Towards a life history explanation of the origin and maintenance of eusociality:

A role specific energy budget for the primitively eusocial paper wasp

Polistes fuscatus

A dissertation

submitted by

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In partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Biology

TUFTS UNIVERSITY

May, 2011

Adviser: Philip Starks

Abstract

Natural selection predicts that each individual should strive to maximize its genetic contribution to the next generation. However, in eusocial organisms, many individuals give up some or all of their reproduction to help another individual. In highly eusocial organisms, like the honeybee, workers are partially or completely sterile and could not found their own colony. However, in primitively eusocial organisms like *Polistes*, workers are capable of reproducing and founding their own colonies. In addition, mated females often accept worker-like roles on new colonies rather than founding their own colonies. This opens the question of why an individual that could reproduce would choose, instead, to help another individual. To address this question I looked at the energetic costs of being a worker or a dominant, solitary, or subordinate foundress, as well as the metabolic differences among these individuals. I measured the cost of different behaviors, including interactions between individuals, flight, grooming and nest care. I found that foraging related behaviors (performed by solitary and subordinate foundress and workers) were quite expensive, while interactions (performed primarily by dominants) were relatively low cost. I also looked at the thermoregulation necessary for some of these behaviors, and found that flying *Polistes* thermoregulate, another energetically costly behavior. When I compared the cost of maintaining ovarian development among these different roles, I found that workers and subordinate foundresses spent more energy to maintain the same level of ovarian development, suggesting that they would need to expend more energy to create a colony, and, therefore, be less able than the average solitary foundress to maintain a successful colony. Finally, I created an energy budget for each role. I found that workers used more energy than any

other role, but, after that, solitaries with workers used approximately the same amount of energy on tasks as subordinates. Solitaries prior to worker emergence would be expected to use even more energy. This suggests that an individual with low resources and a high energetic cost of egg maintenance might be have a low success as a solitary foundress, and, therefore, be better off taking a subordinate role and helping a sister.

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Chapter 1:

Why create an energy budget for *Polistes*?

From honeybees and bumblebees that pollinate our crops to invasive species like the yellow crazy ant (Bos *et al*. 2008), eusocial insects have major ecological and economic impacts. However, there is much we still do not understand about the social system of these important insects. Eusociality is a social system in which multiple generations live together, care for brood together, and display reproductive division of labor. Reproductive division of labor means that one or a few individuals do most or all of the reproduction, while others reproduce much less, or do not reproduce at all. Instead, the animals care for the offspring of the reproducing individuals (Wilson 1971).

This division of labor is something of an evolutionary puzzle as evolutionary theory suggests that each individual should strive to maximize the representation of their genes in the next generation. Darwin himself considered the non-reproductive worker castes of social insects to be one of the greatest threats to his theory of natural selection (Darwin 1859). This is a particularly interesting puzzle in primitively eusocial system like *Polistes*, in which workers are capable of reproducing independently (Reeve 1991). In *Polistes*, all females have multiple reproductive options. Individuals emerging in the summer can either accept a worker role on the maternal nest or attempt to found independently. Individuals emerging in fall can either found alone or in multiple

foundress associations. In these associations, one individual accepts the dominant role, while the others reproduce rarely if at all (Reeve 1991). Since all these individuals are capable of reproducing, it is an evolutionary puzzle as to why they would accept non-reproducing roles.

Despite this conundrum, eusociality has evolved independently many times, particularly in hymenoptera (Andersson 1984). This suggests that there must be substantial fitness advantages to a eusocial living system. Many models have been proposed to explain this. Most models have explained the evolution of eusociality through indirect fitness achieved by the workers due to aiding relatives (reviewed in Reeve & Keller 2001). In addition, recently Wilson and Holdobbler (2005) and Nowak and colleagues (2010) have proposed alternative models of the evolution of eusociality involving eusocial alleles. However, these models do not necessarily explain the joining of foundresses in *Polistes*. Most models that have tried to address this particular question have taken the form of reproductive skew models.

Reproductive skew models

One category of models that has attempted to explain the evolution and maintenance of eusociality is reproductive skew models (Reeve & Keller 2001). Reproductive skew models use ecological and evolutionary data to predict both when associations of reproductively viable individuals should form and how reproduction should be divided within those associations (Reeve & Keller 2001). Most models start from a two-individual nest, with a dominant and subordinate individual. Fundamentally, these models are based on the equation $x \leq p + r(k(1-p)-1)$, where x is the success of a

subordinate founding alone, p is the percentage of reproduction on the nest done by the subordinate, r is the relatedness of the subordinate to the dominant, and k is the reproductive success of the joint nest relative to the reproductive success the dominant would have alone. In order for an association to be stable, the above inequality must be true, meaning that the subordinate's fitness on the dominant's nest is at least as high as it would be if she tried to found alone. These models can be loosely divided into transactional and tug-of-war models (Reeve & Keller 2001, Buston & Zink 2009).

Transactional models assume that one individual--either the dominant or the subordinate—has control over reproduction, although this individual may allow the other to reproduce to maintain harmony. There are two main types of transaction models: concession models and restraint models. In concession models, the dominant controls reproduction, but may allow the subordinate to reproduce in order to prevent the subordinate from leaving (Reeve & Ratnieks 1993). In restraint models, the subordinate controls its own reproduction, but will refrain from reproducing beyond a certain point in order to prevent the dominant from evicting it (Johnstone & Cant 1999).

Tug-of-war models assume that neither individual controls reproduction (Reeve *et al*. 1998). In these models, each individual should cede enough reproduction to the other to maintain group cohesion, but between the ideal of the dominant and the ideal of the subordinate is a zone of conflict (Reeve *et al.* 1998, Tibbets & Reeve 2000). How exactly the reproduction will be distributed within that range will depend on the competitive abilities of the individuals. These models also predict that a great deal of energy will be expended on competition that could otherwise be put towards reproduction, creating a prisoners' dilemma. Buston and Zink (2009) attempted to

resolve this by proposing that the threat of costly competition could be used to negotiate reproductive share, just like the threat of leaving or eviction.

Polistes **as a model system**

Polistes has been a model system for reproductive skew since the first skew models were created (Vehrencamp 1983, Reeve 1991, reviewed in Keller & Reeve 1994). *Polistes* gynes can found colonies either alone, or in multiple foundress associations (Reeve 1991). When they found colonies in multiple foundress associations, a linear dominance hierarchy is formed in which one individual is behaviorally and reproductively dominant (Reeve 1991). This individual does most or all of the reproduction, and initiates most interactions (Reeve 1991). In general, however, foundresses are mated, reproductively viable females. If the dominant foundress is removed, the highest ranking subordinate will generally begin laying eggs, and show behavioral dominance towards any other foundresses present on the nest (Pardi 1948, Field *et al.* 1998, Cant *et al.* 2006).

Since most multiple foundress associations in *Polistes* are two-partner interactions in which both are reproductively viable, *Polistes* has been considered an optimal system for modeling the evolutionary origins of eusociality (Reeve & Keller 2001, West-Eberhard 2006). In addition, *Polistes* are primitively eusocial, which means that the workers are also capable of mating and laying both male and female eggs (Wilson 1971). This means that the queen-worker interactions can be modeled in many of the same ways. It also allows researchers to ask questions about the origins of eusociality that could not be addressed as well in highly eusocial systems (West-Eberhard 2006).

All these facts, along with the ease of observing their behavior (West-Eberhard 2006) made them an ideal system for reproductive skew models (Reeve & Keller 2001). Recent work in *Polistes*, however, has not supported many of the predictions made by reproductive skew models (Nonacs 2006). Early work by Field and colleagues (1998) examined a population in which all foundresses could be full sisters. Their work seemed to support some of the predictions of a staying incentive model, including increasing skew with increasing relatedness. Other work went on to test the conflicting predictions of the different types of skew models (Reeve *et al.* 2000, Tibbets & Reeve 2000, Cant & English 2006) and found support for specific predictions about change in skew or aggression with particular traits such as relatedness, or time of year.

However, more recently, researchers have begun testing the basic prediction that a subordinate accepts a subordinate role because she will have higher overall fitness in that role. Based on the current assumptions of reproductive skew models, these tests have often failed to support this prediction (Nonacs 2006, Nonacs *et al.* 2006, Liebert & Starks 2006). Various explanations have been suggested for this, including inability to discriminate fine-grained variation in relatedness (Gamboa 2004, Nonacs *et al.* 2006), but this is unlikely to entirely address the deviations from the predictions. In order to understand the success necessary for an individual to become and remain a subordinate, it is necessary to understand what she is giving up in doing so.

Hamilton (1964) first explained altruistic behaviors with the inequality rB > C. In this equation, C is the cost to the focus individual, B is the benefit to the receiver, and r is the relatedness of the focus individual to the receiver. Many studies have looked at the relatedness of *Polistes* co-foundresses (e.g. Field *et al.* 1998, Reeve *et al.* 2000, Cant *et*

al. 2006, Nonacs *et al.* 2006), and the advantages of multiple foundress nests (e.g. Nonacs & Reeve 1995, Tibbets & Reeve 2003), but few studies have really addressed what a subordinate is giving up in accepting a subordinate position. Those that have addressed this question have generally assumed that the subordinate would have equivalent success to the average solitary were she to found alone (Nonacs *et al.* 2006). However, this is not necessarily the case. If subordinates do not have the same capacity to lay eggs and build and provision a nest as solitary individuals, they might have relatively low success founding alone, particularly if being a solitary foundress is taxing. To address this, I looked at the energy use of workers and solitary, subordinate, and dominant foundresses.

