality threshold, then they exhibit a sequential choice decision strategy. A distinguishing prediction between these two strategies is that colonies may chose the first nest they found in sequential choice, but not in best-of-n. Finally, colonies may also exhibit a strategy of universal acceptance, which predicts colonies will always raid the first nest they find.

Prior work suggests that slave-maker colonies maximize their net pay-off from a raid (maximizing brood acquired while minimizing mortality) by targeting host colonies of intermediate size (Miller, in prep). To achieve the highest payoffs, I expected slave-maker colonies to display a stabilizing preference function (i.e. where extreme values are considered unfavorable; Edward 2015, Jennions & Petrie 1997) and to enact a best-of-n decision-making strategy that locates these best nests. But, contrary to these expectations, experiments detailed here revealed that slave-maker colonies are non-choosy, and deviate from the best-of-n strategy that is widely used in nest-site selection. These findings raise the additional question of why slave-maker colonies are not more selective in their decisions over where to raid.

Extensive theoretical and empirical work on mate choice (Real 1990, Janetos 1980, Jennions & Petrie 1997), optimal foraging (Stephens & Krebs 1986) and acceptance thresholds (Reeve 1989) have established a general framework to predict which ecological conditions should favor different acceptance thresholds and sampling tactics. According to the hypotheses proposed by the above decision-making models, non-choosiness is expected to evolve under the following conditions: (1) when the search costs are high (Real 1990), which may manifest as low encounter rates (Reeve 1989) or high time constraints (Janetos 1980); or (2) when the variability in the quality of the options is low (Real 1990) or unpredictable across patches (Stephens & Krebs 1986). To test how well *T. americanus* colonies meet the conditions for low-choosiness, I first tested the prediction that encounter rates are low by measuring the latency to discover host colonies and their spatial distribution in the field. To place the encounter rates in a temporal context, and test the prediction that time constraints are high, I also measured the duration of the time period that host brood are available to steal. To account for the possibility that time constraints could also come from the slave-maker colony, I compared the timing of slave-maker worker brood development in relation to host brood availability. To test the non-mutually exclusive hypothesis that low or unpredictable variability in the quality of host colonies favors low choosiness, I mapped and censused the contents of host colonies in the wild.

In summary, I distinguish which strategy of decision-making slave-making colonies employ in decisions over where to raid host colonies. In addition, I evaluate the role of spatial and temporal ecology in shaping selection on the strategy of collective decision-making in *T. americanus*.

Methods

Colony Collection and Maintenance

Temnothorax americanus uses three host species: *T. longispinosus*, *T. ambiguus*, and *T. curvispinosus*. The populations measured in this study consisted almost exclusively of *T. longispinosus* hosts colonies, thus all experimental host colonies were *T. longispinosus*. Slave-make and host colonies were collected at the Huyck Preserve (42° 30' 53.7876", -74° 8' 27.7836"), Cornell Natural Areas (42° 27' 55.2738", -76° 26' 34.299"), Black Rock Forest (41° 25' 11.64" , -74° 0' 33.48") and Bear Mountain State Park (41° 18' 33.976", -74° 0' 14.680") between April and October, 2013 – 2016. Colonies were transported back to Liddell Labs in Ithaca, NY in Ziploc bags and stored in a refrigerator (4ºC) for up to three weeks before being censused for the following: queens, workers, mature sexuals, larvae, and pupae (worker and sexual).

Immediately after being censused, each colony was allowed to move itself into an artificial nest. Artificial nests consisted of a piece of balsa wood or plexiglass (2.5 mm thick) with a circle cut-out to form the interior cavity (22 mm in diameter) and a slit along one side to serve as the entrance (2 mm x 4 mm). This structure was placed between 2 microscope slides (5 cm x 7.5 cm) secured with masking tape and then wrapped in aluminum foil to exclude light. Each colony was housed in a separate plastic Ziploc box (15 cm x 15 cm x 6 cm) with a plaster floor, which was moistened twice per week to maintain humidity levels. Colonies were fed a teaspoon of Bhatkar diet twice per week and housed in a room at 26ºC, with a 14L:10D day:light cycle.

Two-choice Trials

I conducted two-choice tests of slave-maker preference for host colony size in rectangular glass arenas measuring 45cm x 25cm (Fig 2.1A). The rims of the 13 cm high walls were coated with Vaseline to prevent ants from escaping. I placed a slave-maker colony at one end of the arena, and on the opposite wall (~40 cm away), I placed two host (*T. longispinosus)* colonies: one with 25 workers and the other with 50 workers; colonies were matched for brood with a mixture of larvae and pupae for a total of 25 brood items. Host colonies occupied an acorn or hickory nut with a single entry hole that never exceeded 2 mm. In each trial, both options were presented in the same conditions, either in acorns or hickory nuts, but I alternated which corner (left or right) the host colonies occupied between trials.

On the morning of a raiding trial, the room temperature was elevated to 30ºC, and a food dish and wet cotton were placed in the center of the arena to prevent dehydration or starvation during the trials. Immediately after placing colonies in the arena, I video recorded the entire arena from above with a webcam (Logitech C920 HD Pro). By 16:00 EST, if there were no slave-makers recruiting to or attacking a target nest, then the session was terminated and the colonies were removed from the glass arena and returned to their boxes until the next day. The colonies were given a maximum of five days to initiate a raid before the trial was aborted. If there was evidence of recruitment or a raid (i.e. a host nest was vacated, there was a raiding party in the arena, or slave-makers were entering or exiting a host nest), I left the colonies in the arena overnight to complete brood transport and collected them the next morning, so that colonies stayed in the arena for 24 (±4) hours. The raiding room was set to the same 14L:10D lighting regime as the room housing colonies, and I lowered the temperature to 26ºC during darkness. The morning following a raiding attempt, I dissected both host nests and the slave-maker nest to determine which host nest had been raided and whether the raid had been successful. I also watched videos of each raid to determine the time at which each nest was discovered and raided. A raid was defined as initiated when at least one slave-maker ant entered a nest and sent the host colony into alarm. Between trials, the floor and lower walls of the arena were washed with acetone and a paper-towel to remove any chemical cues.

Prior to raids, all slave-maker colonies experienced the same conditions to standardize colony state, since this might influence raiding behaviors. Two days prior to raiding, I removed all brood and some adult slaves from the colony so that there was a 1:1 ratio of slave-makers (including the queen) to slaves. Excess slaves were relocated to a temporary nest without slave-makers until after the raiding trial was complete. During the removals, each slave-maker worker was individually marked with RC Car Pactra Paint, with dots on the abdomen, thorax and head. Ants were isolated in vials for at least four hours while the paint dried before being reintroduced to the rest of the colony. Two days later, slave-maker colonies were used in raiding trials.

To assess *T. americanus* colonies' preference for host colony size, I compared raiding choices using a chi-square goodness of fit test. All statistical analyses were performed in R version 3.1.2. Choice was scored as the first nest the colony chose to raid. To test that the preference function depends on the slave-maker's colony size, I compared raiding preference against slave-maker colony size using a logistic regression.

To determine which decision-make strategy *T. americanus* colonies employ, I recorded searching and nest discovery events prior to each raid. The best-of-n model predicts that slave-maker colonies will sample both nests before raiding, whereas the sequential choice model predicts that slave-makers may raid the first nest they find.

Microcosm

I created a microcosm of the raiding environment by filling plastic collapsible swimming pools (240 cm diameter) with ca. 3 cm of moist top soil and then coating the pool's rim with Vaseline to prevent ants from escaping. I placed a single slave-maker colony in the center and arranged 8 host colonies to surround it (Fig 2.1B). The microcosm included four small host colonies (10 workers, 10 brood) and four large host colonies (50 workers, 50 brood). They were placed either near (38 cm) or far (107cm) from the central slave-maker colony to create four classes of host options. I arranged colonies so that there was an equal number of large and small ones at both near and far distances. Each colony was housed in the plexiglass nests described above. I checked the contents of each colony's nest daily at the same time for three weeks. I judged a raid to have taken place if on a given day both the slave-maker nest contained brood and a host's nest was either empty or had reduced brood.

Prior to placing a slave-maker colony in the arena, all of its larvae or slave pupae were removed from its nest so that newly acquired brood from raids could be readily identified. Care was taken to avoid using host nests from the same collecting locality within a trial to prevent the problem of ants from the same polydomous colony from reuniting in the same nest structure and disrupting the treatment options. To standardize slave-maker colonies, I selected colonies that contained six slave-maker workers and adjusted the *T. longispinosus* slave number to 24.

I positioned eight plastic dishes each containing a tablespoon of Bhatkar diet evenly throughout the arena and replaced them with fresh food once per week. Water was added to the soil ad libitum to keep it visibly moist. Two microcosm habitats were in the same room as the lab colonies so they automatically experienced the same day/night cycles and temperature. The three remaining microcosm habitats occupied different rooms, but all five microcosm arenas experienced the same temperature and day/night for cycle.

To characterize preference function for host colony size, I compared each slave-maker colony's raiding choices using a chi-square goodness of fit test, in which each nest has equal probability of being raided. Although some slave-maker colonies raided multiple host colonies over several weeks, only the host colony chosen on a slave-maker colony's first raid was used to calculate preference. In three trials, the slave-maker colony raided two nests in one day, and both raids were counted in the chi-square tests as separate choices. I also counted the rate at which raids took place to test predictions of different sampling and decision strategies.

Field Plots

I searched for slave-maker colonies in natural areas that had not undergone collection prior to my census to ensure naturalistic measures of nest density and distribution. These areas were the same as my collecting sites: including the Cornell Natural Areas, the Black Rock Forest and Bear Mountain State Park. I mapped each plot by marking the area within a 3 m radius of a slave-maker colony's nest and systematically opening every possible nesting structure. It is unknown at what maximum distance slave-makers will conduct raids, but I have observed natural raids in the field at approximately 3 m from a slave-maker colony's nest; it is possible that colonies raid at greater distances. When either a *T. longispinosus* or *T. americanus* nest was found, I poured the nest contents into a Ziploc bag with a handful of leaf-litter for later census and placed a flag where the nest had been found. After I had searched the whole area, I measured the distance of each flag from the center of the plot, i.e. where the slave-maker colony's nest was located, using a laser distance measure (Bosch, DLR130K) and measured the angle using a digital compass on an iPhone 6. Ziploc bags were stored in a refrigerator (4ºC) for up to two weeks before the contents of each nest were recorded: number of queens, workers, and brood type.

Testing Predictions of Decision Strategies

(1) Encounter rates

To evaluate the prediction that encounter rates will be low for slave-maker colonies under natural conditions, I derived a hypothetical field encounter rate based on lab and field observations. I derived the mean discovery rate in videos of the two-choice trials by calculating the time between when the first scout leaves home and when a host colony is first discovered (n = 13 trials). The density of nests in the two-choice trials in a 45 cm x 25 cm arena was 17.78 nests per square meter. I also calculated the mean density of host colony nests in the field using data from the field plots census by dividing the number of host colony nests at each plot by the area of the plot ($\pi*(3m)^2 = 28.274\ m^2$).

(2) Time Constraints

To evaluate the prediction that time constraints limit the selection of a host colony, I collected host nests from the three locations indicated above over the course of the warm season, between 11 April and 14 September 2013 – 2016. I created a brood development timeline by counting the number of larvae and pupae, and their relative proportions, within each nest at the time of collection. I did the same for slave-maker colonies between 29 May and 25 August during the same years and from the same locations. I pooled the data from all locations and years to maximize the continuity of the data over the course of the season.

To test whether slave-makers are developmentally ahead of their hosts in terms of brood development, I ran a permutation test by doing the following. First, I built a second order polynomial regression (quadratic), using the “lm” function in the R “stats” package, with proportion of brood as the dependent variable and day of the year as the predictor. Using the resulting best-fit equation (intercept and coefficients) using the “polynomial” function in the “polynom” R-package (Venables et al. 2016), I solved for the day at which the curve is a maximum (i.e. when the derivative is equal to zero). I repeated this for both the slave-maker and host nests, and then calculated the difference between peaks (day of host max – day of slave-maker max), such that a positive value indicated host development lagging behind that of slave-makers. To run the permutation test, I randomly assigned species identity across the data-set using the “runif” function in the “stats” package in R, and recalculated the difference between species in 999 permutations to create a null frequency distribution of differences with which to compare the original value.

I also tested whether species identity was a significant predictor of how the proportion of pupae varies with time by running a GLMM and model selection with AICc criteria using the “MuMin” package in R (Barton 2016). The global GLMM had a binomial distribution to allow the response variable to be a two vector proportion. The model’s fixed effects were day, species ID and their interaction and the random effects were year and location. To compare the effect sizes between variables, I standardized beta-coefficients by multiplying the raw beta-coefficients from the model by the variable’s standard deviation.

(3) Quality Distribution within Plots

To evaluate the prediction that variation in the quality of options is low, I calculated the variance-to mean-ratios, the interquartile ranges, and the coefficients of variation for each of the mapped field plots.

(4) Variability between plots (predictability)

To evaluate the level of variability, and thus predictability, between plots, I compared the variation in host colony nest contents between the mapped field plots. I calculated the mean and standard error of the following: the number of nests per plot, the number of workers and brood per nest per plot, and the total number of brood and workers per plot. I also calculated the variance-to-mean ratio for the total brood and workers across plots to describe the variation between plots. Finally, I conducted Levene's to statistically test for homogeneity of variance across plots, using both the total number of brood and the total number of workers between plots.

Results

Choice Trials: Two-choice

Slave-maker colonies demonstrated no preference when presented with a binary choice between large (50 workers) and small (25 workers) host colonies. I conducted 19 trials using 18 slave-maker colonies, but video records of the raids were available for 15 of them. Although video was unavailable for these four trials, slave-maker preference could be determined in three of them because the colony raided only one nest; however, the lack of video made it impossible to determine whether these slave-maker colonies ever found both nests. In 63% (12/19) of trials, both host colonies were raided within a 24 hour period. Considering only the colony's first choice, slave-makers demonstrated no preference in host colony size (Fig. 2.1A; Chi-square = 0, df = 1, p = 1.0), and choice was independent of slave-maker colony size (i.e. number of slave-maker workers; logistic regression, $p = 0.305$, $\beta_{size} = 0.01789$, $R^2 = 0.010$, df = 13). Contrary to the prediction for the best-of-n strategy, slave-maker colonies raided the first nest they found in 73% (11/15) of the trials. When both nests were found prior to the raid (4/15 trials), the larger colony was chosen in three out of four trials. In these three cases, the smaller colony was found first, and the reverse is true in the one case where the small colony was chosen.

Choice Trials: Microcosms

Seven out of the 11 microcosm replicates had evidence of raids. Considering the first raiding choices only (i.e. that occurred within a 24 hour period), four out of seven microcosms raided one nest at a time, but the remaining three microcosms raided two nests in one day, so these raids were pooled for a total of ten raids. Slave-maker colonies did not preferentially raid one type of target nest over the others (Fig. 2.1B; Chi-square = 4.4, df = 3, p = 0.221) and there was no preference for size class regardless of distance (Chi-square = 0.4, df = 1, p = 0.527). Although colonies tended to raid near nests over far ones, this trend was not significant (Chi-square = 3.6, df = 1, p = 0.058).

Indicators of sampling tactic did not support one model over another. On average, slave-maker colonies took 2.7 ± 0.75 days to raid from the start of the experiment (range = 1 – 7), not including colonies that never raided. Five out of the seven slave-maker colonies raided a second time a mean 1.0 day later (range = 0 - 4), and three colonies raided a third time a mean 3.0 days later (range = 1 – 5).

Testing Predictions of Decision Strategies

(1) *Encounter rates*

The hypothetical rate at which slave-maker workers are expected to encounter host nests in the field is low, taking 7 days, 7 hours, 48 min to find one nest (632889 sec/nest) on average (see Table 2.1 for derivation), with a hypothetical minimum of 3d, 7h, 33m and maximum of 11d, 8h, 29m, as derived from the lower and upper confidence intervals of lab search time. The confidence interval of encounter rates is comparable when substituting the upper and lower bounds of field density instead: Lower = 5d, 12h, 20m (476427.5 sec); Upper = 10d, 21h, 51m (942718.3 sec). This encounter rate is relatively low the context of time available to raid, which is detailed below.

(2) *Time Constraints*

The time period during which slave-makers may gain larvae or pupae is limited to about 90 days in New York state (~May 15th to ~August 20th; Fig 2.2C). Although larvae are available throughout this period, the proportion of brood that are pupae is at a maximum for roughly 60 days (~June 15th to ~August 15th; Fig 2.2B). When considering the total quantities of brood (both larvae and pupae), brood numbers peak for ca. 50 days (~30 May to ~ 20th July 20th), and the overlap between peaks in total brood number and the proportion that are pupae occurs for ca. 35 days (June 15th – July 20th).

The timing of brood development in slave-maker colonies coincides with the peaks in pupal abundance in host colonies. The proportion of brood that were pupae was fit to a quadratic function in both slave-maker ($p = 0.011$, $R^2 = 0.039$, $F_{(2,174)} = 4.583$, Fig 2.2A) and host nests ($p < 0.001$, $R^2 = 0.464$, $F_{(2, 313)} = 137.2$; Fig 2.2B). Slave-maker pupae mature earlier than those of their hosts' by approximately 30 days (Fig 2.2 A & B), and this difference is significant according to the permutation test ($p = 0.022$, N=999 permutations). Model selection of a GLMM of the same data confirm that species identity and day of the year, and their interaction, were significant predictors of the proportion of a colony's brood that were pupae (global model: weight = 1, AICc = 8839.4). Species ID also had the largest effect size ($\beta_{Species} = -141.237$, $\beta_{Day} = -0.001105194$, $\beta_{Day*Sp} = 0.07035971$).