Energy Use

One way to understand how "hard" something is for an animal is to look at the energetic costs. Animals are frequently energy limited, particularly during reproductive periods (Masters *et al.* 1988a, b; Villa & Rinderer 1993; Prestwich 1994, Freitas *et al.* 2010). Any energy spent on adult behaviors - such as foraging, interaction and nest defense - is energy that cannot be put directly into reproduction. In addition, even if unlimited energy sources are available, the act of acquiring energy that is foraging is frequently a risky behavior. It can involve going out into open spaces, leaving behind defenseless offspring, and focusing attention away from predators, in addition to involving flying, an energetically costly behavior (Wolf *et al.* 1999, Reeve 1991).

In *Polistes*, early nests are energy limited (Rossi & Hunt 1988). When pre-worker nests were supplemented with honey, the first workers emerged earlier in the

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season (Rossi & Hunt 1988). This is important because most colony mortality occurs in the pre-worker phase, which means that the earlier workers emerge, the more successful a colony is likely to be (Reeve 1991). In addition, foraging is risky in *Polistes*. There is some risk to a foraging foundress from predation and weather and (for a solitary foundress) the defenseless brood are left alone, open to attack by birds or other insects (Reeve 1991). These costs indicate that any extra energy expended by early season foundresses will have a fitness cost. In addition, any winter stores that the foundresses start with could provide some advantage in either lower foraging requirements or earlier brood production.

The differences in energy reserves or energy expenditures between foundresses could help explain the different roles that *Polistes* foundresses adopt. A *Polistes* gyne emerging from hibernation can 1) found a nest alone, 2) join another foundress, or 3) sit and wait, later trying to adopt or usurp another nest (Reeve & Keller 2001, Starks & Fefferman 2006). If she founds with another foundress, she may either accept a subordinate role, or accept only the dominant role and either leave or evict the other individual if she cannot maintain dominance. Dominant, subordinate and solitary foundresses do very different tasks, and, therefore would be expected to have different overall energy expenditure (Table 1.1). Dominant foundresses focus on reproduction and interactions with other individuals, while subordinates do most of the foraging. Solitary foundresses in the preworker phase, however, do all of these tasks, except interactions (Table 1.1).

This differential task performance opens the question of how much energy these tasks use. Since individuals in different roles spend their time differently, we expect them

to also allocate their energy differently. Not only do we expect a dominant to be spending more energy on reproduction, it is possible that the different roles use different amounts of energy overall. Since solitary foundresses do everything, being a solitary foundress might be very energetically costly.   If being a solitary foundress is extremely demanding, it might be easier for a low quality individual to take the role of a subordinate foundress or worker. Even if the subordinate role is not less energetically costly, it might still benefit an individual with low fat stores. If a solitary foundress dies before workers pupate, all her brood dies with her, and she has no reproductive success. However, if a subordinate dies, she has still enhanced the fitness of the dominant. Therefore, even if these weaker individuals have low survival as subordinates, they may not be losing much in accepting this role. If their success as solitaries would be low, the cost of accepting a subordinate role is also low, meaning that the benefit of the subordinate role does not need to be as high.

This work can give us insight into why an individual might accept a subordinate role in *Polistes*, which, in turn, can give us insight into the evolutionary origins and maintenance of eusociality. *Polistes* has been used as a model system for explaining the evolution of eusociality and dominance hierarchies in insects for decades (West-Eberhard 2006). Eusocial organisms play a huge role in the ecology around us and in our economy as pollinators and pests (Moller 1994, Donovan 2003, Ricketts 2004, Hayter & Cresswell 2006). This new perspective provides an opportunity to resurrect reproductive skew models by better incorporating the variation among individuals. This will help us to better understand eusociality and, in doing so, allow us to better understand the eusocial organisms around us.

In the remainder of my thesis I will address these questions. I will start in chapter 2 by discussing laboratory techniques used to address these questions. In chapter 3, I will discuss the energetic cost of reproduction and how it varies among different castes. In chapter 4, I will address the energetic costs of the interactions that take place on *Polistes* nests. In chapters 5 and 6, I will discuss thermoregulation in *Polistes* and its effects on the invasion of *P. dominulus*. In chapter 7, I will discuss the energetic cost of flight in *Polistes*, a behavior that is important for foraging, and rarely performed by dominant foundresses. Finally, in chapter 8, I will compile all of these data into a role specific energy budget for workers and subordinate, solitary, and dominant foundresses. With this energy budget I will address the differences in the roles and the variation among individuals.

Table 1.1 All behaviors associated with the nest in *Polistes*. Behaviors marked with an α are done by the dominant, those marked with a β are done by the subordinate, those marked with an S are done by a solitary foundress. Both the dominant and the subordinate do most behaviors occasionally. Those that are predominantly done by subordinate are only marked with the symbol of that subordinate, and vice versa.

Activity	What it Involves
Foraging	• Flight (β, S) • Killing and butchering prey (β, S) • Collecting nectar (β, S) • Collecting wood pulp (β, S)
Nest Construction	• Adding or repairing cells (α, β, S)
Brood Care	• Feeding larvae (α, β, S) • Checking cells (α, β, S) • Laying eggs (α, S)
Adult Food Exchange	• Trophylaxis (α, β, S) • Prey transfer (β, S)
Adult Interactions	• "Aggressive" behaviors (α) • Tolerant Behaviors (α, β) • Allogrooming (α, β)
Nest Defense	• Attacking non-nestmates (α, S) • Attacking or threatening predators or parasitoids (α, S)
Inactivity	• Resting (α, β, S) • Grooming (α, β, S) • Walking on nest (α, β, S)

Chapter 2

Lab methods in behavioral ecology

Abstract

Behavioral ecology is the study of the ecological and evolutionary bases for variation in animal behavior, answering proximate and ultimate questions of why animals behave the way they do. The laboratory setting enables the isolation and control of specific variables, the removal or randomization of confounding factors and simplifies the tracking of an individual's behavior. Laboratory experiments, in parallel and in comparison to field studies, are valuable for answering specific questions and certainly most ecological investigations can benefit from a combined experimental approach. Here we focus on four model areas of behavioral ecological research: mate selection, nepotism, foraging and dominance. Using both vertebrate and invertebrate examples we consider the advantages and disadvantages of laboratory experiments and the unique information they can provide, including a comparison of three laboratory research contexts; neutral, natural and contrived. We conclude with how laboratory studies can help us to understand the contexts in which behavioral variation occurs in the natural environment.

 Introduction

Behavioral ecology is a popular and active field of research that employs integrative approaches to study both the ecological and evolutionary bases for animal behavior. Behavioral ecology endeavors to determine the role of behavior in enabling individuals' adaptations and constraints to the surrounding environment (Krebs & Davies 1997). The

initial research and observations of behaviorists (ethologists and comparative psychologists) demonstrated that though highly variable, certain behavioral patterns can also be exceptionally characteristic and descriptive at the level of each species (Lorenz 1937, Tinbergen 1963, von Frisch 1974). Together with the development of sophisticated theory and extensive experimentation this has led to the development of the thriving field of behavioral ecology, which connects individual and phylogenetic variation in behaviors with ecological variables and evolutionary history (Krebs & Davies 1997).

In behavioral ecology experimentation, researchers aim to answer four focal types of questions, as famously coined by Tinbergen (1963), and divide research areas into two major fields: proximate and ultimate causes of behavior. Proximate questions look directly at the *causal* and *ontogenetic* factors that influence observed behaviors, whereas ultimate questions, in turn, are concerned with the *functional* adaptations and *evolutionary* processes that may have imparted a selective advantage to a certain behavioral trait (Tinbergen 1963, Krebs & Davies 1997, Drickamer *et al.* 2002). These questions are essential focal points for behavioral ecologists seeking a complete understanding of any and all behaviors observed in nature and examined in the laboratory.

Field research on animals in their natural environment can provide the most appropriate and richest source of information on both the relationships of organisms to individual environmental variables and the relationships between multiple organisms within specific environmental parameters (Simberloff 2004, McGill *et al.* 2006, Johnson & Stinchcomb 2007). However, it is clear that in the field setting there are also countless external variables beyond the realm of experimental control that may be influencing the

particular target behaviors we wish to study. It is in this regard that we take organisms

into a laboratory setting to facilitate a more extensive control over external environmental

and, potentially, internal physiological and motivational variables, as well as limiting the

number of interacting species or individuals that can all impact on behavioral outcomes.