(3) *Quality Distribution*

The quality distribution of nests within a plot tends to be over-dispersed, contrary to the prediction that variation in the quality will be under-dispersed when acceptance thresholds are low. The index of dispersion (variance-to-mean ratio) for each plot is greater than 1 for nest contents in terms of both brood and workers (Table 2.2). Other measures of variation, including interquartile range and the coefficient of variation, are consistent with relatively high levels of variation between nests within a plot.

(4) Variability between plots (predictability)

The variation between plots in the quality and abundance of nest options is moderate, but not statistically significant. Across all plots (n=9), there are 6 (± 0.8 SE) nests per plot and the mean host nest contains 25.7 (±2.6) workers and 35.9 (±3.9) brood items (Fig 2.3). The mean total number of workers per plot is 154.2 (±92.4) and the mean total number of brood is 215.7 (±39.2). The variance-to-mean ratio between plots is 64.13 for brood and is 14.93 for workers. In terms of brood, the variation between plots is marginally significantly different (Levene's test: p = 0.050, df = 8, F =2.0881, n= 63), and not at all significant for the number of workers (p = 0.440, df = 8, F= 1.007, n = 61).

Discussion

The collective decisions-making strategies of slave-making ant colonies were described and classified according to their preference function and sampling tactics. Raiding patterns from the two experiments suggest that slave-makers use a low acceptance threshold for nest size, with a preference function that accepts nests with as few as 10 workers. Given prior work (Miller, in prep), slave-maker colonies were expected to prefer nests containing 50 workers over those containing 25 or 10 because of the expected higher payoff from larger nests. However, slave-maker colonies do not demonstrate a preference for any nest size in both the two-choice and the microcosm experiments. Colonies demonstrate a slight preference for closer nests in the microcosm, suggesting that distance might be a more important attribute than size. In spite of this pattern, this non-significant trend does not distinguish between a bias in encounter probability and true preference, since workers from host colonies may leave chemical cues of their presence by walking around the nest exterior (Wüst & Menzel 2016).

Observations of searching and discovery rates from the same experiments solidly reject the best-of-n decision model. As predicted by the sequential choice model, slave-makers frequently raided the first nest to be discovered in the two-choice experiment (73% of trials) and often raid both if given the time, suggesting instead an opportunistic strategy. It is unlikely that slave-makers are using a best-of-n strategy because of the rarity with which slave-maker colonies discover more than one nest prior to mounting an attack (26% of trials), and thus do not have the opportunity to make comparisons. Results from the microcosm also support either a sequential choice model or a universal acceptance model, in that slave-makers raided multiple nests within a single search period. Although about half of the microcosms had raids spaced several days apart, this may be due to low encounter rates rather than lengthy deliberation periods. While the results point to a sequential choice strategy, they do not firmly distinguish from the universal acceptance model. The experiments here do not explore the lower range of the acceptance threshold, so it is unclear if such a threshold exists and where it lies. Furthermore, the fact that slave-maker colonies rejected the first host colony they found, although in rare cases, more strongly supports a sequential-choice strategy over universal acceptance.

To address the ultimate reasons why slave-maker colonies employ this strategy, I tested for conditions that favor low acceptance thresholds as predicted by mate choice theories. Low acceptance thresholds are predicted to evolve when encounter rates are low (Reeve 1989, Jennions & Petrie 1997), and this is consistent with the rate at which scouts from slave-maker colonies encounter host nests. Hypothetical encounter rates were calculated from measures of host nest density in the field and encounter rates in raiding arenas of the two-choice experiments. These derived rates revealed that it might take on average up to seven days before the colony finds a single nest. At that rate, colonies would need to wait an additional 7 days before finding a second nest with which to make a comparison. Whether this encounter rate is small or large depends on the time period during which colonies have to make such comparisons and reach decisions.

Results on the phenology of brood development show that raiding is bound by high time constraints. Encounter rates are thus very low relative to the time available for raiding. The period during which moderately developed brood is available to steal, lasting about 50 days (Fig 2.2C), is relatively brief compared to the time needed to find multiple nests and make comparisons. This raiding window is even shorter, about 35 days, if slave-maker colonies limit their raiding to periods when the abundance of pupae is at its highest (Fig 2.2B). At the end of this raiding window, the available pupae will have matured into adults, diminishing the value of host colonies by increasing the number of defending workers and reducing the brood to steal (Miller, in prep). If slave-maker colonies take seven days on average to find a nest and they have between 50 and 35 days of optimal raiding time, then colonies could raid between five and seven colonies over the course of this period if they raided every nest they found. This raiding frequency is closely matched by estimates derived from genotyping slave workers in *T. americanus* colonies, which estimates six raids per season (Foitzik & Herbers 2001). Given these calculations, delaying raids in order to make the comparisons required by a best-of-n strategy results in high opportunity costs.

Further evidence that brood phenology is an important feature in the life-history of slave-maker colonies is in the relative timing of slave-maker and host brood maturation. I present correlative evidence that slave-maker pupae mature into adults at the time when host pupae approach maximum abundance. This asynchrony in brood development provides slave-makers a boost in colony size with which to conduct raids, and poises them to take full advantage of this peak in host pupae. The closeness in timing of these phenological events highlights the relevance of timing in the slave-maker system, and has been important in other social parasites, as well (Wcislo 1987).

In addition, low acceptance thresholds are predicted to evolve when there is low variability in the quality of different options (Real 1990). Data on field demographics, however, show that variability is not in the direction predicted for low acceptance thresholds. On the contrary, the quality distributions of host nests are highly variable. As a result, the opportunity to find higher quality nests through continued searching exists and is unlikely to be the reason that slave-maker colonies have low acceptance thresholds. While variability within plots is expected to play a role, so is variability between plots. Low predictability in the number and/or quality of options is expected to favor low acceptance thresholds and reduced sampling rates. This prediction is moderately supported, as the variation between plots is relatively high in terms of the number of brood available per plot.

In summary, these results show that the costs of searching play a major role in shaping decision-strategies, but that the quality distribution is relatively less important. The implications of these results are that slave-maker colonies are optimizing their payoff by raiding as many nests as possible, and not by selecting only the most profitable ones, as was initially expected. It would be of interest to see whether such time constraints are relaxed in other regions within the range of this species (Kleeberg et al. 2015). For example, longer warm seasons in the more southern range could create the opportunity for a second round or extended period of pupal abundance in the host colonies. Reduced time constraints could favor more choosy slave-maker colonies, but this assumes other ecological factors, like food abundance or quality distribution, remain fixed. It would also be interesting to see whether other species of slave-making ants are equally non-choosy and follow a similar decision-making strategy, especially in species with larger colony sizes. As obligate social parasites, *T. americanus* have small colony sizes relative to their non-parasitic counterparts (Wesson 1939), which helps explain their low encounter rates on a mechanistic level. If finding a host nest is so time consuming, it begs the question why slave-maker colonies are not larger to increase discovery rates. However, the advantages of increasing slave-maker worker production may not pay if they outweigh the benefits of the parasitic life-style, in which more energy is devoted to producing reproductives in place of workers.

Although I was unable to find evidence of complex information processing by the colony as a whole, distributed processing may still be of interest in other systems of sequential choice. In some cases, sequential choice may still require that groups integrate and evaluate options from multiple sources, following a threshold preference function. The way in which a threshold is encoded by a distributed system is not intuitive, and could still be of worthwhile study in systems where it is expected. Such systems would include cases where there is a low cost of searching, high variability between options or when the costs of assessing variation are high. For instance, foraging via group recruitment (Dornhaus & Powell 2010) is less likely to be limited by seasonal time constraints and can thus afford to have a higher acceptance threshold.

Future research into the evolution of collective behavior, whether it be decision-making, regulation or construction, should carefully consider the specific currency that selection is optimizing in shaping collective traits. In addition, they should consider how to classify collective behaviors in terms of their behavioral strategies for the purpose of more fruitful comparisons across taxa. Much work is yet to be done to disentangle the mechanistic generalities that give rise to collective behaviors across systems and which mechanisms results from the idiosyncrasies unique to each species.

Figure 2.1: Physical lay-outs of both choice experiments (below) and the results of each (above). In both experiments, choice was defined as the first nest to be raided. (A) The 2-choice experiment gave a slave-maker colony a choice between two host colonies containing 25 workers or 50 workers (n = 16). (B) The microcosm experiment gave a slave-maker colony the choice between colonies of two sizes (50 or 10 workers) placed at near and far distances (n = 7). Close nests containing 50 workers were selected most frequently, followed by close nests containing 10 workers, although there were not significant.

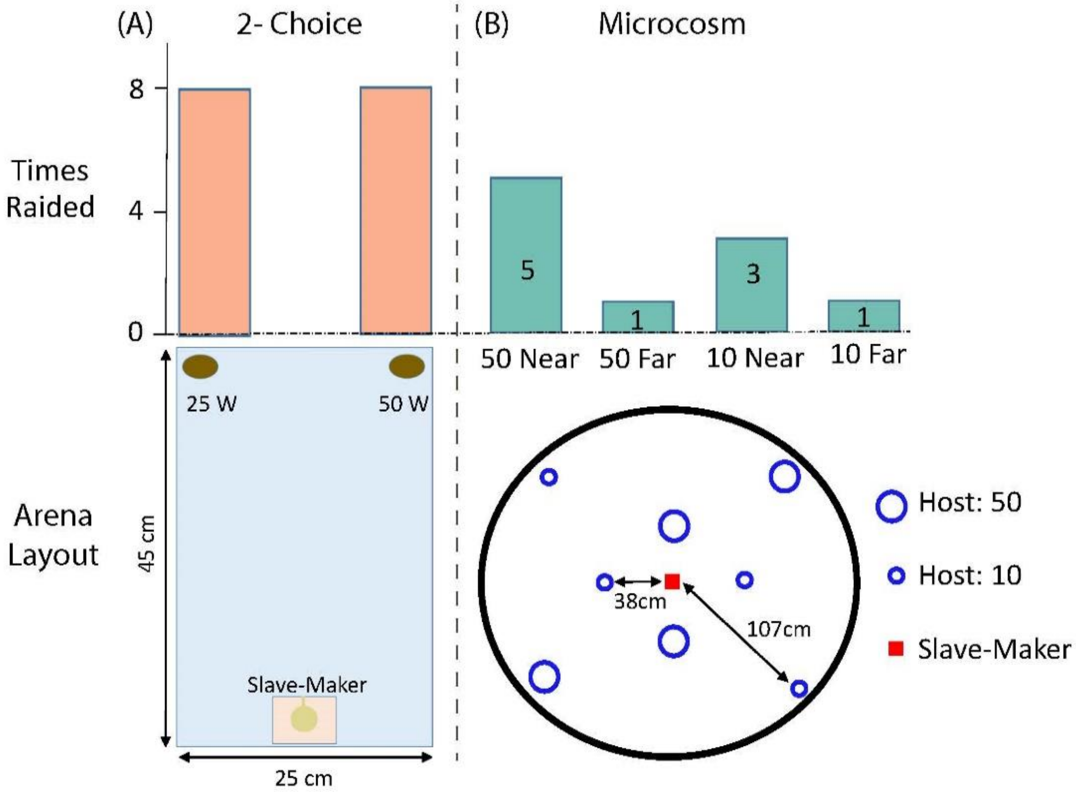

Figure 2.2: Data on brood are plotted with respect to day of the year so that brood abundance can be tracked across the season. Data from three years and three locations are pooled, and location is indicated by color. (A) The proportion of brood that were worker pupae in slave-maker nests is fit to a quadratic function and 95% confidence intervals are shown in grey. The predicted value at which the proportion of pupae are at a maximum is day 168. (B) The proportion of brood that were worker pupae in host nests is fit to a quadratic function. The predicted value at which the proportion of pupae are at a maximum is day 198. (C) The total number of brood, including larvae and both worker and sexual pupae, is plotted over time.

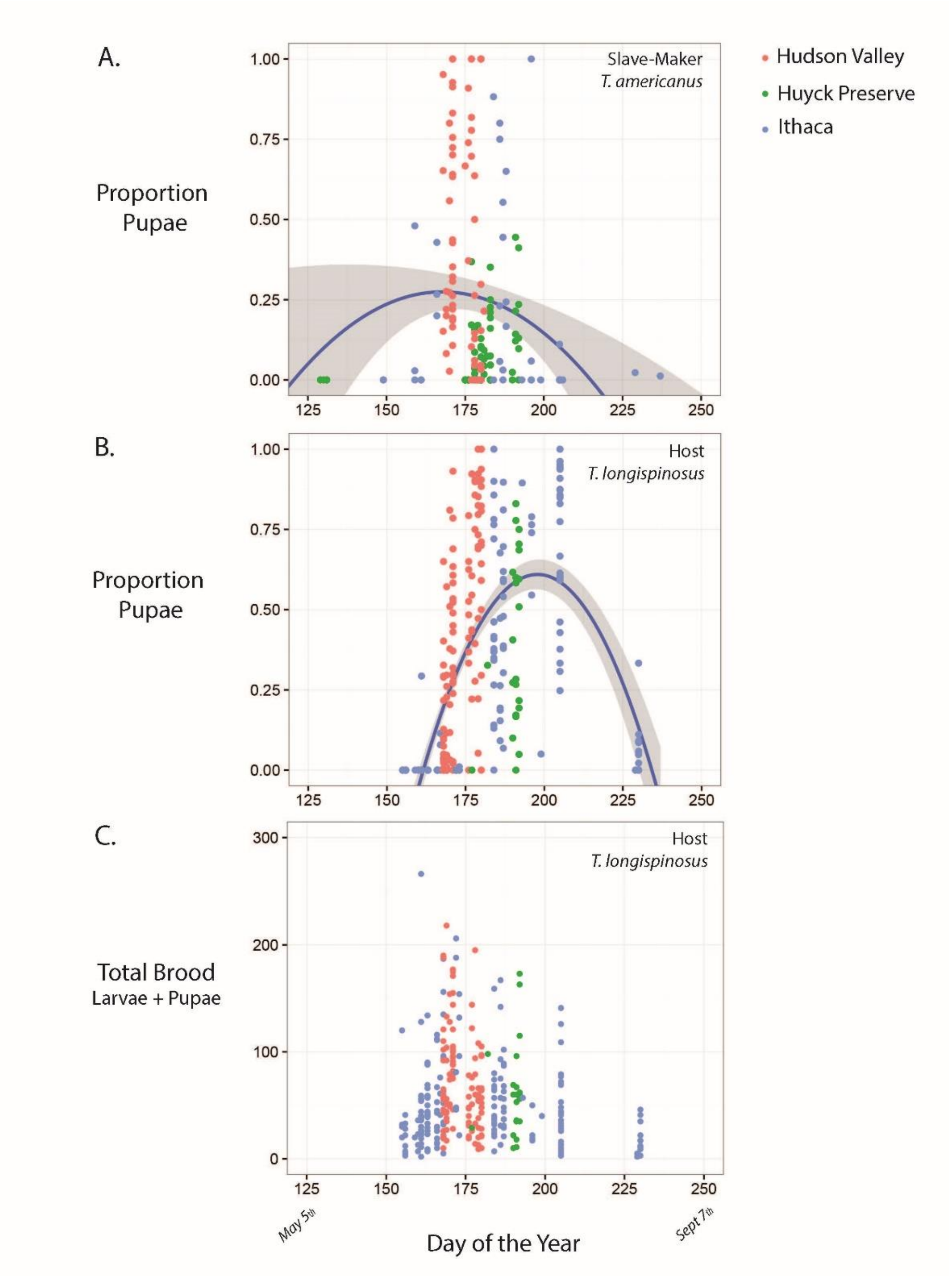

Figure 2.3: Maps representing field plots depict the spatial arrangement and abundance of host nests surrounding a slave-maker nest within a 3m radius.