Of course a captive approach does limit the interpretations and applicability of results

when the target species is isolated from influencing factors that may be present in a

natural field situation (Table 2.1). Therefore, the chosen research setting of field vs.

laboratory must ultimately depend on the question to be answered, although undoubtedly

many research topics in animal behavior can benefit from a combination of both

laboratory and field techniques. Accordingly, when we surveyed the literature of

experimental techniques used in all published articles over 12 months of both '*Animal

Behavior*' and *Behavioral Ecology*' (July 2006 - July 2007) we found similar proportions

of studies that occurred solely in the laboratory (including experiments on captive

populations in reasonably natural enclosures) or solely in the field (research on wild

animals which may or may not have been handled by investigators to individually

mark/tag for identification) and very few studies that looked at the focal animals'

behavior in both settings (Figure 2.1).

Here we will focus on discussing varying settings and methods used solely in the

laboratory under the realm of several major types of behavioral ecological sub-disciplines

that animal behaviorists are actively engaged in investigating. We present both vertebrate

and invertebrate exemplars and discuss what these methods uncover and what possible

limitations the laboratory environment places on interpretation. We also discuss three

varying laboratory research contexts that may be employed, outlining their main

advantages and disadvantages. The methods and topics presented here are not in any way exhaustive; rather they highlight the range of techniques currently available in a laboratory setting for application in behavioral ecological research.

2. Four sample questions in behavioral ecology research

Within the field of behavioral ecology, researchers can set out to answer questions within several major areas of animal behavior that can be explored in the laboratory setting. We selected studies that fall into the four categories of mate selection, nepotism, foraging and dominance. We will present representative examples that highlight the techniques that may be employed when researching these areas, as well as their limitations.

2.1. Mate selection

Mate selection in animals is a well-studied area of behavioral ecological research. It is focused on understanding the factors or recognition facets, such as correct species, sex, genetic relatedness and genetic quality that can lead an animal to select one individual over another for mating and/or pair bonding (Sherman *et al*. 1997). Laboratory mate selection research attempts to dissect the specific features of individuals that may make them attractive or unattractive as potential mates, and to identify what exact perceptual, physiological, and behavioral processes are involved in reaching the final mating decision. Through examples taken from a model system, the zebra finch (*Taeniopygia guttata*) (Zann 1996), we discuss here the use of live stimuli, highlighting findings and limitations of the laboratory setting, including impacts of experimental artifacts and proximal environmental factors on subject ontogeny.

The zebra finch is a small, sexually dimorphic, socially monogamous, Australian passerine. Mate preferences are frequently assessed via the presentation to a subject individual of a selection of live stimuli birds that vary to different degrees in phenotypic or behavioral attributes (Zann 1996), such as bill color (e.g., Collins *et al.* 1994) or song variations (Houtman 1992). These preferences for one individual over another (or several others) in a simultaneous choice scenario are frequently measured by the proportion of choice time spent with a certain stimulus individual (reviewed in Forstmeier & Birkhead 2004), a measurement of passive choice (c.f. active operant choice: e.g., Riebel 2000). This passive spatial association method is widely used across different choice experiments (Hauber *et al.* 2000) but is not without interpretational constraints; the use of a sole preference metric and a lack of physical interactions between subject and stimuli can restrict determination of sexual or social subject motivation (Rutstein *et al.* 2007). The passive choice paradigm does not require context-specific action by the individuals beyond spatial movement, which occurs throughout everyday activities (Hauber *et al.* 2001) and for highly social birds such as the zebra finch, choice could just be indicative of a wish to associate rather than indicating an actual sexual preference (Riebel 2000).

An alternative method to assess preferences is by measuring multiple behaviors displayed by the subjects that are naturally produced in specific contexts, such as female or male courtship displays (e.g., Burley *et al.* 1982, ten Cate 1985, Clayton 1990, Collins *et al.* 1994, Mansukhani *et al.* 1996) and may reveal social, sexual, or pair bonding preferences that spatial associations fail to show (Campbell & Hauber: in press). A further methodological option for confirming sexual motivation is to allow interactions between subject and stimuli in a free-flight aviary paradigm and use detailed behavioral

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observations to document sexual pair bonds formed or ultimately, breeding success (e.g., Burley 1981, ten Cate 1985, Mansukhani *et al*. 1996, Rutstein *et al*. 2007). Most studies though would benefit from a combination of assessment methodologies or a validation of the behavioral metric used in a series of studies to be able to comprehensively measure responses to specific traits in addition to confirming subject motivation and choice context (Rutstein *et al*. 2007).

In addition to mate selection based on varying phenotypic and behavioral traits, artifacts of the experimental environment may also impact on choice decisions. Examples include human alterations of the study species' appearance, such as the application of leg-bands that significantly alter zebra finch attractiveness (Cuthill *et al*. 1997), or factors such as the lighting environment where the absence of ultraviolet wavelengths important in avian vision may influence natural mating preferences (Hunt *et al*. 1997). The physiological stress hormone levels of both the subjects and the stimuli can also influence female choice behavior, which is important to consider in the handling of subjects and giving time to acclimatize to novel testing environments (Roberts *et al*. 2007). However, physiological measures of stress responses, for example after the experimental separation of pair bonded mates, could in some cases provide critical insights into behaviorally cryptic discrimination abilities of individuals (Remage-Healey *et al*. 2003).

Furthermore, it is important to consider how proximate environmental factors may influence subject and stimulus ontogeny. For example the nutritional and stress-related hormonal environment can decrease the quality of males by altering ornamental plumage and song complexity, making them unattractive to females (Spencer *et al*. 2003, 2005, Naguib & Nemitz 2007). A poor developmental history of stimuli, for instance,

may influence zebra finches' selections, irrespective of the phenotypic trait being experimentally manipulated, and poor developmental history of subjects may bias the strength, consistency or direction of their preferences based on their self-perceived poorer quality (Burley & Foster 2006). These factors indicate critically important knowledge of developmental history when testing for mate preferences in a laboratory setting. Finally, it must be considered that although laboratory environments permit experiments not otherwise possible in the field, captive environments may affect the physiology and behavior of research animals leading to different responses than what would be found in their wild counterparts (e.g., Ewenson *et al.* 2001, Rutstein *et al.* 2007).

Laboratory settings, with zebra finches and with many other species, are ideal for measuring individual subject response to specific varying traits that cannot otherwise be assessed in the field, but can present difficulties in determining subject motivation, and the artificial experimental environment may modulate behaviors that would be present in the study species' natural habitat, which needs to be considered for result interpretation.

2.2. Nepotism

Natural selection acts on all living organisms, selecting for alleles and causing them to increase in frequency between subsequent generations. While selection typically occurs on individual survival to reproduction and the success with which individuals pass their own genes on into the next generation, non-descendant kin also share genes with the focal individual in proportion to their relatedness. Therefore, natural selection can select for individually costly behaviors that are preferentially directed at and favor relatives, as long as the benefit to kin (adjusted for relatedness) outweighs the cost to the focal

individual (Hamilton 1963, 1964). In order for kin selection to act, individuals must be able to direct their aid to kin over randomly related (i.e. non-kin) conspecifics in the population. Within the laboratory setting, experiments can be performed to determine whether individuals can and do distinguish kin from non-kin, how they do so, and under what circumstances they show behavioral discrimination (Mateo 2002).

To direct aid towards kin, an individual must first be capable of reliably recognizing or associating with kin. Individuals can be given choices with options that allow them to aid or harm partners that are kin or non-kin, allowing the researcher to discern what features are used for kin recognition by removing or altering cues. In addition, the laboratory setting allows for detailed manipulation of the context of interactions. Frequently, animals exhibit different levels of kin-discrimination in different experimental contexts (reviewed in Liebert & Starks 2004) and these manipulations allow researchers to determine what external variables can affect kin-discrimination.

Neutral arena trials are frequently used in the laboratory to detect kin recognition. Two or more individuals are placed in an arena with minimal natural cues then either resources (e.g., rove beetle larvae *Aleochara bilineata*: Lize *et al*. 2006) or threats (e.g., salamander *Hemidactylium scutatum*: Harris *et al.* 2003) are added to observe competition or defense behavior that favors related individuals.

Studies with contrived contexts can be used to test how nestmate-recognition (a correlate of kin recognition) ability is acquired, as well as the mechanisms and contexts of recognition. To test the context dependence of recognition, proximate environmental cues such as nest fragments can be introduced to arena trials to observe the interactions that result (e.g., paper wasp *Polistes dominulus*: Starks *et al*. 1998). The learning of kin-

recognition can be tested by removing very young animals such as paper wasps (*Polistes fuscatus*), from their parental nests upon emergence and raising them in controlled isolated environments (e.g., single boxes), then subsequently assessing their nestmate recognition abilities (Shellman & Gamboa 1982). These animals may also be reintroduced to unrelated conspecifics or nest material to determine whether they learn those cues from their environment and discriminate in favor of their "adoptive" kin (e.g., *Polistes carolina* and *P. fuscatus*: Pfennig *et al.* 1983).

In colony-living organisms, kin discrimination can be tested by introducing animals to laboratory colonies (e.g., ant *Pachycondyla luteipes*: Kikuchi *et al.* 2007). By varying the animals introduced (e.g., Kikuchi *et al.* 2007) or the circumstances of the colonies to which they are being introduced (e.g., ant *Linepithema humile*: Vásquez & Silverman 2008), the researcher can determine the contexts that lead to acceptance and rejection of nestmates and non-nestmates. These laboratory studies have provided insight into the adaptive value of kin-recognition by increasing our understanding of when animals do and do not display kin-discrimination.

One major flaw with laboratory studies of nepotism is that kin-recognition and discrimination behaviors are frequently context dependent (e.g., Liebert & Starks 2004). In some species, kin-recognition can be as simple as treating all conspecifics in the colony or nest as kin (reviewed in Holmes & Sherman 1982), additionally recognition behaviors are often affected by the social environment (e.g., Fletcher & Blum 1983) or by situational cues (e.g., Starks *et al.* 1998). Therefore, since context can be influential, the unnatural environment of the laboratory can potentially obscure important aspects of kin discrimination or even prevent it from being observed at all (e.g., Starks *et al.* 1998).

One method of partially addressing the lack of an appropriate fitness context in laboratory studies is to augment laboratory kin recognition studies with parallel experiments in the field (Kikuchi *et al*. 2007). Nestmate discrimination studies can be supplemented by studies of relatedness (e.g., ant *Proformica longiseta*: Seppä *et al*. 2008) and aggression (e.g., *Polistes fuscatus*: Gamboa *et al*. 1991) in the field to determine whether animals are discriminating in the contexts and ways that are predicted. In addition, context can be varied within the laboratory setting (e.g., Starks *et al*. 1998) to test for potential effects of a more natural context. Especially when combined with field studies and genetics, laboratory studies can provide valuable insight into the adaptive value and ontogeny of kin discrimination.

2.3. Foraging

All living organisms require food for energy to survive, and evolutionary optimal foraging theories predict decisions to be made during foraging that maximize energy intake (Drickamer *et al*. 2002). There is an extensive variety of foraging tests that can be carried out in a laboratory setting to investigate food preferences, foraging strategies, and the development of foraging proficiency. We present here select examples from research on New Caledonian crows (*Corvus moneduloides*) of how behavioral ecology laboratory research can be used to enhance field findings and provide greater insight into the development and learning of foraging techniques.

New Caledonian crows are omnivorous, forest-dwelling corvids endemic to New Caledonia where field observations have shown these birds to be highly specialized in their manufacture of stick and hooked or barbed tools (exclusively from *Pandanus* spp.

plants) that are used to pry out prey from holes (Hunt 1996, Hunt & Gray 2002). To expand on field findings, researchers in a controlled laboratory setting have specifically explored the development of tool use, the influences of social context, and the limitations of the tool use behavior. Behavioral observations, through the tracking of a particular individual over time, have documented rapid *de novo* shaping and use of novel material (e.g., a piece of straight wire) to obtain food (Weir *et al.* 2002) in the absence of extensive prior experience in tool shaping, thus demonstrating spontaneous tool-making behavior (Weir *et al.* 2002). Further developmental research revealed that laboratory-reared juveniles with controlled ontogeny and no prior experience, observation, or other means of social transmission of information, spontaneously used tools to obtain food (Kenward *et al.* 2005), which provided evidence of an innate predisposition for the tool-use behavior.

The captive environment did, however, severely restrict sample sizes (e.g., Weir *et al.* 2002, one naïve individual of the two captive subjects), which limited the conclusions able to be drawn. In addition, the effects of the surrounding environmental conditions that would be present in the crows' natural habitat but missing in a laboratory setting must be taken into consideration. Field observations revealed that preferential stick or *Pandanus* tool use is highly specialized at the level of the individual (Hunt & Gray 2007) and between different locations (Hunt & Gray 2003). These levels of variability are consistent with a degree of niche partitioning and social transmission important for the cumulative evolution of the tool-use behavior and that is not accounted for in a laboratory setting, which may modulate behavioral patterns (Hunt *et al.* 2007).

Furthermore, while there is evidence for a predisposition for stick tool manufacture, there is little complimentary evidence for innate recognition of the *Pandanus* plant (Kenward *et al.* 2005) over other potential tool materials, which has implications for substrate use when investigating tool manufacture in the laboratory and differential strengths in the role of social learning.

To investigate in detail the possibility of social transmission of information and effects of learning, the full ontogeny of the tool-use behavior was documented (Kenward *et al.* 2006). Specifically, juveniles trained with human demonstrators later showed greater tool-use proficiency than the naïve individuals and preferred to handle objects they had previously seen being handled by their human trainers (Kenward *et al.* 2006). These and other findings suggest the specialized tool-using behavior presents a partially inherited basis followed by individual learning and social influences to perfect tool formation and use (Kenward *et al.* 2005, 2006, Hunt *et al.* 2007) of which the full extent of social behavioral modulation is likely impossible to document in a captive environment.

Such laboratory experiments demonstrate the type of ontogenetic knowledge that can be gained about complex foraging strategies from socially isolated individuals and controlled rearing environments. Nonetheless, the natural ecological context has to be considered and how the artificial environment may modulate results and limit interpretations. Foraging studies such as these presented can benefit from a combination of both laboratory and field studies to have a greater contextual understanding of the observed behaviors.

2.4. Dominance

Many social animals exhibit dominance hierarchies that can be mutually beneficial through reducing conflicts among members of social groups involving the distribution of food (e.g., Appleby 1980), opportunities for reproduction (e.g., Blatrix & Herbers 2004), or the delegation of communal tasks (e.g., Tentschert *et al*. 2001). Since dominance hierarchies mediate factors that are necessary for survival and reproduction (e.g., Pagel & Dawkins 1997), the methods by which dominance hierarchies are established and maintained are important for understanding social behavior in many animals. Within the laboratory setting, both the initial establishment of dominance hierarchies and the interactions within established dominance hierarchies can be observed. The value of dominance to an individual in a hierarchy can also be determined by manipulating environmental or group factors to see whether individuals choose to accept subordinate status, challenge for dominance, or leave the group.

Experimental trials with two or more individuals grouped in a neutral arena, and allowed to contest for dominance (e.g., paper wasp *Polistes dominulus*: Tibbetts & Dale 2004, Tilapia *Oreochromis niloticus*: Gonçalves-de-Freitas *et al*. 2008) allow researchers to isolate environmental predictors of the hierarchy structure such as chemical cues (e.g., Gonçalves-de-Freitas *et al*. 2008) or predictors of dominance in individuals such as female mated status (e.g., *Polistes fuscatus*: Downing 2004).

The establishment and maintenance of dominance hierarchies can be investigated by creating contrived situations within the laboratory and observing the response of the animals. Animals with different traits, such as varying genetic strains, can be grouped in artificial colonies (e.g., rats *Rattus norvegicus*: Ely *et al*. 1997) or given the opportunity

to found their own colonies (e.g., *Polistes fuscatus*: Downing 2004). These studies can allow researchers to determine the effects of specific changes in context on the foundation of dominance hierarchies (e.g., rats *Rattus norvegicus*: Duncan *et al*. 2006).

The fitness component of dominance can also be studied in the laboratory by modifying the social environment within a group and observing the changes in the dominance hierarchy. This can involve removing the dominant individual(s) and observing the re-establishment of the dominance hierarchy after their removal (e.g., ant *Dinoponera quadriceps*: Monnin & Peeters 1999, *Polistes dominulus*: Strassman *et al*. 2004). The dominant individual(s) may then be replaced to observe how they fare in the newly established dominance hierarchy (e.g., ant *Harpagoxenus sublaevis*: Bourke 1988). In addition to behavioral metrics, physiological measures such as sympathetic nervous system activity can be taken from individuals of different dominance status to assess specific physiological correlates of being dominant or subordinate (Ely *et al*. 1997). Additionally, other modifications can be made to the nest or the environment such as removing reproductive-destined eggs in eusocial paper wasps to observe the effects on aggression in the dominance hierarchy (Reeve & Nonacs 1992).

Laboratory experiments allow for detailed manipulations and observations of social structures that are rarely possible in the field. However, laboratory experiments can exclude contextual cues that are important for the establishment and maintenance of a dominance hierarchy. Partners chosen by researchers are unlikely to be the same partners that would have been paired in the field, and may have different interactions. Laboratory studies that may have lacked important cues can be supplemented by replicating the observations in field studies of early dominance contests (e.g., *Polistes dominulus*:

Dapporto *et al.* 2006) and by genetic studies of dominance in established colonies (e.g., paper wasp *Polistes annularis*: Peters *et al.* 1995). When these considerations are taken into account, however, laboratory studies and the creation of unnatural experimental situations can allow observations on and provide information about the establishment and maintenance of dominance hierarchies that cannot be gathered in field studies.

3. Laboratory contexts

Within a laboratory setting, behavioral experiments can be executed in any of three contexts: neutral, natural or contrived. While no laboratory experiment can perfectly replicate field conditions, the different contexts vary in the amount of control they offer and in how well they relate to field conditions.