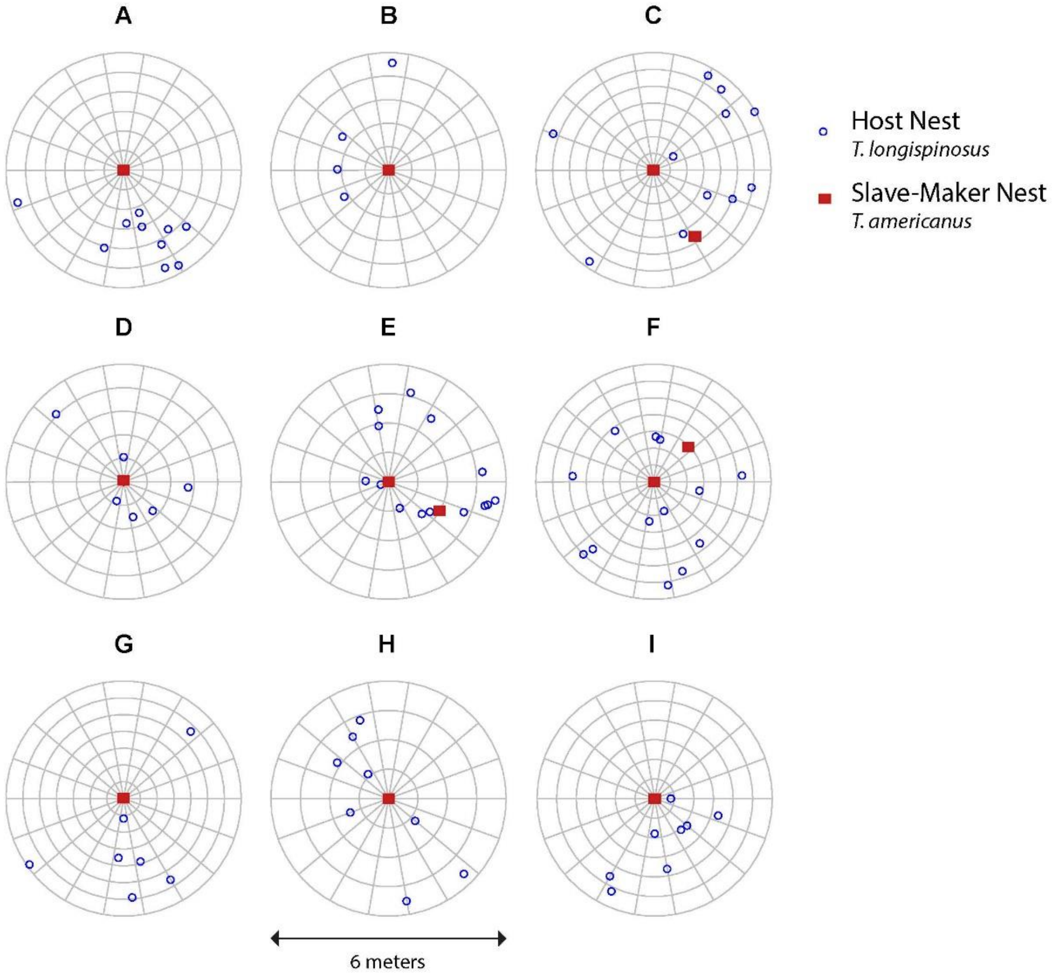

Table 2.1: Values for host density and encounter rates with host nests are tabulated for lab and field data. Encounter rate in the field (in bold) is calculated from the actual values, summarized in this table. The bottom row details the calculations used to derive field encounter rates.

	LAB	**Field**
Host Nest Density	17.78 nests/m^2	0.21 nests/ m^2 SE = ± 0.03
Encounter Rate **(i.e. time to discovery)**	7476 sec/nest SE = ± 1880.5	**632889 sec/nest** Upper 95% CI =980965.7 sec/nest Lower 95% CI = 286394.3 sec/nest
Calculations	**Field Encounter Rate** * Field Density = Lab Encounter Rate * Lab Density $Field\ Encounter\ Rate = 7476\,\frac{\text{sec}}{\text{nests}} * 17.78\,\frac{nest}{m^2} * \frac{1m^2}{0.21\,nests}$	

Table 2.2: For each field plot, three measures of the variation in host nest contents are summarized in terms of worker and brood counts.

Plot ID	**WORKER** Number of workers per nest			**BROOD** Number of brood per nest		
	VMR Variance-to-Mean Ratio	Interquartile range	Coefficient of Variation (SD/mean)	VMR Variance-to-Mean Ratio	Interquartile range	Coefficient of Variation (SD/mean)
A	19.20	22.8	0.914	6.90	17.5	0.516
B	1.49	13.0	0.269	3.38	24.0	0.341
C	2.64	8.0	0.286	8.04	33.0	0.449
D	6.73	19.0	0.807	15.96	36.0	0.636
E	19.78	43.0	0.707	10.41	35.0	0.601
F	13.26	40.5	0.652	43.61	82.0	0.938
G	8.04	25.0	0.680	17.58	51.5	0.743
H	11.96	29.5	0.706	26.80	64.5	0.851
I	24.99	46.0	0.875	34.44	78.3	0.728

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CHAPTER 3

CONFLICTING GENETIC INTERESTS IMPAIR COLLECTIVE RAIDING BEHAVIORS IN SLAVE-MAKING ANT COLONIES

Abstract

The process by which collective behaviors evolve is non-intuitive, since selection most often acts on individual units to favor group-level phenotypes. Theory predicts that cooperation is an essential ingredient to align genetic interests and facilitate selection at the group level. Here I test this prediction by investigating the extent to which collective behavior depends on cooperation levels. Using the collective raiding behavior of a slave-making ant, I explore the corollary prediction that selfish behavior impairs group performance. I manipulated cooperation levels in slave-maker colonies by elevating worker reproduction, and measured their performance in raids staged in the lab. I also characterized the shape of the benefit function for raiding and describe the sub-tasks of ‘herding’ and ‘door guarding’ associated with raiding in an attempt to link changes in individual behaviors to collective outcomes. Elevated worker reproduction led to a shift in raiding strategy: scouts raided solitary rather than as a collective. Although selfish colonies did not recruit nest-mates, they gained commensurate quantities of brood as control colonies. The raiding benefit function adopted a concave down shape, with the gains at their greatest when there were at least two slave-maker workers. However, the frequency of door guarding was a more important predictor of raiding success than was raiding party size. The results here illustrate that selfishness can interfere with the expression of collective behavior, but that the resiliency of a collective behavior to selfishness may stabilize defection and limit selection at the group level. Establishing if and how conflicting genetic interests impairs

collective behavior will be a critical first step in understanding how collective behaviors evolve as group-level adaptations.

Introduction

Despite extensive work to elucidate the mechanisms that give rise to collective animal behaviors, relatively less is understood about how collective behaviors evolve. By classifying collective behaviors as either ‘cooperative’ and ‘coordinating,’ Sumpter (2010) brings to light the different ways selection may shape collective behaviors as they relate to individual behaviors. In cooperative collective behaviors, individuals have evolved to perform a costly task because they gain a net benefit from the outcome of the collective behavior itself. This type of collective behavior is characteristic of social insect colonies, like ants or termites, where altruistic workers gain indirect benefits from novel functions provided by a collective behavior (Hölldobler & Wilson 2009). In the other class, coordinating collective behaviors, individuals produce a collective behavior as a by-product of acting in their own self-interests. The costs that an individual pays to perform the behavior are repaid as a direct benefit from coordinating with the group, but individuals have not evolved costly behavior specifically to optimize group performance. Some examples of coordinating collective behaviors include the synchronized movement of schools of fish and flocks of birds, where an individual reduces its chances of predation by coordinating its movement with the group, but has no genetic interests in how well another fish blends in with the group’s movement (Ioannou et al. 2012). Sumpter’s (2010) classification system is useful in that it clarifies the role of aligning genetic interests for the evolution of collective behaviors. Similar points have been made in in the context of the major evolutionary transitions, in which aligning genetic interests is thought to facilitate the joining of lower level units into groups that are defined by their collective traits (Maynard-Smith & Szathmáry 1995). One of the major drivers of this shift in selection is the promotion of cooperation and the elimination of competition (Michod & Herron 2006).

Despite these theoretical arguments, competition is still a divisive feature common to many social groups (Bourke 2011, Ratnieks & Reeve 1992), and still exerts influence even in highly cooperative groups (Boomsma & Franks 2006). Given the abundance of examples where individual genetic interests are neither completely aligned nor completely opposing, the dichotomy proposed by Sumpter (2010) fits better as two ends along a spectrum. The effects of cooperation and competition on collective behaviors in the middle regions of this spectrum are almost completely unstudied and it remains unknown how collective behaviors take shape in the face of the opposing genetic interests. While a few studies have established that conflict has costs to group-level efficiency (in social insects – Mattila et al. 2012, Cole 1986; slime molds – Foster et al. 2002), there has been no work to date that analyzes the influence of conflicting genetic interests on the expression of collective behaviors in cooperative groups. In cases where conflicting interests in collective behavior have been considered, studies are limited to classes of ‘coordinating’ collective behaviors, where individual level selection dominates. For example, studies on how troops of baboons (Strandburg-Peshkin et al. 2015) reconcile conflicting preferences about the direction of group movement have shown that individuals compromise to maximizes group cohesion to maintain the benefits of group living; but the benefits of group living are not achieved via the movement per se. Existing empirical work, therefore, cannot tell us how such conflict interest will affect the evolution of collective behaviors in otherwise cooperative groups.

In the theoretical realm, however, there has been more progress on this question. Within-group variation in costly cooperation has been the central focus in models of public goods games and collective action problems (Frank 2010, Gavrilets & Fortunato 2014), but this work has not been extended to the study of collective behaviors specifically. Furthermore, predictions from these models are limited in their scope because they do not take in to account biologically relevant factors, like the effect relatedness within groups (Gavrilets 2015). Most existing models predict that the highest ranking individuals should invest the most cooperative effort in collective action problems (Olson 1965, Frank 2010, He et al. 2014); however, this pattern contradicts the common pattern in cooperative groups, where the dominants contribute the least to the public good (Keller & Reeve 1994).

If collective behaviors are sensitive to the group's mean cooperative effort in the first place, the degree of sensitivity will likely depend on the mechanistic details of the particular collective behavior. The nature of this dependency can be represented more abstractly as a benefit function, which depicts the rate at which total group cooperative effort is converted into group payoff (Fig 3.1; West et al. 2015, Gavrilets 2015). A collective behavior that become less effective in direct proportion to the loss of cooperative effort would have a linear shape (Fig 3.1 – dotted line). In contrast, a collective behavior that is only achieved after some threshold of cooperative effort would have a nonlinear shape (Fig 3.1 – blue and orange lines). This occurs when the cooperative investments of the group members combine synergistically to produce the collective behavior, and its associated payoff to the group. These relationships have been described by Maynard-Smith & Szathmary (1995) using the metaphor of rowing versus sculling. In rowing, two passengers in a boat can each paddle on only one side at a time and can only move forward if both passengers paddle, so their interactions are synergistic. Using this metaphor, rowing produces a nonlinear benefit function with respect to cooperative effort. In sculling, each passenger has a pair of paddles, so the boat will move forward even if only one passenger paddles, although they will move slower than if both paddled; this is characteristic of a linear relationship. The shape of a nonlinear benefit function will further dictate the impact of variation in a group's cooperative effort. To extend this paddling metaphor further, if ten passengers find themselves in a very large boat, then all of them will need to paddle to make the boat go forward. In this imaginary scenario, if one passenger refrains from paddling, then the boat will be immovable, as is represented in the orange, concave-up curve in Fig 3.1. On the other hand, if ten passengers are in a very small boat, and one passenger refrains from paddling, the boat will continue to move forward at about the same speed. Only after seven of the passengers stop rowing will the boat begin to slow, as is represented by the blue, concave-down curve in Fig 3.1. This means that collective behaviors are expected to vary in what level of cooperative effort they require (the size of their boat) and how threshold-like those requirements are, since collective behaviors can span the range between the extreme cases illustrated here. The implications of these dependencies are that changes in cooperative effort will not have identical effects on all collective behaviors, but will depend on how the cooperative efforts of group members combine to produce a collective behavior. Despite the implications for these different payoff structures for the evolution of collective behaviors, we are lacking empirical evidence that explicitly shows how the benefits from collective behavior are realized in relation to the overall cooperative effort of the group (but see Michod 2007).

To test the hypothesis that collective raiding behavior depends on levels of cooperative investment by group members, I investigated collective raiding behavior in the slave-making ant, *Temnothorax americanus*. Slave-making ant colonies attack the nests of their hosts in coordinated group raids, during which they gain possession of the host's brood to populate their slave workforce (Buschinger 2009). Slave raiding is a collective behavior that can be broken down into the cooperative efforts of individual and easily measured for its payoff to the group as number of brood stolen. Slave-making ants are known to have naturally high levels of worker reproduction (Blatrix & Herbers 2004, Foitzik & Herbers 2001), which means some individuals use group resources for selfish gain (i.e. direct reproduction of males) while reducing their cooperative effort. Prior work on these ants and other slave-making ants with similar life-histories has shown that reproductive workers are weak cooperators; they leave the nest to search for hosts less often (Pohl et al 2011, Blatrix & Herbers 2004) and partake in fewer raid-related activities, including following scouts and fighting (Bourke 1988), however, their effect on coordinated behaviors and colony-level outcomes are untested.

I manipulated levels of cooperation by activating worker ovaries, but measure cooperative investment by characterizing each worker's costly participation in raids. First, I measured the impact of cooperative investment on the expression of collective behavior, i.e. at a mechanistic level (Birch 2012). Then, I described how the benefits of raiding change in relation to a colony's total cooperative effort in raiding activities by mapping the shape of the benefit function for raiding. In addition to the global view provided by the benefit function, I characterized how selfish behavior manifested during raids. To do so, I described the function of different sub-tasks during raiding in an attempt to link individual behaviors to the benefit function. I then measured the effects of cooperation on these sub-tasks, thereby relating individual behaviors to the group's behavior.

Methods

Colony Collection & Maintenance

Colonies of *T. americanus* and their host species, *T. longispinosus*, were collected at the Huyck Preserve (42° 30' 53.7876", -74° 8' 27.7836"), Cornell Plantations Land (42° 27' 55.2738", -76° 26' 34.299") and Bear Mountain State Park (41° 18' 33.9768", -74° 0' 14.6808") between May and July, 2014 – 2016. Colonies were transported back to Liddell Labs in Ithaca, NY in Ziploc bags and stored in a refrigerator (4C) for up to three weeks before being censused. Following census, each colony was placed inside a nest box containing an artificial nest. Artificial nests consisted of a balsa wood or plexiglass U-shape structure placed in between 2 microscope slides held together with masking tape. Nest were then wrapped in foil to exclude light and housed in a plastic Ziploc box (10 x 10 cm) with a plaster floor, which was moistened twice per week to maintain humidity levels. Colonies were fed a teaspoon of Bhatkar diet twice per week and housed in a temperature controlled room at 26 C, with a 14L:10D daylight cycle.

Raid Set-up

In all trials, the temperature was elevated to 30C the morning of the raid, and a plastic dish of food and moistened cotton were placed at the center of each glass arena to prevent dehydration or starvation during the trials. The upper rims of the 13 cm high walls were painted with Vaseline to prevent ants from escaping. If by 4pm, there were no slave-makers recruiting to or attacking the target nest, both colonies were removed from the glass arena and returned to their boxes until the next day. The colonies were given a maximum of five days to initiate a raid before the trial was aborted. If there was evidence of recruitment or a raid (i.e. the target nest had vacated, there was a raiding party in the arena, or slave-makers were entering or exiting the target nest), I left both colonies in the arena overnight and collected them the next morning, so that colonies stayed in the arena for 24 (±4) hours. The raiding room was set to the same lighting regime as the room housing colonies, and I lowered the temperature back to 26C to coincide with darkness.

Cooperation Experiment

To test the effect of cooperation on the collective raiding behavior of *T. americanus,* I artificially boosted the level of selfish investment in reproduction in experimental colonies. I then compared their performance during raids to that of control colonies. To elevate the level of selfish reproductive investment, I performed a temporary sociotomy, i.e. split the colony into two equal parts, to give middle-ranking slave-maker workers the opportunity to ascend the dominance hierarchy. Previous work in other ant species has shown that middle and high ranking individuals ascend the dominance hierarchy when the queen or most dominant worker was removed, and that their reproductive systems respond in kind to these social changes through enhanced ovary development (Ito & Higashi 1991; Heinze & Smith 1990). For the purposes of keeping splits even, queens were counted towards slave-maker workers. I then allowed the two colony fractions to exist separately for 3 weeks. Three weeks was a conservative estimate of how long it would take subordinate workers to develop their ovaries, as this effect took 5-6 days in another slave-making ant with similar life history (*Harpagoxenus sublaevis*, Bourke 1988), and 2 weeks in *Diacamma* ants (Peeters & Tsuji 1993).

I confirmed that the treatment had the intended effect of elevating worker reproduction by dissecting each worker's abdomen under a dissecting microscope. For each worker, I measured the length of each ovariole using a micrometer eyepiece at 20x magnification and summed the length of each ovariole to assign each worker an ovarian development score. I then computed a mean ovarian development score for the colony by summing each score and then dividing by the number of workers in that colony. Using this metric, I compared mean ovarian development between treatment and control colonies using a Mann-Whitney U test.

After the separation period, I marked each slave-maker on the abdomen, thorax and head with RC Car Pactra Paint, and let each dry in a vial for at least four hours. I then re-untied the two halves of the colony into a single nest with a fraction of their host workers to standardize the host-to-slave-maker ratio at 1:1 and removed any brood. The ants were allowed to move in to an observation nest (same as described below for hosts) and left them overnight. On the following morning, I observed colonies in a dissecting microscope in three 10 minute sessions to record dominance interactions, which included: antennal boxing, food solicitation, biting, or standing on top of another ant.

One to three days before a raid, host nests were prepared by randomly selecting 25 workers and 25 brood items (a mixture of larvae and pupae) from an existing *T. longispinosus* lab colony. When source colonies contained insufficient brood, I added brood from other lab colonies, but always from colonies collected from the same population, and they were readily accepted. The workers were then allowed to move in to an observation nest of standard size, consisting of 2.5 mm thick balsa wood with a 22 mm diameter circle and 1 mm entry way placed between two 5 cm x 7.5 cm microscope slides. A piece of clear red plastic filter was taped to the top microscope slide to make it appear dark to the ants but visible to the researcher.

Raiding trials took place in containers consisting of 2 glass rectangular boxes (45 cm x 25 cm) connected by a paper bridge measuring 10 cm in length and bottom of the glass arenas were lined with white construction paper. On one end of the arena, I placed the slave-maker colony and on the other side, the host colony, such that they were 1 meter apart. Raids were filmed using a webcam (Logitech C920 HD Pro), with cameras positioned above each nest so that behaviors inside the nests were observable.

On the day following a raid, I opened each nest and counted the contents of each to determine the number of brood the slave-maker colony had stolen. I then placed all slave-maker workers in a freezer (-18ºC) for later dissection. I watched the recordings of each nest to determine if recruitment had occurred in the slave-maker nest following host nest discovery, and if so, the size of the raiding party that arrived at the host nest prior to attack.

To test the effect of worker reproduction on raiding strategies, I compared control and treatment colonies in (1) their frequency to initiate attacks alone versus as a group. I used a one-tailed Fisher's Exact test in both comparisons. To measure the effect of worker reproduction on raiding performance, I compared the number of brood stolen between control and treatment trials using a Mann-Whitney U test with the "wilcox.exact" function in the exactRankTests R package.

Benefit Function for Raiding

To characterize the benefit function for raiding, I compared number of brood stolen with the size of the raiding party that arrived at the host nest. I included additional data from raids conducted for other purposes, but for which methods were similar. These additional raids were conducted in smaller arenas (25 cm x 45 cm) without paper-lined floors. In 7 of these additional trials, slave-makers were offered a single host nest containing 20 workers and 20 brood, housed in Plexiglas observation nests. In 9 of these additional trials, slave-makers were offered two host nests consisting of acorns or hickory nuts, one containing 25 workers and 25 brood, the other containing 50 workers. For these trials, I only selected cases where the slave-makers found the 25 worker nest only. The host nests in the all of the trials used for this analysis contained a 1:1 ratio of workers and brood, and were very similar in their absolute sizes (25 or 20), a difference that is unlikely to make a large difference in slave-maker success (Miller, Chapter 1). All trials followed the same pre- and post-raid procedures with the exception of the details described above.

I first compared the raiding success between solo attack strategy (raiding party = 1) and the group raiding strategy (raiding party > 1) using a Mann-Whitney U test. To generate the benefit function, I then characterized the relationship between raiding party size and the proportion of brood stolen with a variety of regression models and selected the one with the best fit. I ran the following regressions using the "glm", "glm.nb" and "nls" functions in the lme4 and stats R packages : (1) linear model with a binomial distribution and two-vector response variable, (2) Linear model with a negative binomial distribution, (3) linear model with logit transformed response variable, and (4) non-linear model using Michaelis-Menten equation. I compared the goodness of fit between models using mean squared error (MSE), the correlation between the predicted and actual data and AICc values.

To more robustly measure the effect of raiding strategy (solo vs group) on group payoff, I compared the outcomes of solo raids to group raids in terms of brood stolen and mortality rates using the same larger dataset as described above. I compared the mean proportion of brood stolen and the mean mortality rate between strategies using a t-test.

Mechanisms of Raiding

To characterize the behavioral mechanisms slave-makers use during attack, I observed videos of host nests from the cooperation experiment. I focused on the behavior of slave-maker workers from the time a slave-maker worker initiated a raid by entering the host nest until the host nest was evacuated of all host workers, not including brood. I used GriffinVC (Singh & Ragir 2015), an ethogram data collection tool, to code behavioral events and assign time-stamps.

There were two stereotyped behaviors, ‘herding’ and ‘door guarding’, on which I focused my measurements. In addition, I recorded the time at which each brood item and/or host worker exited the nest during the raid. Using these data, I calculated the proportion of the raid that herding or door guarding occurred, and for when they occurred simultaneously.

To determine how raiding success is affected by these raiding-specific tasks, I constructed a linear model of the number of brood stolen as a function of the following factors: proportion of time spent guarding the door, proportion of time spent herding, raiding party size and all possible interactions. Using the “dredge” function in the MuMin R package, I selected the top models with a delta AICc <7. There was more than one model that met this criteria, so I averaged the top models using the “model.avg” function (Table 3.1).

To test the hypothesis that door guarding functions to keep brood from being carried away by host workers, I compared the rate of brood exiting the nest during door guard presence to absence. The data were non-normal, so I used a Mann-Whitney U test in all the comparisons described in here. To test the hypothesis that herding accelerates evacuation, I compared the rate at which host workers exit during herding to when there was no herding. To determine whether the level of cooperation in a colony affects these raiding-specific behaviors, I compared the following metrics between control and treatment colonies: the effectiveness of door guarding (i.e. rate of brood exit) and the proportion of time that herding and door guarding co-occur.

Results

Cooperation Experiment

I conducted 17 successful trials (8 treatment, 9 controls) with 14 different slave-maker colonies, such that three colonies were tested in both the control and treatment conditions. The mean ovarian development score for treatment colonies (23.9 ± 15.8 µm) tended to be higher than control colonies (8.4 ± 4.5 µm) for control colonies, however this difference was not statistically significant ($p = 0.146$, W = 12).

Treatment colonies differed in their raiding strategies relative to control colonies (Fig 3.2; $p<0.001$, odds ratio = inf.). In general, even if a scout initiated an attack alone, it might still return home to recruit a raiding party but the host colony would already have begun evacuation. In all of the treatment trials, the slave-maker scout that discovered the host nest initiated the attack alone (solo attack strategy), without the aid of other slave-maker workers. In contrast to this solo strategy, control colonies initiated the attack as a group in eight out of the nine trials, such that the scout return home to recruit a raiding party before entering the host nest. In only two of the treatment trials did the scout return home after initiating attack; in one case there were no other slave-maker workers at home to recruit and in the other case, slave-maker workers were present, but the slave-maker did not perform the behavioral excitement characteristic of recruitment and elicited no followers. In the one case of solo attack in the control trials, the slave-maker scout returned home and performed the regular recruitment behaviors, eliciting a raiding party to follow. Despite the fact that treatment colonies differed in their attack strategy, they did not steal significantly less brood than control colonies (Fig. 3.2; $p = 0.248$, W = 28.5, $\bar{x}_{\text{control}} = 11.22$, $\bar{x}_{\text{treat}} = 8.25$), although treatment colonies tended to steal fewer brood.

Benefit Function for Raiding

Results from raids in the larger data-set show that the group raiding typically leads to higher rates of brood acquisition than the solo attack strategy ($p = 0.039$, W = 86, $\bar{x}_{\text{solo}} = 0.356$, $\bar{x}_{\text{group}} = 0.525$). The benefit function describing how gains in brood increase with participation level, i.e. the size of the raiding party, was best approximated by a concave down function. The model of best fit was the non-linear regression using the Michaelis-Menten function $y=(\alpha*x)/(\beta+x)$, which had had the smallest mean squared error and AICc values, and the highest correlation between predicted and actual data (Fig 3.3, Table 3.2). The parameter estimates for the non-linear model are $\alpha = 0.634$ (± 0.160; $p<0.001$) and $\beta = 0.715$ (±0.602; $p=0.244$).

In general, raids initiated as a group stole a higher proportion of brood than raids that initiated raids using a solo attack strategy (Fig 3.4A; $p = 0.011$, $df = 30.2$, $t = -2.39$, $\bar{x}_{\text{solo}} = 0.34$, $\bar{x}_{\text{group}} = 0.56$). Similarly, raids that initiated raids using a group suffered lower mortality than raids initiated using a solo strategy (Fig 3.4B; $p = 0.021$, $df = 30.6$, $t = 2.13$, $\bar{x}_{\text{solo}} = 0.17$, $\bar{x}_{\text{group}} = 0.05$).

Mechanisms of Raiding

When a raid was initiated, at least one slave-maker worker entered the host nest and rapidly moved around the interior. During this rapid movement, which I term ‘herding’, a slave-maker worker approached groups of host workers, which promptly fled from the slave-maker. Host workers occasionally oriented towards an attacking slave-maker, but the slave-maker prodded these hosts with her head or mandibles, leading the host workers to retreat. The slave-makers do not appear to be moving to evade host worker aggression, but rather they move towards host workers, even re-orienting towards other clusters of workers when the targeted cluster had moved out of physical contact. In addition to ‘herding’, a slave-maker worker also stood at the nest entrance during the raiding process, a behavior I term ‘door guarding.’ This slave-maker was usually positioned inside the nest, with her head facing towards the exterior. The door guard appears to permit empty-handed host workers exit the nest, but she bites and chases brood-carrying workers, preventing their exit. Slave-maker workers do not begin to carry brood back to their home nest until all of the resident host workers have vacated. The attack, including the events described above but not including brood transport, last a mean 8.23 (± 1.78) minutes. Herding takes place during 64.5 (± 0.07) % of the attack and at least one door guard is present during 34.7 (± 0.06) % of the time, however a door guard often stays in position after all the host workers have evacuated. The raiding party that initiated an attack was on average 2.4 (± 0.2) slave-maker workers, but was as few as one and as many as 8.

The results of model selection and model averaging show that the most important predictor of the number of brood stolen was the proportion of time spent guarding the door (Table 3.1; $p = 0.011$, relative importance = 1.00, $\beta = 0.784$). The size of the raiding party was the next most important predictor ($p=0.375$, relative importance =0.27, $\beta = 0.385$), followed by the strongly negative effect of herding time ($p=0.799$, relative importance =0.12, $\beta = -0.983$). The extent of overlap between these two tasks was a significant positive predictor of brood stolen ($p = 0.022$, $F_{1,13} = 6.803$).

The hypothesis that door guarding prevents host evacuation of brood was supported. When the door was being guarded, fewer brood escaped than when no door guard was present (Fig. 3.5A; $p < 0.001$, $W = 36$, $\bar{x}_{\text{present}} = 0.010$, $\bar{x}_{\text{absent}} = 0.038$). The level of cooperation in a colony had no significant effect on slave-maker’s tendency to guard the nest entrance during a raid ($p = 0.661$, $W = 29$, $\bar{x}_{\text{control}} = 0.32$, $\bar{x}_{\text{treat}} = 0.395$) nor did it affect their effectiveness at keeping brood inside ($p = 0.697$, $W = 23.5$, $\bar{x}_{\text{control}} = 0.006$, $\bar{x}_{\text{treat}} = 0.016$).

The hypothesis that herding accelerates host evacuation was supported, as host workers exit at a greater rate during herding (Fig. 3.5B, $p < 0.001$, $W = 51.5$, $\bar{x}_{\text{herd}} = 0.071$, $\bar{x}_{\text{no.herd}} = 0.026$). The level of cooperation had a slight, non-significant effect on the amount of time herding and door guarding occurred simultaneously ($p = 0.058$, $W = 47.5$, $\bar{x}_{\text{control}} = 0.232$, $\bar{x}_{\text{treat}} = 0.019$), with greater task overlap in control trials.

Discussion

Cooperation levels had a measurable and significant impact on the expression of collective behavior. Colonies with elevated levels of selfishness adopted different raiding strategies than control colonies. Instead of recruiting colony-mates and raiding as a collective, scout ants from selfish colonies abstained from recruitment and attacked alone. Despite these behavioral differences, colonies with elevated selfishness did not perform significantly worse than control colonies, although there was a tendency to steal fewer brood (Fig 3.2). Solo attacks generally result in fewer stolen brood, as shown by data from a larger sample of raiding trials, so it is noteworthy that conflict colonies employing this strategy did not suffer the same penalty. The reason for the lack of disparity between treatments is unclear, but it suggests that workers from selfish colonies behaviorally compensated for their handicap of raiding alone, or alternatively, that there was not adequate power to detect a difference.

The benefit function of raiding behavior follows a concave down shape (Fig 3.3), corresponding to low sensitivity to selfishness (Fig 3.1). The additional benefits to raiding parties greater than two were marginal, with the largest increase between raiding parties from one (solo attack) to two. Even though there were apparent benefits of attacking as a raiding party, the benefits of the collective strategy are not as large as expected. Since the act of raiding does not always require a group, the success of the colony may be less dependent on group performance during raids, thereby relaxing selection on this collective behavior.

The descriptive measures of raiding presented here illustrate that slave-makers can be relatively effective as solo attackers. This is particularly true if they spend ample time guarding the door rather than herding, and thus prevent much of the brood from leaving with escaping host workers (Fig 3.4A). Having an additional slave-maker to assist during the attack offered an advantage when one ant herds while the other guards the door. These two tasks appear to act in synergy, and lead to greater quantities of stolen brood when done simultaneously rather than sequentially, and this was the case for control colonies.

While herding behavior accelerates host evacuation, it also accelerates evacuation of brood, unless there is a door guard. Thus, the benefits of raiding as a pair are achieved only when two workers coordinate by adopting complementary actions, and may help explain why there are diminishing returns after raiding parties reach a size of two workers. Additional slave-makers beyond the two may help with either herding or door guarding, although redundancy in these tasks was not essential for success. However, having back-up may help ensure at least one slave-maker is in position if there is more than one nest entrance or when slave-makers are immobilized by the defending host workers (Jongepier et al. 2014).

The observations here shows that cooperation creates conditions for task coordination to take place, but does not guarantee that it will. Whether selfishness affects the skill with which individuals perform their specific tasks or the degree of coordination could not be analyzed because selfishness led only to solo attacks. Further investigation into the mechanics of raiding is needed to determine what facilitates coordination and which cues are used to initiate these tasks in real-time. Another important behavior during slave-raids is the release of propaganda pheromone (Brandt et al 2006), but it was unclear from lab observations when or by whom such compounds were emitted, so specific tasks could not be linked to pheromone release behaviors. It is clear, however, that workers from high conflict colonies are less likely to return home to recruit nest-mates. This implies that workers are keeping track of the cooperation levels in the rest of the colony, but there may be other social mechanisms at play that alter a slave-maker's threshold to recruit.

The resiliency of *T. americanus* raiding behavior to low levels of cooperation implies that worker selfishness may reach relatively high levels before incurring significant fitness costs. Elevated worker reproduction is well documented in other slave-making ants (Heinze 1996, Herbers & Stuart 1998) and has been attributed to conflict over sex-ratios (reviewed in Bourke 1989), however the results here suggest an alternative explanation. Worker reproduction may incur minor costs to the group when raiding does not necessitate a large fraction of the colony in order to be successful. It is unclear if raiding behavior is equally resilient to worker selfishness in other species, and it would be interesting to see if their benefit function for raiding matches the concave down shape found here.

The association between worker reproduction and the benefit function found here simply establishes an interdependency between the two, but does not distinguish an order of evolutionary causality. In other words, it remains unclear whether elevated worker reproduction in slave-making ants is an evolutionary response to the low cooperative efforts required of raiding, or instead whether the shape of the benefit function is an adaptation to high rates of worker reproduction permitted by the slave-maker lifestyle. Nevertheless, these experiments do suggest that cooperation has the potential to impact the expression of a collective behavior, and vice versa. As long as selfishness disrupts the benefits gained through collective behavior, then selection ought to favor groups that are more cooperative (Gardner 2015).

The findings here raise further questions about what relationships we should expect between cooperation and collective behavior in other social insects and other cooperative groups. Collective behaviors that require more components or larger numbers ought to be more sensitive to lapses in cooperation, assuming that selfishness leads to non-involvement as it did here. This suggests that more complex collective behaviors may only be stable in groups that are already highly cooperative. On the other hand, collective behaviors may still evolve in groups where conflict is common, but the benefit functions are likely to differ and instead exhibit lower sensitivity (e.g. linear or concave down). Future empirical and theoretical investigations of collective behavior and within-group heterogeneity should consider cooperation for both its mechanistic and ultimate level consequences.

Figure 3.1: Hypothetical benefit functions depict the relationship between cooperative investments by the group and the payoff dispensed to the group. Here, the benefit functions for three different hypothetical collective behaviors are drawn to illustrate the implications of curve shape. A collective behavior's benefit function will dictate how sensitive or resilient it is to cooperation. Different hypotheses for how cooperation levels will impact collective behavior are depicted with different colors. Collective behaviors with (blue) concave down benefit function are expected to have low sensitivity to loss of cooperation, and exhibit minimal loss of function. Collective behaviors with (orange) concave up benefit functions are expected to have high sensitivity to loss of cooperation, and exhibit large loss of function. The linear dotted line depicts an intermediate level of sensitivity, in which loss of cooperation corresponds directly to loss of function.

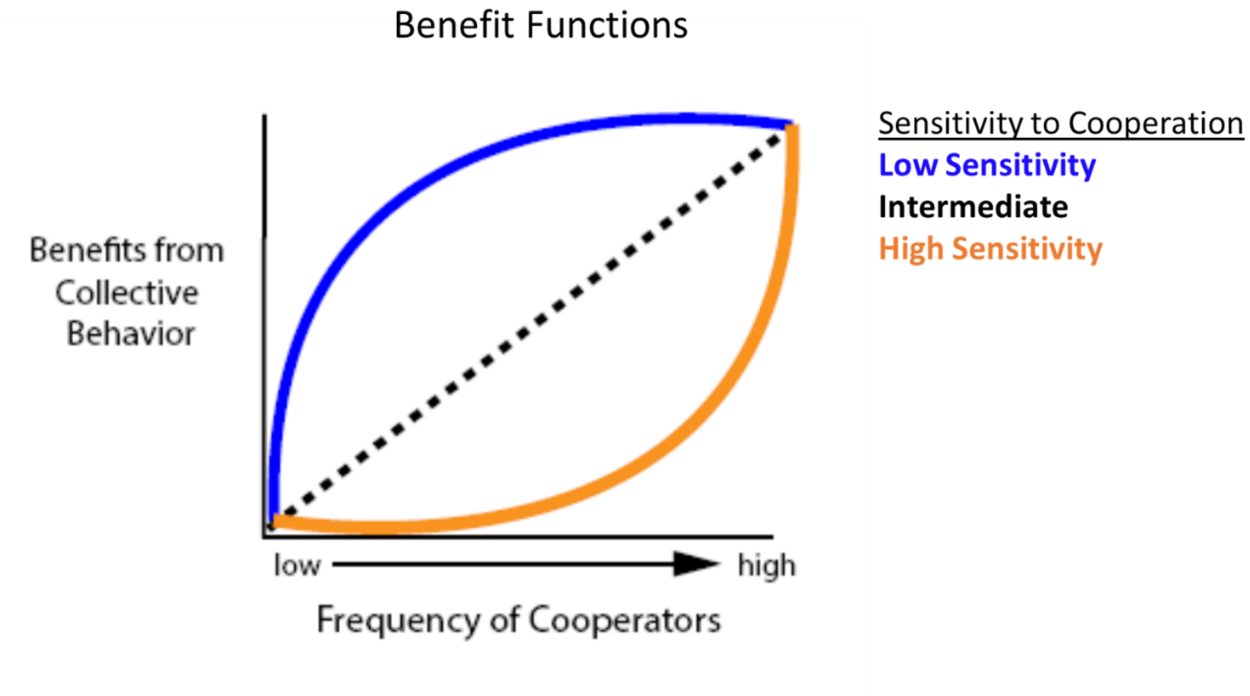

Figure 3.2: Selfish and control colonies acquire a similar quantity of brood from raids at host nests containing 25 workers and 25 brood. Although control colonies gain slightly more brood, this trend is not statistically significant ($p = 0.248$). Control colonies are much more likely to raid as a group (green data points) than selfish colonies, which exclusively adopted a solitary raiding strategy (orange data points; $p < 0.001$).