A neutral context is an experiment in which the animal is removed from natural cues and placed in a simple neutral arena. While in the arena, the animal has very few cues outside of those specifically provided for the experiment. These experiments are artificial and far removed from natural conditions, but permit close observation, fine control of variables and response to selected stimuli that are expected to provoke a reaction including, for example, a conspecific, a food item, or a predator cue.

Neutral arena trials with conspecifics can be used to study a variety of behavioral systems such as mate choice (e.g., cockroach *Blattella germanica*: Lihoreau *et al.* 2007, parasitic wasp *Aphidius ervi*: Villagra *et al.* 2007), kin selection (e.g., halticine bee *Lasioglossum erythurum*: Kukuk & Crozier 1990), dominance hierarchies (e.g., *Polistes dominulus*: Tibbetts & Dale 2004, *Oreochromis niloticus*: Gonçalves-de-Freitas *et al.* 2008) or food preferences (e.g., crayfish *Cherax destructor*: Meakin *et al.* 2008). They

can also be used to study the mechanisms (e.g., Gravel *et al.* 2004), ontogeny or evolutionary history (e.g., Kukuk & Crozier 1990) of behavioral traits by presenting carefully controlled stimuli. But with the arenas being artificial environments they lack the variety of cues that animals may normally use, and may themselves provide cues that modulate the animal's behavior. In addition, they remove context that may be important for making decisions, such as the distance to home or other resources, or the presence of predators. As such, they may not detect behavior that would naturally be present (e.g., Starks *et al.* 1998) or may cause behavior that would not normally occur (Weir *et al.* 2002). In order to compensate for the artificial nature of the environment, neutral arena trials should be followed with trials in more natural environments to confirm their findings. Despite these concerns, neutral arenas allow for very tight control over many variables, and can tease apart effects of interwoven factors.

A natural context experiment, in comparison, is an attempt made within the laboratory to replicate some aspect of a natural environment as much as possible. While a laboratory environment can never truly replicate field conditions, this style of experiment attempts to bridge the gap between field and laboratory experiments, by providing much of the control of a laboratory experiment with some of the realism of a field experiment.

Behavioral observations are frequently applied to natural contexts to document behaviors such as foraging (e.g., *Polistes dominulus*: Brown *et al.* unpubl) or mate-choice (e.g., *Blattella germanica*: Lihoreau *et al.* 2007). Observing the time spent performing different behaviors can suggest what factors are limiting to the reproductive success of an organism. In this way, behavioral observations may give insight into the adaptive value of different behaviors. They can also be used to study ontogeny by observing how

behavioral patterns change over the lifespan of individual animals. But while behavioral observations carried out in laboratories can add significantly to the range of observable behavior, the behaviors may differ from those observed in the wild. Animals supplied *ad libitum* food, for example, may engage more frequently in energetically costly behaviors (Kotiaho 2001) and spend less time foraging.

A laboratory invertebrate nesting colony is an example of a natural context that can be manipulated by removing or adding individuals or brood (e.g., *Polistes fuscatus* Reeve & Nonacs 1992) or altering the nest itself (e.g., ant *Temnothorax curvispinosis* Pratt 2005). This then allows for detailed manipulations of one factor of the social environment or microhabitat of the animals and can be used to observe habitat selection (Pratt 2005), changes in the dominance hierarchy or changes in interactions among animals (e.g., Nonacs *et al*. 2004).

However, nests kept within a laboratory setting will experience a different environment from those in a field setting and animals may respond differently to nest manipulations in the presence of abundant food and the absence of predators and conspecifics. Laboratory nest manipulations therefore, can provide a first step for testing hypotheses but benefit from being supplemented by observations and complementary experiments carried out in a field setting.

Finally, in a contrived context experiment, the researcher is neither trying to replicate a natural environment nor make the environment as neutral as possible. Instead the researcher provides an artificial environment with novel cues that do not resemble the natural environment. Contrived context experiments can be excellent for determining the factors of the environment that have the largest impact on particular behaviors. For

example, an organism can be introduced to an entirely novel environment where their reactions can be used to provide information about their environmental perceptual processing. Novel environment experiments are frequently used to study foraging behavior (e.g., honeybees *Apis mellifera ligustica*: Sanderson *et al*. 2006) as many animals will take food from novel sources, or they can test the ability of organisms to distinguish among different food sources (e.g., Sanderson *et al*. 2006), or to secure other resources such as nesting material or nest locations. However, it is difficult to know whether animals perceive and interact with an artificial stimulus (e.g., a honeybee feeder) in the same way they do with a natural stimulus (e.g., a flower).

These studies can be used to determine the mechanisms organisms use to make decisions. They can also be applied to study the ontogeny of decision-making by exposing an organism to novel environments at different points in its life cycle. The major concern with studies using novel environments is that the environment may be so remote from natural as to make the results inapplicable. Novel stimuli may also leave out important cues, such as scents or polarized light and similar studies should be carried out presenting natural stimuli to see if the same responses are observed.

4. Conclusions

In this review, we have detailed select questions of behavioral ecology research to which extensive laboratory methods have been applied, to understand the evolutionary and social context of animal behavior in vertebrates and invertebrates. Different laboratory contexts have their advantages and disadvantages and none of them are complete alone.

Laboratory experiments can be greatly informative for explaining the behavioral patterns observed in the field and can provide extensive information on details of behaviors that cannot be tracked in other ways. Yet, despite their experimental and ontogenetic appeal, such laboratory experiments cannot stand alone. In general, laboratory experiments have more control but less realism than field experiments and therefore the goal of the laboratory should alternatively be to better understand animal behavior in the wild. Many of the best experimental progressions of behavioral research take work carried out in the field and elaborate on it in the laboratory or vice versa. Our literature review suggests that future studies should focus on combining both field and laboratory based data to increase theoretical appeal and experimental validity.

Table 2.1. The costs and benefits of laboratory experiments in comparison to field studies with both vertebrate and invertebrate examples provided.

Laboratory experiments vs. Field studies	
*The **Benefits** of laboratory experiment in comparison to field studies*	*Examples*
Controlled settings allowing the manipulation of environmental variables.	Controlling water quality to modulate chemical cue concentrations in Tilapia (Gonçalves-de-Freitas *et al.* 2008).
Experimental control for testing the influence of single factors only.	Quality of artificial ant nests (Pratt 2005).
Controlled social development for captive-reared animals allowing assessment of innate responses and effects of social learning.	Tool-making in New Caledonian crows (Weir *et al.* 2002, Kenward *et al.* 2006).
Isolation and tracking of individual development and testing of reactions at an individual level.	Assessing effects of varying ontogeny on the quality of male zebra finches as perceived by female conspecifics (Spencer *et al.* 2003, 2005, Naguib & Nemitz 2007).
Ability to limit or exclude conspecific and/or heterospecific interactions.	Isolation of newly emerged *Polistes* females (Shellman & Gamboa 1982).
Ease of physiological manipulation for testing specific reactions.	Altering diet quality to assess effects on plumage characteristics and song development in zebra finches (Naguib & Nemitz 2007).
*The **Costs** of laboratory experiment in*	*Examples*

comparison to field studies	
Laboratory strains of animals are often domesticated and therefore may behave differently from their wild counterparts.	Physiological condition and mate preference behavior in zebra finches (Ewenson *et al*. 2001, Rutstein *et al*. 2007).
Artificial food supply can alter foraging behavior.	*Ad libitum* food sources lead animals to engage in more costly behaviors (Kotiaho 2001).
Mate selection experiments often don't allow subject and stimuli interaction to accurately assess subject motivation.	Spatial association measures in zebra finch mate preference choice trials (Rutstein *et al*. 2007).
Captive environments may increase subject and stimuli stress levels modulating behavioral results.	Mate preferences in zebra finches (Roberts *et al*. 2007).
Artifacts of the experimental environment can influence behavioral choices.	Leg bands and lighting conditions in zebra finch mate preference trials (Cuthill *et al*. 1997, Hunt *et al*. 1997).
Specific experimental results can be context-dependent and the unnatural laboratory environment can potentially obscure their observation.	Studies of kin recognition in the paper wasp, *Polistes dominulus* (Starks *et al*. 1998).
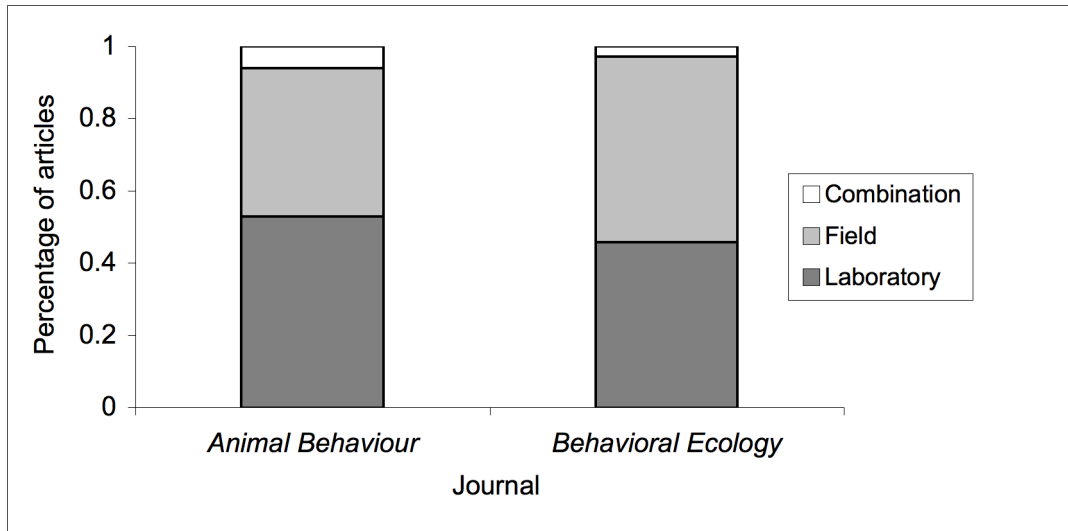
Figure 2.1. The percentage of surveyed experimental articles in *Animal Behavior* (*n* = 246) and *Behavioral Ecology* (*n* = 208) that were carried out in the 'Laboratory', 'Field' or a 'Combination' of Laboratory and Field settings. Although similar in overall proportions, *Behavioral Ecology* published more field only and fewer lab and combination studies (X^2 = 6.34, *df* = 2, *p* = 0.042).