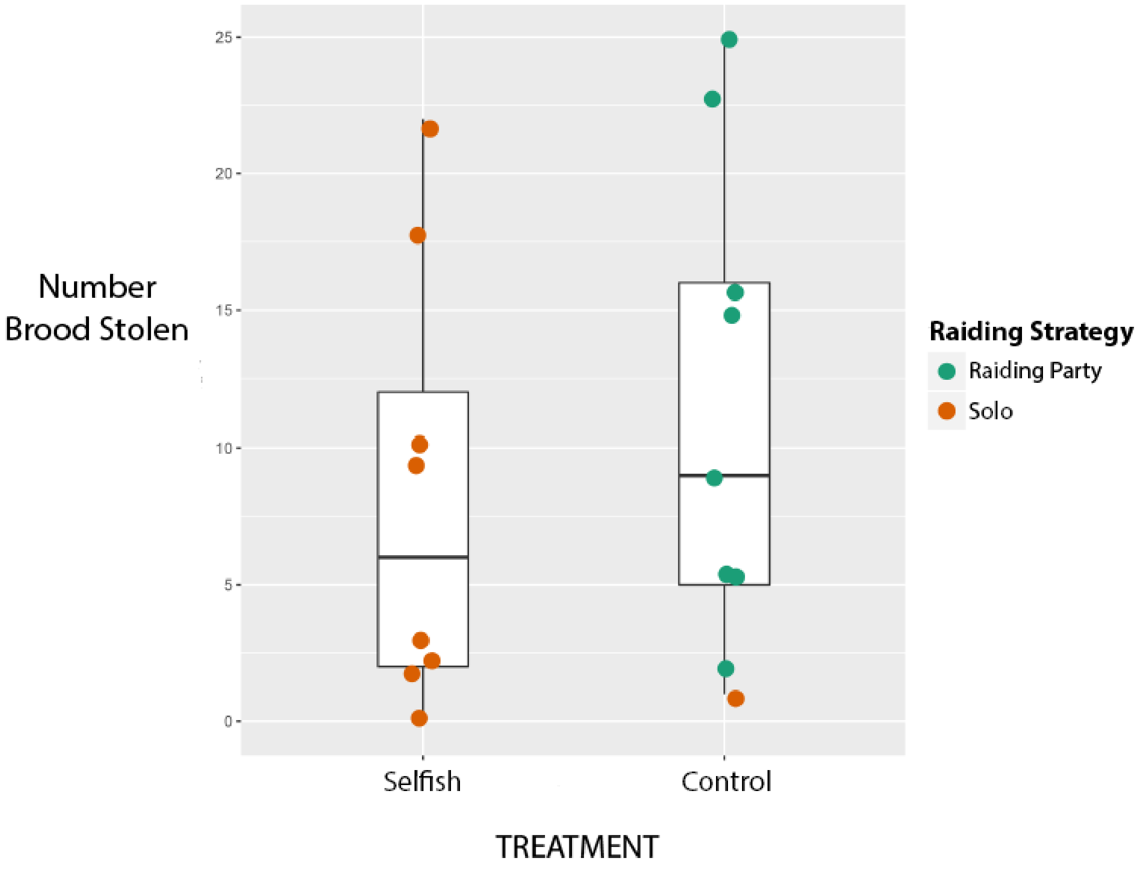

Figure 3.3: The benefit function for collective raiding is depicted as a function of participation level, or raiding party size. The data were fit to a non-linear regression and the curve and 95% confidence intervals are plotted [y=0.634*x/(0.715+x)].

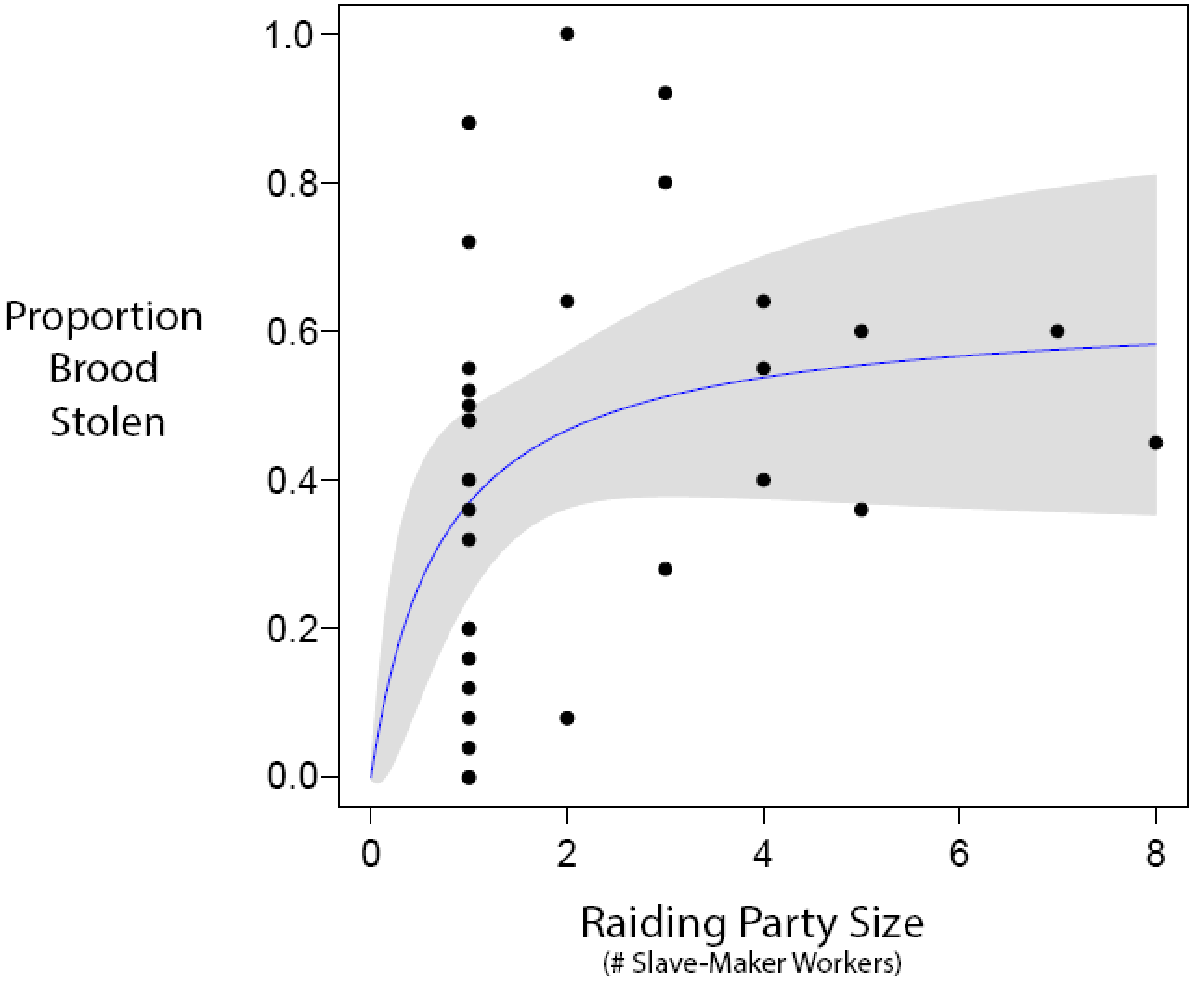

Figure 3.4: The payoffs of solo versus group raiding strategies in terms of the (A) benefits, as the mean proportion of brood stolen (p = 0.011) and (B) the costs, as mean mortality rate (p = 0.023), measured as the proportion of the colony's slave-maker workers that died. All raids conducted at host colonies containing 20 or 25 workers and brood, where brood and worker number were matched.

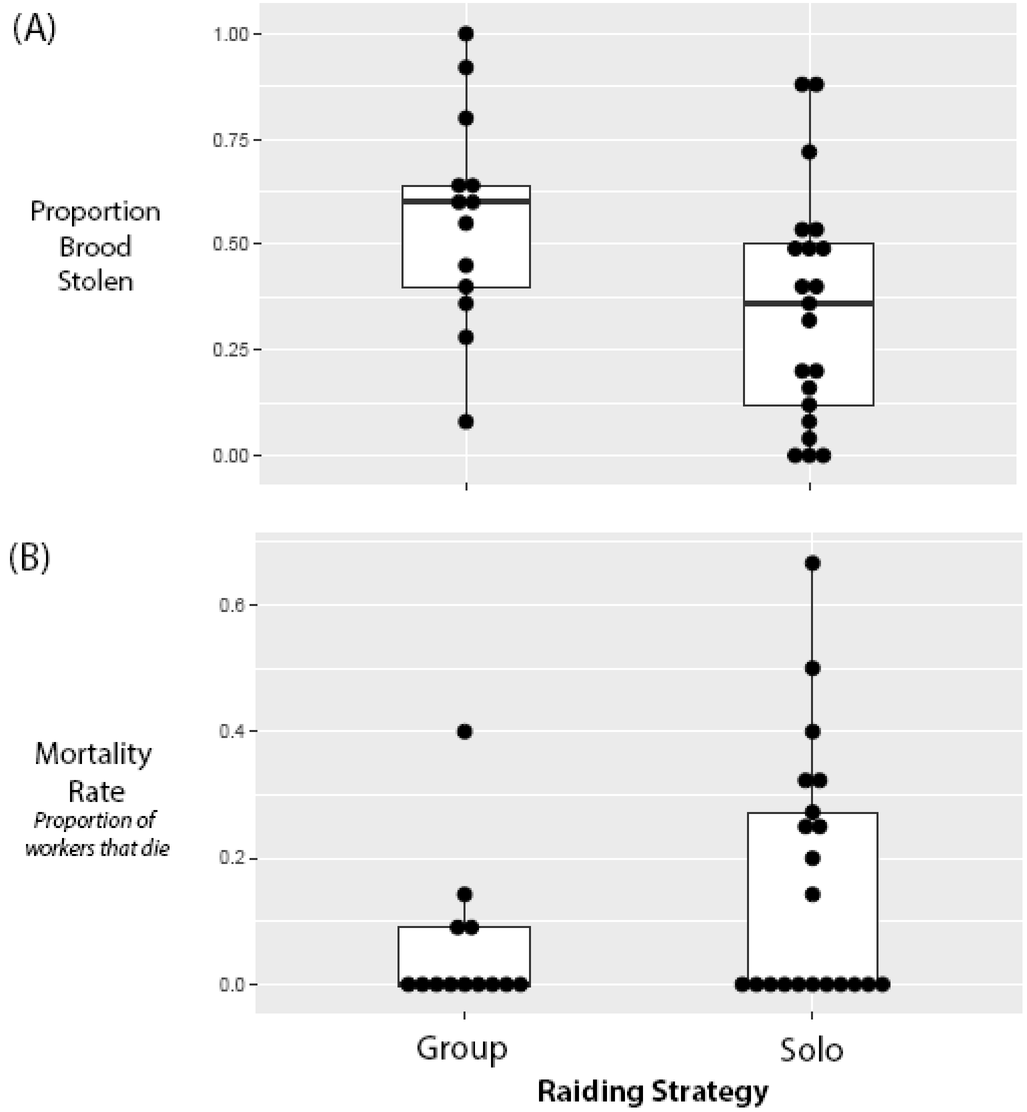

Figure 3.5: The function of sub-tasks that occur during raiding are summarized in these two plots. (A) The presence of a door guard effectively prevents brood from leaving the attacked nest ($p < 0.001$) and (B) the activity of herding within the nest interior accelerates host evacuation ($p < 0.001$).

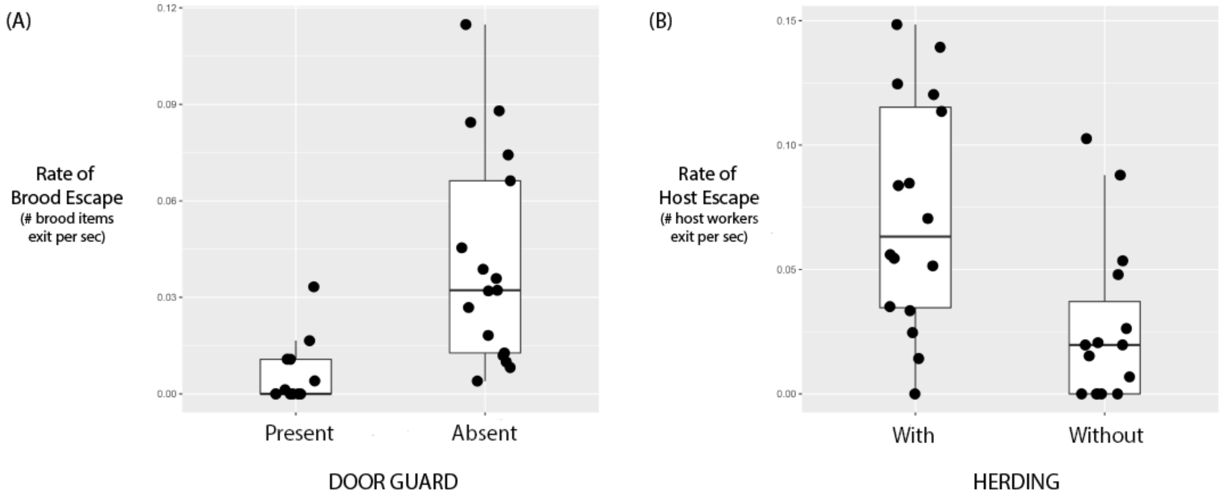

Table 3.1: What takes place during raids that determine success or failure? (A) Results of model selection (ΔAICc<7). (B) Results of model averaging. Predictor abbreviations: **Herd** = Proportion of time spent herding; **Door** = Proportion of time spent guarding door; **RP** = Number of slave-makers in raiding party.

(A) Global Model: Number brood stolen ~ Herd * Door * RP						
Candidate Models		**df**	**logLik**	**AICc**	**Delta (ΔAICc)**	**Weight**
Door		3	-47.81	103.8	0.00	0.613
Door + RP		4	-47.25	106.5	2.70	0.159
Door + Herd		4	-47.78	107.6	3.76	0.093
Door + RP + Door* RP		5	-45.60	107.87	4.07	0.08
Door + RP + Herd		5	-46.98	110.63	6.84	0.02
(B) Model-Averaged Results						
Parameters		**Estimate**	**SE**	**Z**	**P-value**	**Importance**
Door		0.784	0.290	2.534	0.011	1.00
RP		0.385	0.409	0.887	0.375	0.27
Herd		-0.059	0.209	0.255	0.799	0.12
Door*RP		-0.983	0.598	1.464	0.143	0.08

Table 3.2: Different regression models for the benefit function are listed along with different measures of goodness-of-fit. AICc refers to the information criteria of the model; Correlation refers to the correlation between the predicted and actual data, and MSE is the mean squared error. The models analyzed raiding party size as a predictor of the proportion of brood stolen.

Model		AICc	Correlation	MSE
Non-Linear (Michaelis-Menten)		12.8	0.2891	0.0721
Linear: logit transformed		128.6	0.2257	2.4040
Linear: binomial distribution (success, failures)		396.5	0.2255	8.9357
Linear: negative binomial distribution		52.8	0.7162	0.7162

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CHAPTER 4

THE EFFECT OF COLLECTIVE BEHAVIORS ON THE EVOLUTION OF COOPERATION: WHEN EMERGENT PROPERTIES PROVIDE NONLINEAR BENEFITS

Abstract

I construct a nonlinear public goods game to investigate the effects of collective behaviors on the evolution of cooperation. I argue that sigmoidal benefit functions capture the payoffs from many self-organized collective behaviors and I borrow a model from biochemistry, the Hill equation for cooperative binding, to approximate the way individual contributions can combine non-additively. I develop a model in which cooperation and competition trade-off with one another in a tug-of-war structure. This structure differs from former models of public goods which assume separate costs that are independent of other group members' actions. The model also incorporates variable group size and relatedness to investigate how these factors interact with the benefit function. The model predicts that sigmoidal benefits enhance cooperation, and that steeper increases promote cooperation. On the other hand, early inflections and diminishing returns reduce cooperation, particularly in groups with high relatedness. Interestingly, the boosts to cooperation provided by sigmoid benefit functions are predicted to become stronger as group size increases. The implications for the role of collective behaviors in the evolution of cooperation are as follows: (1) the influence of collective behaviors may be limited to groups that are already large. (2) Collective behaviors that are fitness enhancing will not necessarily increase cooperation, however they will if they are both fitness enhancing and arise in a non-linear fashion. (3) On the other hand, collective behaviors that are fitness enhancing will reduce cooperation if they are both fitness enhancing and bring inflection points earlier. More empirical

work is needed to measure the parameters defining the shape of benefit functions of various collective behaviors to test the validity of these predictions.

Introduction

Theoretical models of public goods games predict that complete defection is inevitable, however cooperation is widespread in biological examples of shared public goods. This contradiction has been the focus of many behavioral ecologists, and has led to the discovery of several mechanisms that stabilize cooperation and minimize the tragedy of the commons, including policing (Frank 1995), relatedness or population structuring (Hamilton 1964), reciprocity (Axelrod & Hamilton 1981) and between-group competition (Reeve & Hölldobler 2007). More recently, however, researchers are recognizing the power of non-linear returns or synergistic interactions to promote cooperation in public goods games (Archetti et al. 2011; and earlier, Queller 1985). Nonlinear benefits are probably as common as linear benefits and recent reviews have highlighted the possible importance of nonlinear returns to the stability of cooperation within and between species (Riehl & Frederickson 2016, Archetti et al 2011).

The earliest attempts to treat nonlinear returns theoretically were intended to model specific cases of group hunting or attack (Packer & Ruttan 1988, Berryman et al. 1985, Aviles 1999), however these efforts laid the ground-work for more general models of non-linear public goods games. Recently, there has been a surge in the analysis of multi-player public goods games with non-linear benefit functions (Peña et al. 2015, Van Cleve & Açkay 2014, Zhang et al. 2013; Archetti & Scheuring 2010). Despite differences in their approaches, they converge on the same basic prediction that nonlinear benefits can create a stable a mixture of cooperators and defectors. However, existing models have restrictive assumptions and more nuanced predictions about the dynamics of cooperation are still relatively unexplored.

Prior efforts to capture non-linear benefit functions consider cases of either uniquely diminishing returns, uniquely accelerating returns with no bounds (Frank 2010, Foster 2004, Van Cleve & Açkay 2014, Peña et al. 2015), or step-functions (Archetti 2009 JEB, Boza & Szamado 2010), none of which are likely to represent most group behaviors. Step-functions are limited if one wishes to explore the continuous variation in curve steepness, and a combination of gradually accelerating and decelerating returns, i.e. a sigmoid, is probably closer to reality. There is evidence for sigmoid benefit functions across taxonomic groups and social contexts, including cooperative hunting in mammals (Packer et al. 1990, MacNulty et al 2011), cooperative nest defense in birds (Niemasik personal communication, Rabenold 1984), and in cooperative production of chemical compounds in yeast and bacteria (Gore et al. 2009, Greig and Travisano 2004, Pearson et al. 1997). In turn, several authors have considered sigmoidal benefit functions in public goods games, confirming that sigmoidal benefit functions yield predictions that are distinct from the other benefit functions described above (Pacheco et al. 2009, Vásárhelyi & Scheuring 2013, Zhang et al. 2013, Chen et al. 2012, Cornforth et al. 2012). These prior attempts to model sigmoidal benefits, however, fail to incorporate other aspects or applications of the model presented here.

In particular, I apply this modeling framework to the study of collective behavior. In addition to the cooperative behaviors mentioned above, public goods games with nonlinear returns may be especially relevant to collective or self-organized behaviors, where interactions result in emergent properties at the group-level (Couzin & Karuse 2003). For instance, researchers in statistical mechanics have noted that group-level behaviors undergo a ‘phase transition’ or are poised at a ‘point of criticality’, in which a group suddenly acquires a new property under the right conditions (Miramontes 1995, Bialek et al. 2012, Leonard 2014). Such collective actions are distinct because they lead to unique phenotypes or benefits otherwise unattainable as individuals (Camazine et al. 2001, Sumpter 2010), thus providing a public good. While a phase transition is the idea of an emergent property arising suddenly, emergent properties could also arise more gradually, depicting a sigmoidal curve with a more gradual rise.

These rates of change will depend on the specific nature and/or the frequency of interactions, which may be constrained or shaped by the ecology or physiology of a particular species, such that each collective behavior may produce its own benefit function. Aside from theory, nonlinear benefit functions have been identified in a handful of self-organized, collective behaviors so far, including the following examples. Foraging increases at a nonlinear rate in response to recruitment via chemical trails in the Pharaoh's ant (Beekman et al. 2001). Collective cognition also improves at a nonlinear rate with group size when tested in *Temnothorax* ants over a discrimination task (Sasaki et al. 2013). The emergence of small world networks has also been found to arise in a nonlinear fashion with minor changes in connectivity (Watts & Strogatz 1998, Robson & Traniello 1999). In a swarm-founding wasp, the effectiveness of group defense increases nonlinearly as the number of participants increases (London & Jeanne 2003).

Analogous threshold dynamics are well described and quantified in the literature on biochemistry, as researchers have aimed to describe the cooperative binding properties of various enzymes and drugs (Whitty 2008, Frank 2013). Cooperative binding is often described using the example of oxygen binding hemoglobin. Once one oxygen molecule binds one of the four binding-sites, the affinity of the other binding sites increases, a result known as cooperativity. When the binding rate is plotted against the total concentration of oxygen, the result is a sigmoidal curve, in which binding is at first slow, but the rate of binding increases in a non-linear fashion as binding affinity rises. Eventually, the binding rate slows again as un-bound sites become less abundant. The equation developed to describe this process is called the Hill equation (Hill 1910), and sometimes referred to as the dose-dependent curve (De Lean et al. 1978).

The binding characteristics of chemicals and the behavioral interactions in collective behaviors are obviously quite different in a literal sense, however the dose-dependent quality of each makes for an apt analogy to collective behaviors. As the ‘dose’ of a relevant behavior (i.e. the cooperative investment) increases, there is a non-linear increase in the pay-off for performing those behaviors. In order to carry this analogy mathematically, I adopt the Hill equation to approximate collective behaviors that require cooperation.

Framing collective behaviors as a public goods game in this way will progress our understanding of how and when collective behaviors evolve. Intrinsic group factors, such as individual phenotypes, average cooperation or group size, are all expected to play important roles in the evolution of collective behaviors (Marnard-Smith & Szathmary 1995, Dornhaus et al. 2012, Modlmeier et al 2012, Pinter-Wollman et al. 2011), however that is not to discount the importance of extrinsic factors, like ecology, as well (Gordon 2014). The multi-player nonlinear public goods game provides one of the first theoretical frameworks to explore the connection between a group’s cooperative investment and its collective behaviors.

There are a couple reasons to suspect that cooperation may be especially relevant to the evolution of collective behaviors. First, many classes of collective behaviors require efforts by group members that do not immediately lead to benefits for the actor. Group behaviors are often, but not always, cooperative endeavors that require an upfront investment, but yield some benefit to the group. Second, cooperation can facilitate selection for group-level traits, like collective behaviors, that enhance a group’s competitive ability (Maynard-Smith & Szathmary 1995, Bourke 2011, Corning & Szathmary 2015).

To clarify the assumptions and applications of this model, it is important to emphasize that this model only applies to cases where participating in a collective behavior brings about an immediate, direct cost to the actor, and thereby represents an altruistic act. For instance, this model does not accurately represent a flock of birds, in which coordinating movement with the group results in a simultaneous immediate benefit to the actor and a coincident benefit to the rest of the flock, as there is no social dilemma. Likewise, the nonlinear benefit curves should be distinguished from allee effects. An allee effect often refers to the beneficial but passive by-product of conspecific presence, as in positive frequency dependence (Stephens et al. 1999). The behavioral interactions required for the class of collective behaviors to which this model applies require both active and initially costly behavioral responses from conspecifics. It is also important to note that the analysis here does not make any assumptions about the absolute payoffs of any given benefit function. Linear functions and sigmoid functions are assumed to produce the same payoffs at complete cooperation.

Here, I incorporate cooperation and competition into my model by breaking with two of the standard assumptions of a public goods game in which (1) there are individual costs to cooperating, and (2) benefits are shared by all group members equally. Instead, I allow for continuous degrees of cooperation, allowing players to adopt variable cost burdens. In addition, each player receives a different quantity of the group resource based on their competitive investment relative to the efforts of others, so that players are in a tug-of-war over the group’s resources. Each individual must therefore make an allocation decision between cooperation and competition. Such a trade-off is likely to characterize many cooperative behaviors, and be especially true of collective behaviors which require an individual be present or involved in a place or activity that precludes acting in self-interested ways. Thus, the costs are not explicitly set at any given value or even allowed to vary according to some coefficient. Instead, the costs are simply a degradation in the ability of the focal individual to acquire those group-level resources for oneself.

Finally, this model incorporates group size and within-group relatedness to facilitate more biologically relevant predictions. While other models analyze the effects of relatedness (Van Cleve & Açkay 2014, Zhang et al. 2013) or population viscosity (Peña et al. 2015, Vásárhelyi & Scheuring, 2013) on cooperation, their conclusions are limited because their analyses were specialized to extract the unique effects of each. My aim here is not to repeat these analyses, but rather examine how relatedness interacts with other model parameters in non-intuitive ways, and the same is true of group size. Here, I assume that groups of any size can produce benefit functions of any shape, however it is plausible that group size imposes constraints or has independent effects on the shape of the benefit function (Dornhaus et al 2012).

Here, I present a more generalized version of the nonlinear public goods game for the purposes of analyzing the effects of collective behaviors on the evolution of cooperation. I argue that sigmoidal benefit functions are an approximation that captures the generality of many self-organized collective behaviors and I borrow a model from biochemistry to approximate the way individual contributions can combine in a synergistic or non-additive manner. This form of the model allows investigation of how and to what extent collective behaviors can drive cooperation. To address this, I incorporate the following parameters into a model: (1) collective behavior traits, encompassing the rate at which the behavior and its associated benefits emerge; (2)

cooperation and competition between group-members; and (3) other group traits, including group size and within-group relatedness.

I show that a sigmoid benefit function can elevate cooperation beyond linear benefits, but not universally. The steepness of sigmoid curve promotes cooperation, with steeper increases predicting higher rates of cooperation, and such boosts to cooperation become stronger with increasing group size. Although models of public goods games are more often focused on identifying mechanisms that promote cooperation, nonlinear benefits reduce cooperation relative to additive benefits due to the diminishing returns part of the sigmoid.

The model

This model describes a group of "n" symmetrical individuals that produce a shared group resource, *G*. Each group member extracts a fraction, *F*, of that shared resource, so an individual's direct fitness is simply determined by their personal share of the group resource. Therefore, direct fitness is depicted as the size of the group resource multiplied by the selfish fraction (a percentage):

$$W = F * G \qquad \textbf{(Eqn 1)}$$

I will now walk through how the selfish fraction, *F*, and the group resource, *G*, are built. I will consider two different benefit functions for *G*, and compare the outcomes for each.

Each individual has an identical starting energy budget of 1, which they can devote to either cooperative or selfish efforts. Individuals can vary the degree to which they allocate their energy budget to the two options, but face a trade-off between the two. Cooperative efforts, *x*, contribute to the group's total resources, G. Selfish efforts, or *1-x*, are used to consume/extract the group's resources. This structure assumes that each group member has the same ability to extract from or produce the group resource, but that individuals can vary in how much they chose to invest in each.

I analyze the model for stable rate of cooperation that results in the optimal fitness benefit at the individual level. In order to evolve, the decision to cooperate is assumed to be under genetic control. I include a parameter for relatedness, r, between group-members, such that cases where groups consist of kin can be considered. If there are kin in a group, they will adopt the same strategy with probability 'r', i.e. the value of relatedness.

The structure of this model uses the neighbor-modulated fitness, which is the original Hamiltonian formulation of kin selection (Hamilton 1964). Neighbor modulated fitness accounts for the effects that related group members dispense onto the focal individual. Thus, W (Eqn 1) is the direct fitness of a mutant, not its inclusive fitness. Unlike the simplified version of inclusive fitness, neighbor-modulated fitness makes no assumptions about the costs and benefits being additive, rendering this form especially appropriate for an analysis of non-linear benefits to cooperation.

This model follows a tug-of-war structure, where the total size of individual's fraction of the group resource will depend on both its own selfish effort ($1-x$) and the selfish efforts of all other group members. To represent this relationship, an individual's selfish effort is divided by the sum of the selfish efforts of n other group members. A rare mutant who adopts the selfish strategy ($1-x$) in a population playing the population strategy ($1-x^*$) will have the following selfish fraction, F:

$$F = \frac{1-x}{(1-x) + r\,(n-1)(1-x) + (1-r)(n-1)(1-x^*)} \qquad \textbf{(Eqn 2)}$$

The denominator in Eqn 2 consists of the sum of the mutant's selfish effort (*1-x*), the selfish efforts of those playing the same mutant strategy and the selfish efforts of those playing the population strategy (*1-x**). The number of individuals playing the same mutant strategy is determined by *r (n - 1)*, or the number of group members that share the allele for the mutant strategy. Relatedness, *r,* is the probability that two group members share the allele for the mutant strategy, whereas individuals lacking the mutant allele will play the population strategy (*1-x*),* and (*1-r)(n-1*) is the number of individuals playing that strategy.

Increases in selfish efforts come at the expense of the group's cooperative efforts, thereby diminishing the total group resource available to everyone. In this public goods game, there is no direct cost to acting cooperatively, as there are in standard public goods games (Frank 2010); only a coincident decrease in selfish investment and thus a decrease in the actor's fraction of the group resource.

Benefit Function: Linear

The remaining cooperative efforts, *x*, are combined and converted into group resources (*G*), the way in which those efforts combine varies according to the benefit function. In its simplest form, cooperative efforts can combine additively, producing a linear benefit function, with group output increasing linearly with increasing cooperation. In the case of linear rate of returns, a rare mutant playing the cooperative strategy *x* in a population playing *x** would yield the following group output:

$$G_L = [(x) + r\,(n - 1)(x) + (1 - r)(n - 1)(x^*)] \qquad \textbf{(Eqn 3)}$$

Benefit Function: Nonlinear

Cooperative efforts can also combine in a non-linear fashion, so that increasing rates of cooperation lead to accelerating or diminishing returns in group output, or some combination of the two to form a sigmoid. These classes of non-linear rates of return are of special interest because they most closely approximate collective behaviors of interest in this model.

To characterize collective behaviors mathematically, a function is required to explicitly describe how individual behaviors accumulate and culminate in benefits at the group level. Here, I adapt the Hill equation (Hill 1910) used to describe the dynamics of cooperative binding from biochemistry as an analogy for how cooperative behaviors combine to produce emergent benefits. To carry this analogy mathematically, I make the following substitutions:

Hill Equation	Group Output Function for a Collective Behavior
$\% \, Binding\ Sites\ Bound = \frac{1}{1+\left(\frac{K}{[L]}\right)^{H}}$	$G_N = \frac{1}{1+\left(\frac{n*i}{\sum x}\right)^{s}}$

In the Hill equation, the percent of sites bound increases non-linearly with the concentration of the ligand, [L]. In the group output function for a collective behavior (G_N), group output increases non-linearly with the total contribution of cooperative efforts, represented by the sum of *x*-values. The shape of these curves are controlled in two ways: (1) the steepness of the curve at its point of inflection can vary as determined by the exponent *H* or *s* (Fig. 4.1A), and (2) the point along the x-axis where inflection occurs, *i* (Fig. 4.1B).

In the Hill equation, curve steepness is determined by the Hill coefficient ‘H’. Each biomolecule has its own unique value according to its physical properties (Hemoglobin’s Hill coefficient is between 2.3 and 3.0). In the group output function (G_N), *s* determines the steepness in the rate of return. As with each type of biomolecule, the steepness of the group output curve, *s*, is expected to vary based on the type of collective behavior and on the biology and ecology of species in question. The s-value for a given collective behavior can be quantified and measured by plotting a group's level of cooperation against the payoff to the group (although this data is rarely collected). In the equation used here, *s* can be any value greater than or equal to one.

The point of inflection in the Hill equation is captured by the disassociation constant, K, or the concentration of ligand at which the rate of binding and disassociation are equivalent. This can also be visualized as the point of inflection, *i*, on the curve. To mathematically represent this, I multiply the group size, *n*, by the inflection value, *i*, which is a value between 0 and 1. The expression '*n*i'* accounts for groups of variable size, but the important term here is '*i*': small values of '*i*' mean that an inflection comes early and values closer to 1 mean the inflection comes later. Early inflection result in concave down functions, late inflections result in concave up functions, and intermediate values result in sigmoid curves.

Ultimately, the group output function (G_N) converts cooperation into a benefit or resource for the group, but at rates that a determined by the values *s* and *i* (Fig. 4.1). In order to evaluate the effects of non-linear benefits on the evolution of cooperation, it is critical to isolate the effects of curve shape in the analysis. Due to the structure of the benefit function, altering the shape reduces the maximum possible group output, which will have a secondary influence on evolutionary stable outcomes. To overcome this limitation and standardize the group output, I multiplied the group output by $1 + i^s$. This modification sets the maximum group output to the group size, regardless of output function's shape.