Chapter 3

Caste determination cascade: Energetic constraints on reproductive dominance

Abstract

Eusociality has long been an evolutionary puzzle since, in eusocial systems, some individuals accept non-reproducing helper roles. Attempts to explain this have recently taken the form of reproductive skew models, which try to explain the fitness payoffs necessary for an individual to accept a subordinate role. Recent tests of reproductive skew models, however, have frequently failed to support predictions, particularly in *Polistes*, the system in which many of these models were developed. If, as skew models assume, subordinate foundresses were equivalent to solitary foundresses in their founding ability, a subordinate foundress would need a fitness payoff equivalent to that of a solitary foundress. In general, however, subordinates have been found to be less successful than solitary foundresses. Our new results offer a possible solution to this conundrum by questioning the assumption that solitary foundresses and subordinate foundresses are equivalent. We show that *Polistes dominulus* subordinates must spend more energy to maintain ovarian development than solitary or dominant foundresses. Subordinates, therefore, may not be able to found a nest alone, or at least would likely have very low success doing so. We propose that subordinate foundresses are locked into the subordinate role by a caste determination cascade starting with maternal manipulation, followed by dominant and subordinate interactions. If skew models are to be resurrected for *Polistes*, they must take into account variation in the quality of foundresses. This approach could lead to a new understanding of the evolution of eusociality in *Polistes*.

Introduction

The evolution of eusociality has long been a puzzle. Darwin considered the sterile workers of social insects one of the greatest difficulties to his theory of natural selection (Darwin 1859). Evolutionary theory suggests that organisms should strive to get as many genes as possible into the next generation. Eusocial organisms, however, have reproductive division of labor, meaning that one or a few individuals do most or all of the reproduction (Wilson 1971). In highly eusocial societies, such as honeybee colonies, most of the individuals cannot found their own nests, and may not be able to reproduce at all[2]. In primitively eusocial societies, however, all individuals are capable of reproduction. This raises the question of why an individual that is capable of reproducing would accept a non-reproducing "helper" role.

Many models have been proposed to explain self-sacrificing behavior (Keller & Reeve 1994, Reeve & Keller 2001, Wilson & Holdobbler 2005, Buston & Zink 2009). These models have included trait-group selection, in which intergroup competition can select for traits that are disadvantageous for intragroup competition (Wilson & Holdobbler 2005), "selfish gene" adaptation by a eusociality gene (Nowak *et al.* 2010) and maternal manipulation, in which the mother manipulates her offspring into accepting roles that generally benefit her, but decrease the fitness for some offspring. One major category of models that attempt to explain the evolution of eusociality is the reproductive skew model (Keller & Reeve 1994, Reeve & Keller 2001, Buston & Zink 2009). These models combine ecological and genetic data to predict the circumstances that would be required for individuals to accept subordinate roles and surrender some or all of their

reproduction. The models further predict the degree of reproductive partitioning, or skew, within a colony. In order for a subordinate role to be beneficial the subordinate must reap at least as much reproductive success as it would by reproducing alone. Generally, this is predicted to be achieved through some combination of kin-selected benefits and individual reproduction. Individual reproduction may be achieved either by reproducing as a subordinate or by inheriting the nest (Keller & Reeve 1994, Reeve & Keller 2001). Recent tests of skew models, however, have frequently failed to support these models (Reeve & Keller 2001, Nonacs *et al.* 2004, Liebert *et al.* 2006, Nonacs *et al.* 2006), particularly in *Polistes*, the system in which many of these models were developed[6-8].

Polistes is considered an ideal model system for studying the evolution of eusociality, as it is primitively eusocial, meaning that all the individuals are capable of reproducing independently (Wilson 1971, West-Eberhard 1996). A great deal of the work on modeling the evolution of eusociality has been based on *Polistes* multiple foundress associations (Keller & Reeve 1994, Reeve & Keller 2001, Buston & Zink 2009). In these associations, two or more reproductive females found a nest together. Within these associations, a linear dominance hierarchy develops, and the dominant individual lays most or all of the eggs (Reeve 1991). Skew models have attempted to explain how these hierarchies are established and why a subordinate would accept that role.

In this study, we addressed a fundamental assumption of reproductive skew models: that the subordinate, were she to found alone, would have equivalent success to a solitary foundress. If subordinate foundresses are less able to found alone than the

average foundress, they may benefit from adopting a solitary role, even if their total fitness payoff is much lower than that of a solitary foundress. We tested this by measuring the amount of energy used by workers, dominant, subordinate, and solitary foundresses to maintain egg development. We predicted that, if non-reproducing individuals were "weaker", they would use more energy to maintain the same level of ovarian development.

Methods Summary

Animals

P. dominulus colonies were collected during the founding phase of the colony cycle in 2007 and 2008 from Carlisle, MA, Andover, MA and Southampton, NH. Animals from both years were kept with their corresponding nests in wasp boxes (Weiner *et al.* 2010a,b). Wasps were taken from a total of 54 colonies, with 25 singly founded nests and 29 multiply founded nests. Any wasps emerging post-collection were assumed to be workers, as all measurements were taken before any males emerged.

Respirometry

Each individual wasp was placed into a 23-cm^3-glass respirometry chamber kept at 27±1.5°C that was covered in foil in order to discourage wasp activity. Carbon dioxide production by individual wasps was measured by differential open-flow respirometry using a Li-Cor 6262 CO_2/H_2O analyzer (Li-Cor, Lincoln NE, USA) for 1 hr (Weiner *et al.* 2009). Resting ventilation patterns were determined by preliminary measurements to distinguish periods of rest from other more energetically costly behaviors (Weiner *et al.*

2009), and animals underwent an additional 20-60 minutes of testing if rest patterns were not achieved during the first hour. Data were transformed and analyzed in Datacan V (Sable Systems, Las Vegas NV, USA).

Dissection

Each wasp was dissected, and the ovaries were extracted. The length of the 6 largest oocytes was measured using ImageJ (Wayne Rasband, National Institute of Health, Bethesda, MD) and the average lengths of the 6 largest oocytes were used as a measure of ovarian development (Sledge *et al.* 2001).

Data Analysis

Data on energy expenditure and ovaries from the two seasons did not display significantly different slopes of CO_2 output against mass (ANCOVA, F= 0.021, df=1,80, p> 0.88). Therefore, the data were adjusted for intercept differences and combined. Mass correlated with energy use ($r^2 = 0.234$, p <.0005), so mass specific data were used in order to allow comparisons of wasps of varying sizes.

Results

We found that standard metabolic rate (SMR), as measured by CO_2 production, increased with increasing egg size in *P. dominulus* (Figure 3.1), thus allowing us to calculate the energy cost of maintaining eggs. However, egg size varied dramatically between workers and foundresses, and between subordinate, dominant and solitary foundresses (Figure 3.2, ANOVA, F =133.7, df = 128, p < 0.0001; Tukey's Post-Test, all

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comparisons p < 0.01). When SMR was measured relative to egg size, both subordinate foundresses and workers used more energy for an equivalent level of ovarian development than did solitary or dominant foundresses (Figure 3.3). While subordinates had smaller egg sizes than solitary foundresses, they actually used more energy at rest, thus indicating that they do not have equivalent reproductive potential. This was not explained by the mass-specific differences expected among differently sized animals, since workers, which are smaller than subordinates, had a lower mass specific SMR than subordinates did (Figure 3.4).

Discussion

Our results indicate that both subordinates and workers require more energy to maintain ovarian development than dominant and solitary foundresses. From these results, we suggest that subordinate foundresses and workers are not fully totipotent, but are, in fact limited in their reproductive options. Tests of skew models have assumed that a subordinate foundress would have had the same success founding alone as an average solitary foundress. Our work suggests that this is not the case, and thus decouples the success required for a subordinate foundress to join a nest from the success of a solitary foundress in the population. Since a subordinate foundress would require increased energetic input to produce and provide for her own brood, she is making the best of a bad situation by joining a dominant foundress, and would benefit by staying to help even at fitness levels far below those of an average solitary foundress.