Fitness Functions

Now that both the selfish fraction and the group output functions have been defined, the fitness equation for both a linear and a non-linear context can be built by taking the product of the two components. The following is the fitness function for a rare mutant in the case of a linear benefit function:

$$W_L = \frac{[(x) + r\,(n-1)(x) + (1-r)(n-1)(x^*)] * (1-x)}{(1-x) + r\,(n-1)(1-x) + (1-r)(n-1)(1-x^*)} \qquad \textbf{(Eqn 4)}$$

The following is the fitness function for a rare mutant in the case of a non-linear benefit function:

$$W_N = \frac{(1-x)}{(1-x) + r\,(n-1)(1-x) + (1-r)(n-1)(1-x^*)} * \frac{1}{1+\left(\frac{n*i}{x+r(n-1)x+(1-r)(n-1)x^*}\right)^s} * n * \ (1 + i^s) \quad \textbf{(Eqn 5)}$$

Solving for the ESS

Using the above fitness equations, the evolutionary stable strategy, x_{ESS}, can be determined for each scenario. This is achieved by determining the rate of cooperation at which fitness is maximized while all group members adopt the same strategy (*x=x*).*The expression for maximum fitness, W, is determined by taking the partial derivative of W with respect to x, and setting the derivative equal to 0 to find the maxima and minima.

$$\left.\frac{\partial W}{\partial x}\right|_{x = x^*} = 0$$

The maximization expression above is then rearranged to solve for the value of *x* (cooperation) at which individual fitness is maximized. I then verified that the solution is a real maxima and not a minimum or end-point using the second-derivative test. The linear form of the fitness function yields an analytical solution, in which the evolutionary stable rate of cooperation is:

$$x_{ESS\ Linear} = \frac{1 - r + nr}{n}$$

The non-linear fitness function cannot be solved analytically, so I analyzed this part by plotting numerical solutions given a range of values for each parameter {n = 2-10^7 ; r = 0-1; i = 0-1; s = 1-9}. I examined how the numerical stable rates of cooperation changed with respect to each parameter independently, and with respect to the linear solution.

Results

Non-linear returns to cooperation can increase the stable rates of cooperation in a group, however, they do not do so universally. Only benefit functions with later and steeper inflections will boost cooperation beyond the case of linear benefit functions (Fig. 4.2). For other curve shapes, non-linear returns instead reduce rates of cooperation. In order to more thoroughly evaluate the effect of the benefit curve on the stable rates of cooperation, I summarize the effects of each parameter on the shape of the curve and compare their outcomes to the linear case.

Curve Steepness

Increasing the benefit curve's steepness '*s*' generally increases the ESS cooperation rate (Fig. 4.2A). At most *s*-values, non-linear returns provide a boost to cooperation beyond the linear condition. Cooperation is higher when the curve is sigmoidal (at intermediate values of s) than when it is concave-down or step-like. When *s* is small, the benefit function adopts a concave-down shape, which always yields higher payoffs than the linear curve for all rates of cooperation. Therefore low s values require less total cooperation to achieve equivalent benefits than when benefits accrue linearly.

As *s*-values get increasingly large, the curve approaches a step-function. The rate of cooperation stops increasing when the curve adopts this step-function and begins to decrease mildly. This mild decrease in cooperation at high s-values is most pronounced in smaller groups, but becomes negligible as groups get larger. This mild decrease in cooperation is due to the fact that the incremental pay-off for additional cooperation becomes ever smaller after the steepest part of the curve is reached. After this point, incremental benefits for additional cooperation get smaller, and decrease in a non-linear fashion. To summarize, the higher the s-value, the more step-like the benefit function, the more rapidly diminishing are the benefits to additional cooperation once the ‘step’ in the curve has been cleared.

Point of Inflection

As the point of inflection moves to the right along the x-axis, the stable rate of cooperation increases (Fig. 4.2B). This can be explained by the fact that as inflection points come later, more cooperation is necessary to achieve the benefits brought about by cooperating. Conversely, as inflection points come earlier, relatively less cooperation is required to achieve those benefits, and stable rates of cooperation are consequently lower.

Even though shifting the inflection point towards higher values predicts an increase in cooperation generally, nonlinear benefits only provide a boost to cooperation beyond the linear condition at later inflections. In this manner, linear returns to cooperation actually result in higher rates of cooperation than non-linear benefits that have early inflections.

Curve Shape Interactions

The effects of curve steepness and the inflection point interact to influence the stable rates of cooperation. In general, the inflection point exerts a stronger influence over stable rates of cooperation than curve steepness. Early inflections always reduce cooperation below that of linear returns, regardless of steepness. Increasing curve steepness at early inflections (i.e. concave down curves) will actually decrease cooperation, which is the opposite of s's effect when inflections come later. Curve steepness only begins to exert greater control over cooperation rates after inflection points occur at higher values (i.e. sigmoid curves).

Relatedness

Increasing relatedness among group members increases the stable rate of cooperation in both linear and nonlinear benefit curves, such that groups with higher relatedness have higher rates of cooperation (Fig. 4.4A). Non-linear benefit curves elevate cooperation beyond the linear case, but only at relatedness values in the lower range. This effect is reversed at higher levels of relatedness, with non-linear benefits yielding lower rates of cooperation than the linear case. This result is due to the fact that the potential advantages for further cooperation are greater for non-linear returns than for linear returns at lower relatedness (and thus lower total cooperation). Conversely, at high relatedness, the potential advantages of further cooperation are greater for linear benefits.

More precisely, the advantage of being slightly more cooperative depends on the slope of the benefit curve at that group's level of cooperation. The cooperation level at which linear and non-linear returns have equal slopes is the point at which the incentive for further cooperation is the same (for a given relatedness value and group size). This effect is depicted in the Figs. 4.4 (B & C) and Fig. 4.5, where the curve representing the rate of per capita benefit for non-linear returns meets that of the linear case. Cooperation decreases relative to the linear case when slope of the non-linear curve in Fig. 4.4C falls below the linear curve at higher relatedness values (or higher rates of cooperation). This leads to the non-intuitive prediction that nonlinear benefits can elevate cooperation in groups with low relatedness, but that nonlinear benefits can actually reduce cooperation in groups with high relatedness or that are already highly cooperative.

Group Size

For both the linear and non-linear forms of the model, increasing group size reduces stable cooperation rates. In the case of linear returns, the rate of cooperation approaches the value of relatedness as group size approaches infinity, however these effects are realized at group sizes starting at about 20. In the case of nonlinear returns, the boost to cooperation beyond linear benefits grows as group size increases (Fig. 4.3). At very small group sizes, linear returns always yield higher rates of cooperation than the nonlinear case, regardless of curve steepness. Thus as groups get smaller, the rates of cooperation for linear and non-linear models become more similar. In brief, the cooperation boost provided by non-linear benefits increases as groups get larger.

In addition, group size and relatedness interact in a complex way to influence stable rates of cooperation. As described above, at low relatedness values, non-linear returns lead to higher rates of cooperation than the linear case, but this boost to cooperation increases as group size increases. However, as relatedness increases, increasing group size promotes cooperation less. These interacting effects can be visualized in Figure 4.3, where the difference in cooperation between a linear and a non-linear benefit function for a group of 3 individuals is only about 10%, whereas that difference doubles to about 20% as groups reach a size of 20 individuals. In summary, the boost to cooperation provided by non-linear returns in large groups is diminished in groups with higher relatedness.

Discussion

The goal of this model was to evaluate how synergistic interactions that lead to non-linear benefits can influence cooperation in a group. I attempt to capture the features of synergism that are especially prominent in collective behaviors by adopting a model from biochemistry, the Hill equation for cooperative binding. By allowing cooperation and competition to evolve in response to the shape the benefit function, group size and relatedness, I uncover the following predictions.

Sigmoid Shape

This model confirms the results of others that nonlinear returns to cooperation have the potential to enhance cooperation beyond additive returns (Boza & Számadó 2010, Archetti & Scheuring 2010), however I find this result is not universally true. The sigmoid benefit function has the effect of driving cooperation higher in some cases, but destabilizing cooperation in others. Other models of nonlinear public goods make different predictions based on the benefit function they use. When accelerating or diminishing returns are analyzed independently (Peña et al 2015, Van Cleve & Açkay 2014, Frank 2010, Hauert et al. 2006, Foster 2004), the power of nonlinearities to drive cooperation is underestimated. Sigmoid curves, which include both accelerating and decelerating components at once, predict higher rates of cooperation than curves of diminishing returns only. Alternatively, when a step-function is used in place of a smooth sigmoid function (Bach et al 2006, Archetti 2009), cooperation is predicted to only go as high as the point at which the public good is produced, but no farther. While this result is similar to the one outlined here, it fails to incorporate the important effects of curve steepness or relatedness outlined below, which will shift the stable point of cooperation away from the step-function's point of threshold (Figs 4.4 & 4.5).

The cooperation-promoting effect of the sigmoid derives from the accelerating portion of the curve. If relatively low cooperation is predicted by classic, linear public goods games (because of low relatedness or large group size), a sigmoid benefit function has the effect of boosting cooperation in such groups. The strength of this result is also determined by the location of the inflection point, with later the inflection points leading to greater boosts to cooperation. On the other hand, the diminishing returns portion of the sigmoid after the inflection point will conversely reduce cooperation. When the rate of return drops below that of the linear curve, the incremental benefits of additional cooperation fall below those achieved through linear returns (Fig. 4.4C, 4.5B). As such, the earlier the inflection point, the stronger the deterrent to cooperation. Thus, the combined effects of the sigmoid curve lead to the non-intuitive prediction that nonlinear benefits can elevate cooperation in groups with low cooperation, but reduce cooperation in groups that are already highly cooperative.

This prediction for reduced cooperation may explain the high frequency of worker reproduction in slave-making ants (Heinze 1996, Foitzik & Herbers 2001, Brunner et al. 2005). While sex-ratio theory has been invoked to explain elevated worker reproduction in slave-maker workers (Herbers & Stuart 1998, Bourke 1989, Trivers and Hare 1976), patterns of worker reproduction could also be attributed to diminishing returns to cooperation of the slave-maker lifestyle. Aside from conducting raids, obligate slave-maker workers are liberated from colony maintenance, so the benefits to staying active and sterile outside the raiding season are likely to have few advantages. In addition, the benefits of increasingly large raiding parties or additional raids beyond some threshold may produce diminishing yields, as is the case for *T. americanus* (unpublished data). Furthermore, slave raids usually make use of far fewer slave-maker works than are available. The number of workers required for a successful raid can therefore be viewed as the point of inflection, with additional slave-maker worker participation yielding diminishing returns. One prediction of this hypothesis is that species which require a smaller fraction of their colony for raids will have higher frequencies of reproductive workers, although there are currently insufficient data on raiding behavior to test this. This explanation is not necessarily an alternative to the sex-ratio hypothesis, but could explain why these reproductive workers are not policed by the queen.

Curve Steepness

The model also predicts that cooperation will increase as the benefit curve increases in steepness. This prediction has also been identified by Archetti & Scheuring (2010) for sigmoid functions, and by Foster (2004) and Frank (2010) for accelerating or decelerating functions, however the implications of these predictions have not been thoroughly unpacked, especially with reference to collective behaviors.

This prediction is satisfied by social spiders in the *Anelosimus* (Theridiidae) group, which are known to capture prey cooperatively on a large shared web. In social spiders, different prey sizes effectively give rise to different benefit functions. Cooperative capture of larger prey items leads to a steep rise in resources for the colony, approximating a sigmoid function (Kim et al. 2005, Yip et al. 2008). On the other hand, cooperative capture of smaller prey items yields benefits at a linear rate, as increasing cooperative effort yields proportionally more prey items. Thus, the availability of larger prey items creates conditions for non-linear returns to cooperation, and under these conditions, colonies are predicted to exhibit higher rates of cooperation according to the model here. This prediction is satisfied by Powers & Aviles (2007) and Purcell & Aviles (2007), who show that low elevation colonies that have access to larger prey are more cooperative than colonies at higher elevations that have access to smaller, but more numerous prey.

Evidence for this prediction is also found in a comparison of two sympatric social spider species which differed in their body and group size. As group size increased, the benefit function for the small species (*A. domingo*) is steeper than it was for the larger species (*A. eximus*; Guevara & Aviles 2011). The model predicts that cooperation will therefore be greater in *A. domingo* colonies, and indeed more individuals participate in prey capture in groups and there is greater participation across all age classes. *A. domingo* also forms smaller groups, which is also consistent with the model's prediction that cooperation declines with group size; however there were no group size differences in the population sampled for this study.

Group Size

The model predicts that cooperation declines as group size increases, a result that is consistent with other tug-of-war models with linear returns (Reeve & Hölldobler 2007) and other models of non-linear public goods (Boza & Számadó 2010). This pattern is due to a greater reliance on other group members to produce benefits as groups get larger, resulting in a 'free-loader' phenomenon. However, the sigmoid curve mitigates this free-loader problem at high group sizes, as free-loaders pay a greater cost to defecting if the steepest part of the benefit function hasn't been cleared. As a consequence, the boosts to cooperation provided by sigmoid benefit functions are predicted to become stronger as group size increases.

This prediction has the strongest implications for collective behaviors that emerge through self-organizing. The expression of such collective behaviors are often group-size dependent and occur predominantly in larger groups (Dornhaus et al 2012). Thus, the role of such collective behaviors in regulating cooperation may be limited to groups that are already large. Although models with linear benefits predict that cooperation declines as group size increases, this prediction suggests that collective behaviors of this variety may help maintain cooperation as groups increase in size. In addition, if nonlinear benefits provide the strongest boosts to cooperation in larger groups, then self-organized collective behaviors may play a larger role in driving the elaboration of sociality rather than its origin, consistent with the conclusions of Avila & Fromhage (2015).

Group Output

All the predictions made here assume that the maximum possible group output is equivalent regardless of the shape of the benefit function. Ultimately, the benefit functions favored by selection will be the ones with the greatest per-capita pay off when everyone plays the stable rate of cooperation. Identifying which benefit functions will yield the highest payoffs is not encompassed by the model here, but this model does provide some guidance if such data were available. For instance, if behavioral innovations that create steeper inflections result in greater outputs, then the model predicts that cooperation will increase in those cases. Consider instead a case where behavioral innovations with the greatest outputs are produced by early inflections, then the model predicts that cooperation will decrease in those systems. More generally, selection favoring a more productive set of interactions, and the coinciding benefit function, will carry cooperation along with it. When the payoffs from cooperation exceed any losses, then the interaction more closely resembles a mutualism and there is no longer the social dilemma of a public goods game (Clutton-Brock 2002).

It is unlikely that behavioral innovations that alter the shapes of the benefit functions will leave the pay-offs unaffected. There is no precedent, however, for assuming that certain shapes should lead to greater or lesser group outputs. It is plausible that interactions that lead to a steep increase in the benefit function (like a phase-transition) will coincide with an increase in the group's resources. For instance, collective organization or coordination is expected to improve group efficiency or reliability through division of labor (Oster & Wilson 1978) or it may expand the range of resources available to a group, for instance through coordinated carrying (McCreery & Breed 2014) or attack (Berryman et al. 1985). The key point here is that collective behaviors that are fitness enhancing will not necessarily increase cooperation, however they will if they are both fitness enhancing and arise in a non-linear fashion.

Future Directions

The findings from this model highlight the importance of curve steepness in understanding the evolution of cooperation and collective behaviors, however more focused data collection on the rate of returns are needed. Researchers working on cooperative species can collect this data by measuring the pay-offs resulting from variable levels of a group's cooperative investment. This could include measuring the effort of individuals towards a collective task (e.g. time spent hunting) or the degree to which individuals invest in their personal fertility (e.g. ovarian development). Collecting a continuous measure of this relationship will reveal the rate at which benefits accrue, yielding *'s'* values that are specific to a given population or species, or which pertain to certain behaviors or ecologies. Empirical measures of a behavior's inflection point *'i'* would also result from the same data collection method. Natural variation in cooperation is often overlooked in place of averages or such variability is removed through controlled experimental groups, even though such continuous variation may be instructive.

The predictions from this model can also be applied more specifically to the study of collective behavior. First, more data is needed on which benefit shapes most commonly characterize coordinated behaviors that lead to emergent properties or collective actions. At what level of participation are the benefits from a collective behavior realized and at what point are returns diminishing? Second, one could gather data on how subtle changes in interactions or individual behaviors alter the steepness of a benefit curve. For instance, does the use of a communication signal to coordinate the timing of an action raise the group output or generate a steeper benefit function? One could also gather data on how external factors, like ecological conditions could also alter the shape of the benefit function. Such variation could be captured, for instance, by looking at geographic gradients (as was demonstrated above with the social spiders) or through changes in the seasons. Classifying collective behaviors according to their benefit functions will facilitate comparisons between species that lead to a more general understanding of the role of collective behavior in social evolution.

Furthermore, more work is needed to understand how collective behaviors arise in relation to group size. The relationship between group size and self-organized behaviors is reviewed in Dornhaus et al. (2012), but there are still relatively few empirical studies that determine how group size relates to the expression of collective behaviors. To what extent are nonlinear benefits limited to large groups, and to what extent are small groups limited to additive benefits? Patterns relating group size to different benefit curves would help disentangle their independent effects on cooperation.

While many examples of collective behaviors are in species that have extremely high levels of cooperation and thus invariable in their levels of cooperation, such species will not be informative for testing the predictions of this model. However, understanding the selective forces and evolutionary processes that promote cooperation will help explain the trajectories that led to these more extreme social forms. As such, the predictions from this model will only be testable in species that retain some variation in their levels of cooperation. Possible study groups include simple eusocial groups, (e.g. Ponerine ants, Halictid bees, bumble bees, and Polistes wasps), and cooperative or communal breeders. Case studies need not be limited by reproductive cooperation, but could include cases where groups require cooperative effort to complete some task, such as when bark beetles overwhelm plant defenses as a group (Raffa & Berryman 1983). Any instances where cooperative groups engage in group-level or highly coordinated behaviors, such as the 'war dances' in meerkats (Jordan et al. 2007), the formation of fruiting bodies in social amoeba (Strassmann et al. 2000), or cooperative nest construction in sociable weavers (Leighton 2014) offer contexts to test the model's main predictions.

Future attempts to model the co-evolution of cooperation and collective behavior should focus instead on evolving collective behaviors that give rise to *s*- and *i*-values. Agent-based simulations of collective behavior would allow individual decision or interaction rules to evolve given some group's level of cooperation, such that benefit functions arise as a byproduct of optimal rules. Another extension of this model would be to permit players to differ in their competitive or reproductive efficiencies. The symmetrical structure of the model here most directly applies to groups where all individuals share an equal ability to take and use the communal group resource, either breeding independently or communally. However, many cooperative groups are not symmetrical, and instead include a dominant breeder who is more efficient at monopolizing or converting group resources into fitness than subordinate helpers, as exemplified in cooperatively breeding vertebrates (Koenig & Dickinson 2004) or social insect colonies (Wilson 1971). In order to model the evolution of collective behaviors in these asymmetrical groups, the current model would require a two roles with an efficiency coefficient to differentiate dominants from subordinates, and may predict different outcomes (Rubenstein et al. 2016).

Implications and Conclusions

Understanding the role of nonlinear benefits in the evolution of cooperation will more fully provide an explanation for why cooperation is maintained in biological systems. In addition, nonlinearities may be instrumental in explaining the co-existence of cooperation and conflict. The influence of nonlinear returns to promote cooperation are enhanced when such returns are steeper and in larger groups. However, nonlinear returns can discourage cooperation when thresholds (i.e. inflections) come early or groups are highly related.

The importance of nonlinear returns to cooperation may be especially relevant to the evolution of collective behaviors, which are likely to produce benefits in a nonlinear fashion. The role of synergistic or nonlinear interactions have been suggested to evolve in a positive feed-back loop with cooperation (Corning & Szathmary 2015), such that nonlinear interactions promote cooperation and cooperation promotes selection for more efficient interactions. The model here provides the first formal treatment of collective behaviors in the context of cooperation, providing theoretical support for the first step in this feedback loop. More empirical work is needed to confirm the effects of nonlinear benefits on cooperation, and their relevance to specific collective behaviors.

Figure 4.1: Group output curves for differently shaped benefit functions. Group size is fixed at ten (n=10) and the maximum group output is standardized to group size. The red dotted-line depicts the group output for linear returns to cooperation in both. **(A)** Three superimposed nonlinear benefit curves, which differ in their steepness (inflection, i = 0.5.) **(B)** Three nonlinear benefit curves, which differ in their point of inflection (curve steepness, s = 4).

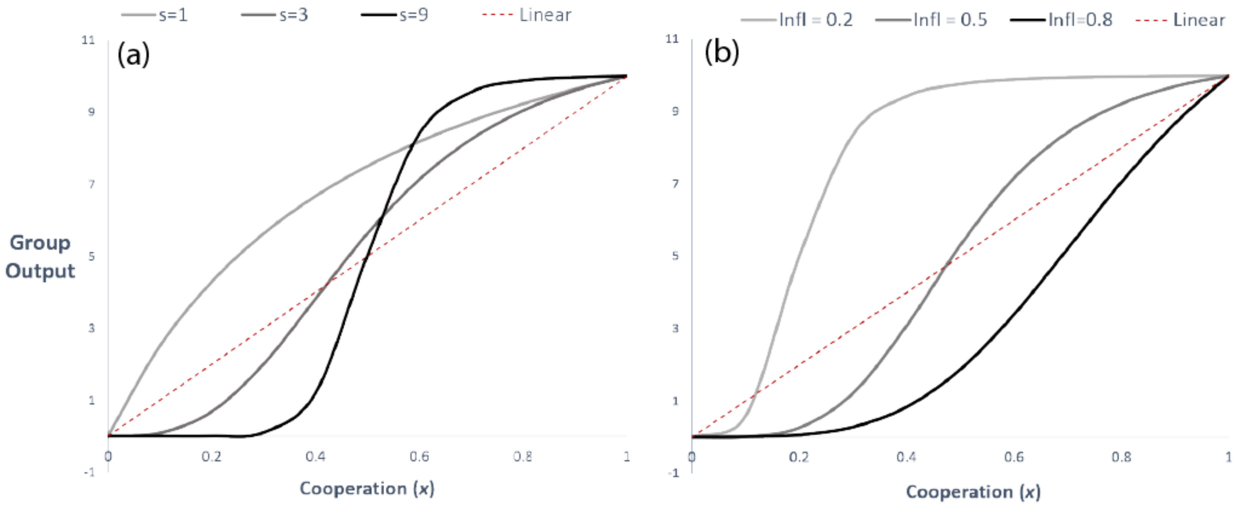

Figure 4.2. (A) The evolutionarily stable rate of cooperation rises as the steepness of the benefit curve (s) increases [relatedness (*r*) = 0.5, inflection (*i*) = 0.5, group size (*n*) = 10^7]. (B) The evolutionarily stable rate of cooperation rises as the point of inflection increases [relatedness (*r*) = 0.5, curve steepness (*s)* = 4, group size (*n*) = 10^7]. For comparison, the stable rate of cooperation for linear returns is plotted as the dotted line for equivalent group size and relatedness parameter values.

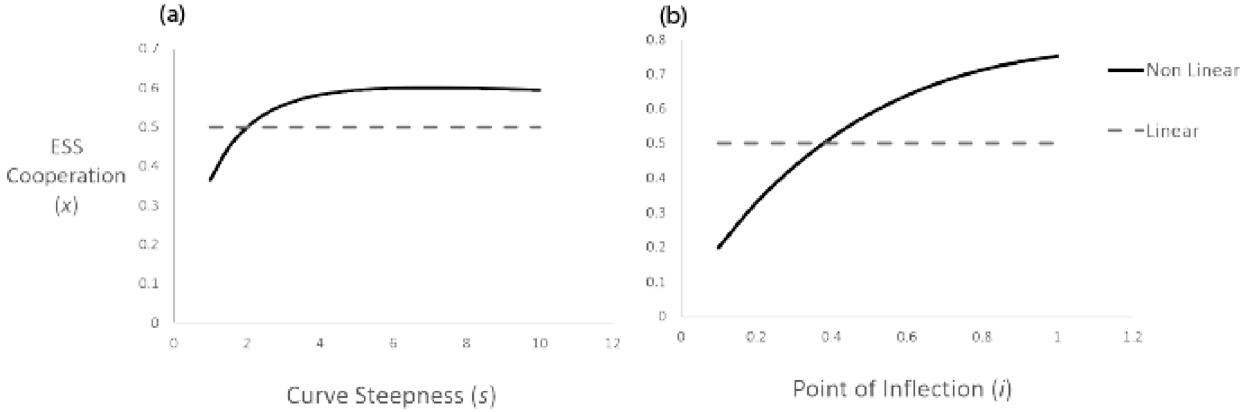

Figure 4.3: The stable rate of cooperation decreases as group size increases for both linear (dotted-lines) and nonlinear benefit functions (smooth lines). Three pairs of plots are depicted for different values of relatedness {*r*=0.2, 0.5, 0.8}, and are derived from an equivalent sigmoidal nonlinear benefit function [inflection (*i*) = 0.5, curve steepness (*s*) = 4]. For *r*=0.2 (green) and *r*=0.5 (blue), cooperation stays higher for nonlinear benefit functions, and the difference between linear and nonlinear ESS cooperation rates increases with group size. For higher relatedness, *r*=0.8 (grey), cooperation stays higher for linear benefit functions and the difference between the two stay relatively unchanged as group size increases.

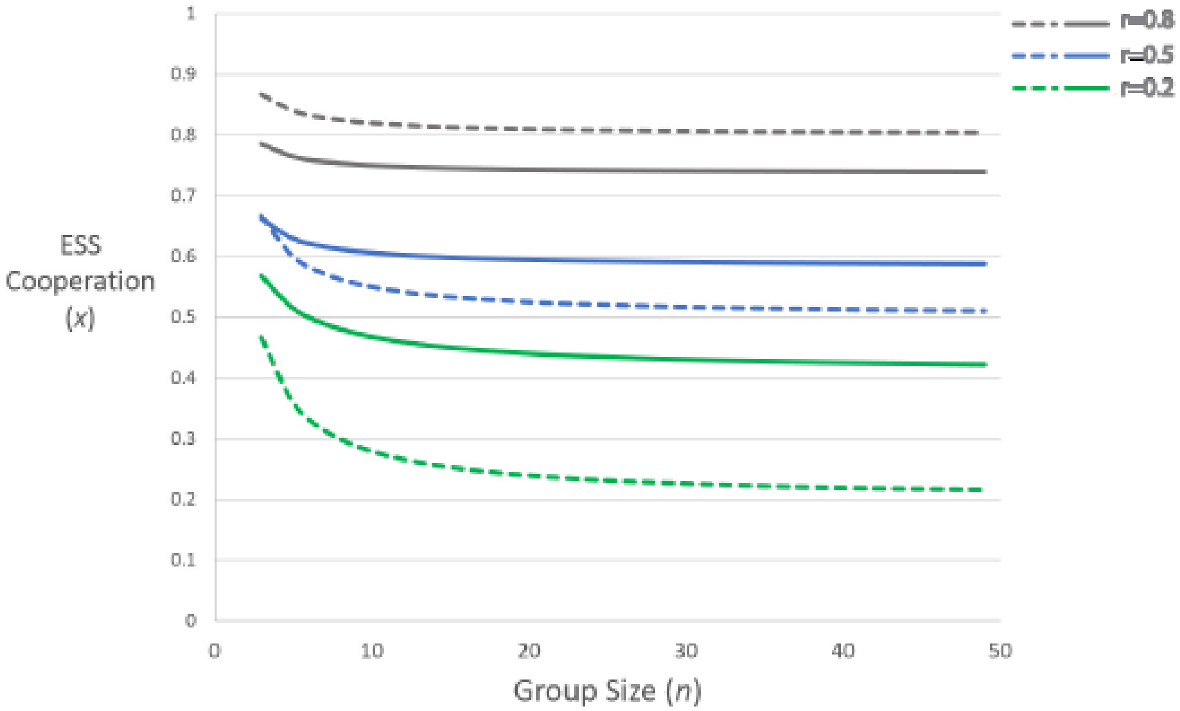

Figure 4.4: (A) The stable rate of cooperation increases with relatedness for both linear and nonlinear benefit functions. As relatedness increases, stable rates of cooperation start above the linear solutions and transition to below. (B) Benefit functions used to derive the lines in part (A). Each dot superimposed onto the benefit function represents the stable rate of cooperation for each benefit function at different relatedness values. (C) The instantaneous slope of the benefit function at the ESS cooperation rate for different relatedness values (r ={0.1, 0.3, 0.5,0.7, 0.9}). This figure depicts the relatedness values at which the nonlinear rate of return (i.e. slope) falls below the linear slope, illustrating why nonlinear benefits predict a decrease in cooperation at high relatedness. All benefit functions assume large group size (*n*) = 10^7 and the nonlinear benefit function has a curve steepness (s) = 5, inflection (*i)* = 0.5.

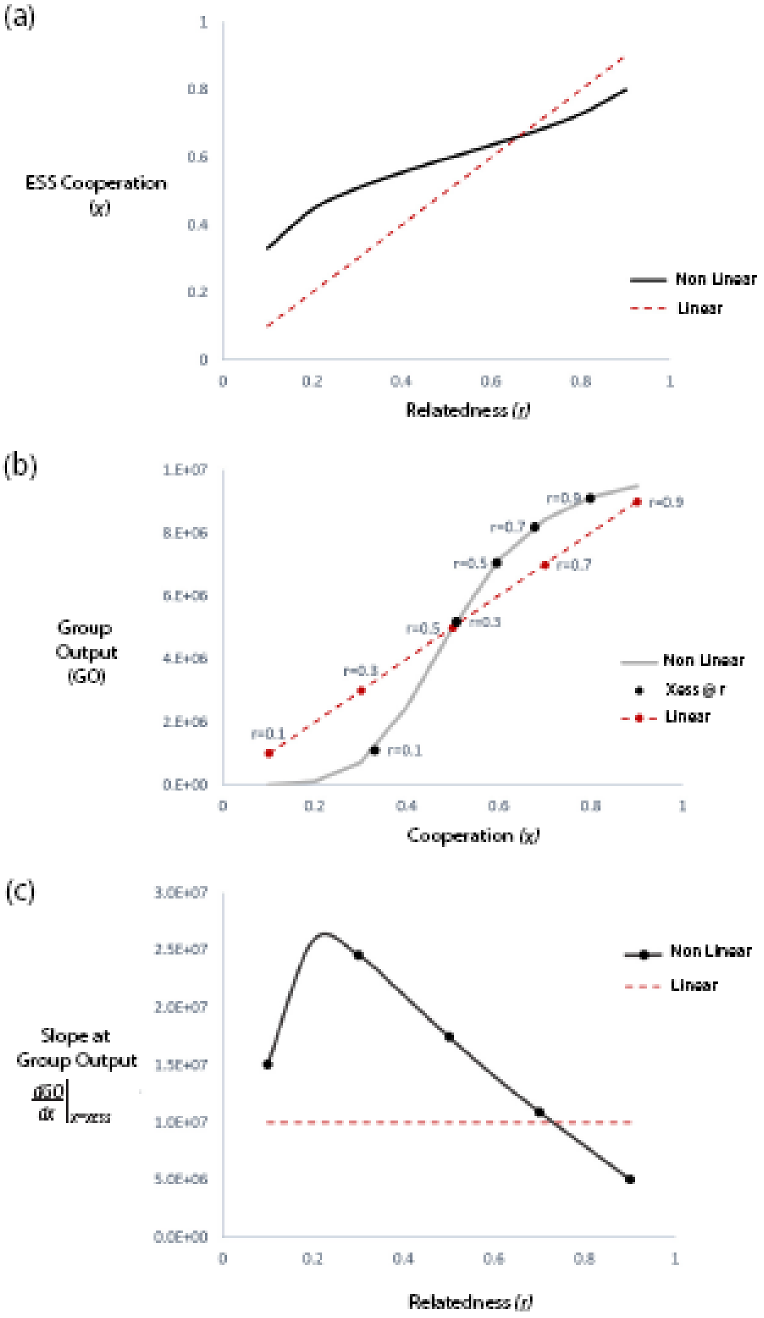

Figure 4.5: (A) The two nonlinear benefit functions here differ in their point of inflection (grey, i = 0.2; black, i = 0.8), and in the center is the linear benefit function (red dashed-line). Each dot superimposed onto the benefit function represents the stable rate of cooperation for different relatedness values (0.1-0.9). The position of the dots, or ESS cooperation rates, illustrates how curve shape shifts cooperation up or down, especially in relation to the linear case. (B) The distribution of ESS cooperation rates depends partly on the instantaneous slope of the benefit function. For the nonlinear curve with infl = 0.2, the slope lies above the linear case, but falls below at approx. r= 0.6. This reduction in slope below the linear case leads to a decrease in cooperation at high relatedness. Conversely, the slope of nonlinear curve with infl = 0.8 stays above the linear slope after passing the steepest part of the curve, and ESS cooperation is consistently higher than the linear case. All benefit functions assume large group size (*n*) = 10^7 and a curve steepness (s) = 5.

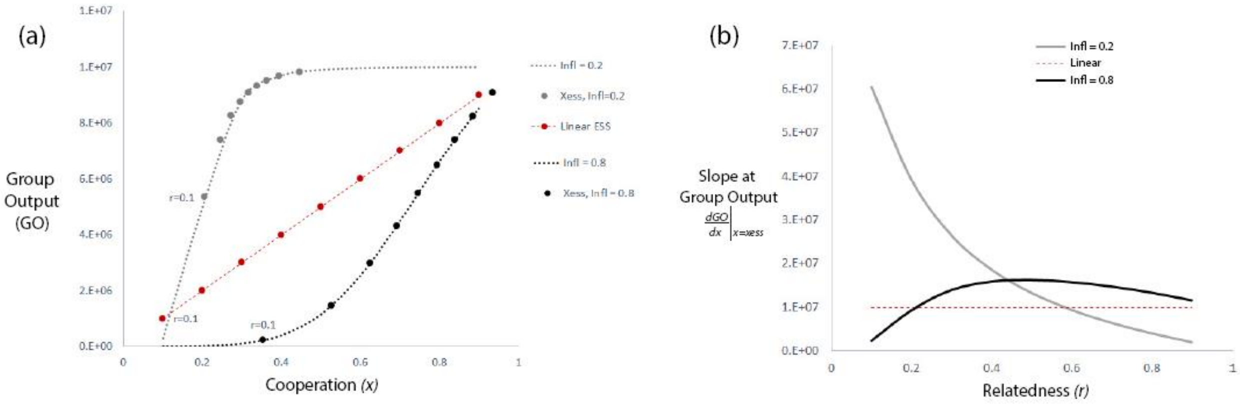

Table 4.1: List of model parameters and their definitions.

Variable	Definition
n	Number of individuals in the group
r	Relatedness between group members
G	Group Output, or shared resource produced by the group. GL refers to additive or linear accumulation of resources. GN refers to a nonlinear accumulation of resources.
F	The fraction of the group resource acquired by the focal group member. This is the result of selfish effort relative to the selfish efforts of the rest of the group
x	cooperative effort (0<x<1)
1-x	Selfish effort
x*	Population cooperative effort
s	Curve steepness - this is analogous to the Hill Coefficient. Each biomolecule or collective behavior will have a unique value.
i	Point of inflection – the proportion of group cooperative effort at which the curve steepness is greatest.

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