As we were measuring subordinate foundresses after the initial founding period, we do not know when the energetic differences between these individuals emerge.

Subordinate foundresses could be much weaker from eclosion, or from emergence from hibernacula, or from the stress of being a subordinate. Based on these results and significant previously published work, we suggest that subordinate status is determined by a caste determination cascade during which the eventual role is gradually cemented. While we expect that each step of this cascade affects eventual role determination, it is not necessarily the case that every step is required for an individual to become a subordinate.

1. **On the maternal nest**: some larvae are fed more than others, creating a size disparity in the reproductive brood (Hunt 2007). Since subordinates are smaller than dominants, this sets up a disparity in who is likely to win in dominance struggles (Reeve 1991).

2. **In pre-hibernation aggregations:** dominance struggles take place that determine the place of individuals in the pre-hibernation hierarchy (Dapporto *et al.* 2006). Some of the individuals that lose these conflicts go on to perform worker tasks in the pre-hibernation aggregations (Dapporto *et al.* 2005). Losing these conflicts and performing worker tasks causes these individuals to use more energy and go into the winter with lower stores, as well as, potentially "priming" them to accept low-status positions (Hunt 2007).

3. **On emergence from hibernacula**: individuals that entered hibernation with lower stores are expected to emerge with lower food stores and might emerge earlier due to having exhausted their internal stores (Starks, unpublished data).

4. **During the founding phase:** these weaker individuals would, therefore, be more likely to attempt to join another foundress, rather than found alone, and will lose

the initial founding conflicts, due to their relative weakness (Reeve 1991, Reeve

1998).

This cascade is similar to the track that produces summer workers (Hunt 2007),

suggesting that both subordinate foundresses and workers are following the same

trajectory. As in this "caste determination cascade," summer workers are fed less than

gynes (Reeve *et al.* 1998) and work on the nest after emergence (Hunt 2007). These

combined factors, along with maternal signals (Strassman 1993), are thought to drive

caste determination in *Polistes* (Hunt 2007). As such, we suggest that subordinate

foundresses should be considered "spring workers" rather than foundresses.

By the time larvae begin hatching on the nest, the cascade of factors has made a

subordinate not just a "weak queen" but, effectively, a type of worker. Like summer

workers, she has been subject to maternal manipulation (Hunt 2007, Jeanne 2009) and

performed worker tasks (Reeve 1998). The whole cascade may not be needed: an

individual might emerge fully fed, but suffer a hard winter, and leave the hibernacula

weak, for example. These factors could conspire to cause her to require more energy to

maintain ovarian development. According to this interpretation, a subordinate would

have much more difficulty maintaining her own nest than an average solitary foundress.

As such, she would benefit from taking a subordinate role even with a very discounted

level of reproductive success.

Accordingly, all that is necessary for the production of subordinates is for it to

benefit a foundress for some of her offspring to become subordinates on their sisters'

nests. This approach conceptually combines some of the trait group selection ideas

proposed by Wilson and Holdobbler (2009) and expanded upon by Nowak and

colleagues (2010) with previous work on reproductive skew models. In this case, while indirect fitness is likely to be necessary to keep the subordinate on the nest, her total fitness does not need to be equivalent to that of a solitary foundress. We propose a maternal manipulation pathway to eusociality, in which the mother's fitness drives the social behavior of the next generation through a caste determination cascade. These data could allow the resurrection of skew models through taking into account the variability of individuals' reproductive potential.
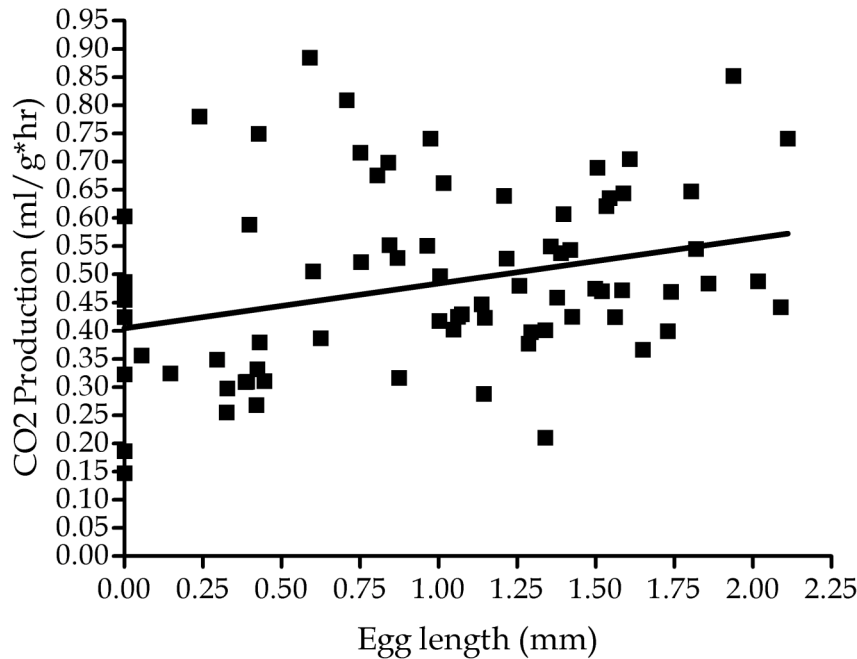
Figures

Figure 3.1. Mass specific standard metabolic rate of *P. dominulus* by mean egg size.

Each data point represents one individual (regression, n=74, r^2= 0.087; p<0.001).
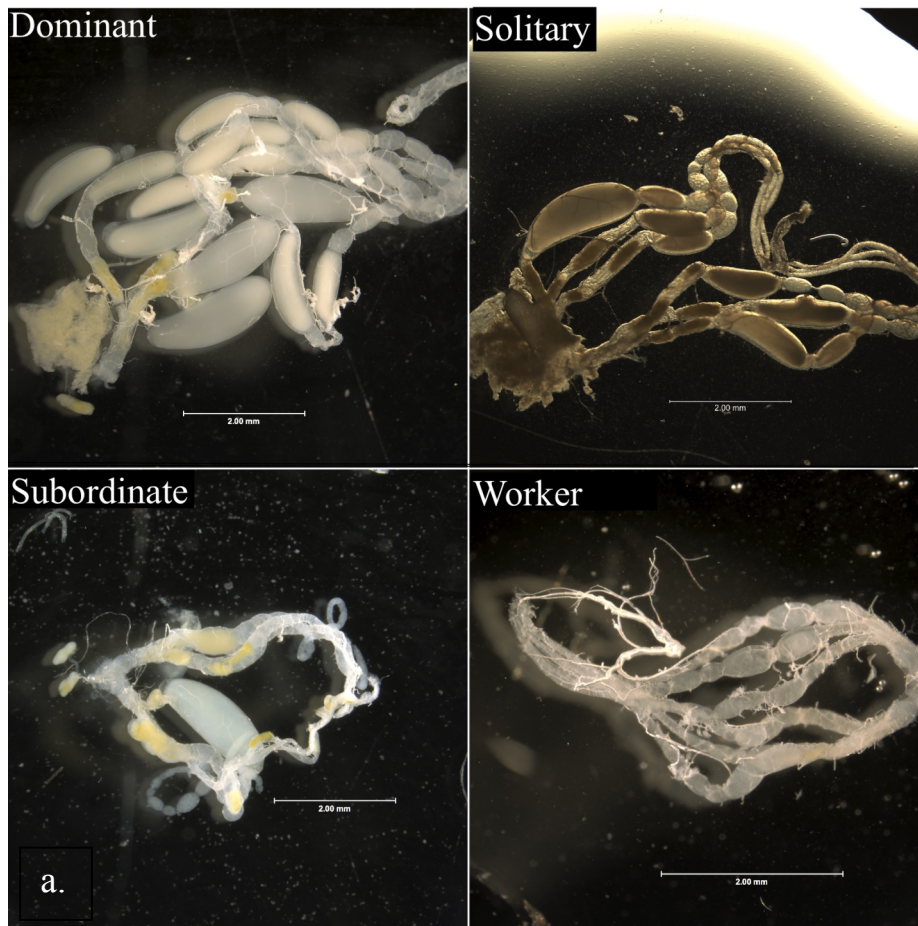
Figure 3.2. a) Representative pictures of ovarian development by role. Each picture

shows the extracted ovaries of one individual with approximately median ovarian

development for their role. b) Bars show means, error bars show standard error (ANOVA, df = 128, F = 133.7, p < 0.0001).
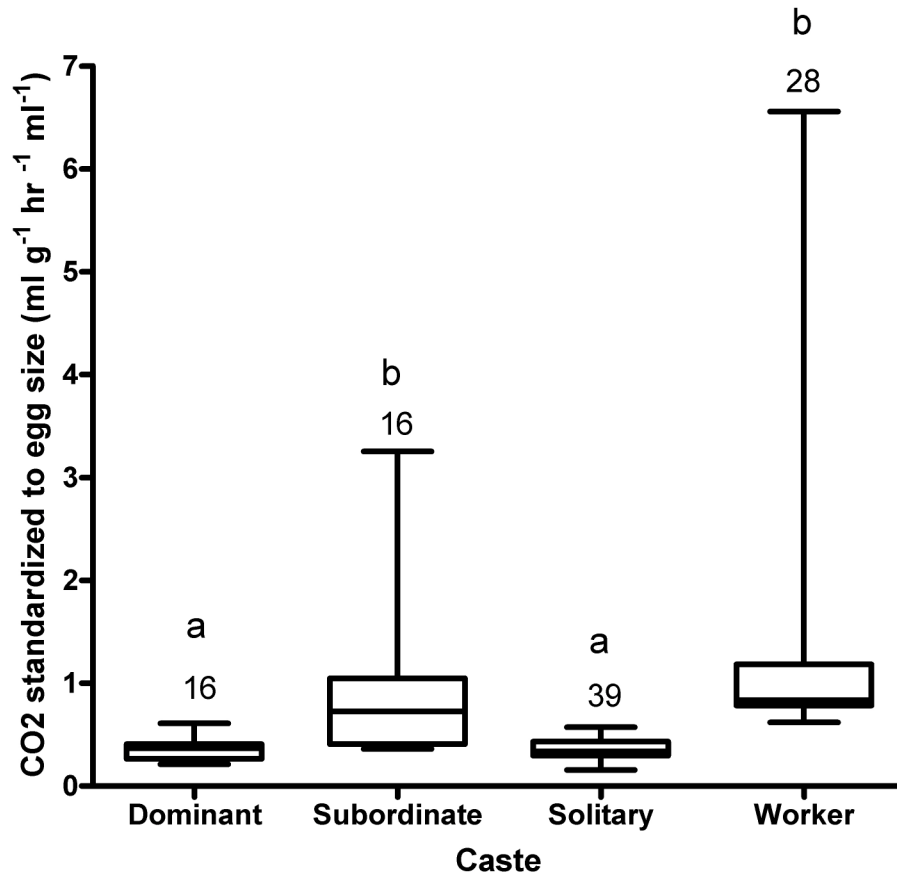
Figure 3.3. Standard metabolic rate relative to egg development. Each shows the median mass specific metabolic rate relative to the median egg size. Different letters indicate significant differences (Kruskal-Wallis = 63.86, P < 0.0001, Dunn's Multiple Comparison Test, all significant differences P <0.001).
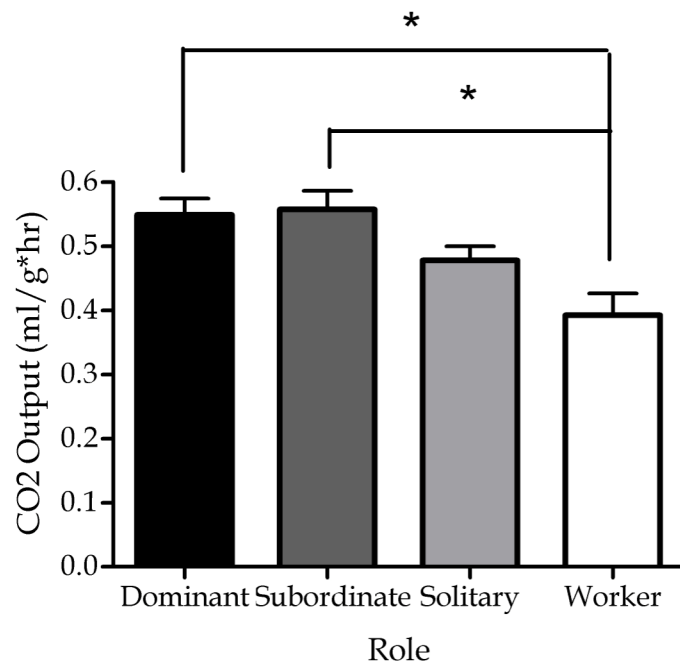
Figure 3.4. Resting metabolic rate by role in *P. dominulus.* Bars show means, error

bars show standard error (ANOVA, df = 130, F = 7.29, p < 0.0001). Asterisks indicate p

<0.05 from Tukey's Test.

Chapter 4

The energetic costs of stereotyped behavior in the paper wasp, *Polistes dominulus*

Abstract

Polistes wasps engage in many behavioral interactions. Although there has been debate over the meaning of these interactions, these stereotypical behaviors can be used to determine a colony's linear dominance hierarchy. Due to the implicit relationship between behavioral and reproductive dominance, behavioral interactions are commonly used to distinguish the reproductively dominant alpha foundress from the beta foundress. It has been suggested that, in order to maintain reproductive control, the alpha foundress is forced to remain at a physiologically constrained activity limit. This, in turn, may allow aggressive interactions to be used as determinants influencing reproductive partitioning between cooperating individuals. Energetic costs can place important limitations on behavior, but the energetic cost of the interactions has not previously been measured. To address this, we measured the CO_2 production of 19 non-nestmate pairs displaying interactive and non-interactive behavior. The rate of energy use during interaction behavior was positively associated with published rankings of aggression. However, our results indicate that interactions are not very energetically costly in *Polistes*, particularly when compared to the likely cost of foraging. These data suggest that maintaining reproductive dominance is not very energetically expensive for the dominant, and that the dominant foundress expends energy at a lower rate than the subordinate foundress.

Introduction

Dominance hierarchies are central to social systems in many animals, including humans (e.g. De Bruyn *et al.* 2005, de Waal 1986). Dominance hierarchies can mediate distribution of food (e.g. Appleby 1980), opportunities for reproduction (e.g. Blatrix and Herbers 2004), and delegation of communal tasks (e.g. Tentschert *et al.* 2001). Dominance hierarchies are often set and maintained by displays of aggression (reviewed in Forkman & Haskell 2004). In eusocial insects, dominance hierarchies are particularly important since most or all of the eggs in the colony are usually laid by the dominant individual (Keller & Perrin 1995).

Dominance hierarchies are central to the social biology of *Polistes* wasps (Pardi 1948). *Polistes* wasps are considered primitively eusocial because the castes are totipotent (Wilson 1971, Hunt 2006); meaning that workers can mate and lay diploid eggs and foundresses can adopt subordinate roles that are similar to worker roles (Reeve 1991). In temperate species, including *P. dominulus,* foundresses produce workers early in the season that remain on the nest, and later in the season produce reproductive gynes that mate and overwinter to found colonies the following year (reviewed in Reeve 1991).

Some *Polistes* gynes found colonies in multiple foundress associations (Pardi 1948). In colonies founded by multiple reproductive females, a linear dominance hierarchy forms between foundresses, and this hierarchy determines distribution of tasks and reproduction (West-Eberhard 1969). Due, in part, to this colony founding behavior, *Polistes* wasps are considered models for insights into the origins of eusociality (reviewed in Reeve 1991; Turilazzi & West-Eberhard 1996, Starks and Turillazzi 2006).

In multiple foundress *Polistes* nests, the dominant individual reproduces more than subordinates, while the subordinates perform the riskier tasks, such as foraging

(reviewed in Reeve 1991). The dominant foundress does not leave the nest as often, thus avoiding exposing herself to predators and other hazards. This likely explains why a dominant female has a better chance of surviving than do subordinates or lone foundresses (reviewed in Nonacs *et al.* 2006). This leads to increased rates of survival for nests founded by multiple females by improving the chances that at least one foundress will survive to worker emergence (Reeve 1991).

Cooperative foundresses have a dominance hierarchy, and this hierarchy can be determined by calculating one of two factors. One factor is behavior; the dominant animal initiates the majority of interactions (e.g. Sumana & Starks 2004). The other factor is reproduction; the dominant foundress lays the majority of the eggs (e.g. Reeve *et al.* 2000). With genetic techniques, the reproductive dominance hierarchy can be determined fairly easily (e.g. Liebert & Starks 2006). However, researchers often use behavioral interactions to determine the reproductive hierarchy, due to the strong correlation between the two, and due to the ease with which behavioral interactions can be observed (Reeve 1991, Reeve & Ratnieks 1993, but see Röseler & Röseler 1989). Perhaps due to this relationship, it has been assumed that interactions are used by foundresses to set and maintain reproductive dominance hierarchies (Reeve & Nonacs 1992, but see Nonacs *et al.* 2004).

Tibbetts and Reeve (2000) tested a model that suggested that, if the relative distribution of reproduction is mediated by aggressive behavior, subordinates should "fight back" to maximize their own reproductive opportunities. One might assume that, since the subordinate does most of the foraging (reviewed in Reeve 1991), the dominant may not need to expend much energy to maintain control. Tibbetts and Reeve (2000),

however, suggested that the dominant is forced to remain near her physiologically constrained activity limit when maintaining reproductive dominance. Since wasps are poikilothermic, the authors reasoned, the physiological limit should increase with the surrounding temperature. Because an increase in aggression by the dominant in higher temperatures was observed, Tibbets and Reeve (2000) suggested that the dominant was at or near her physiological maximum. If this is true, aggression would be an honest signal of quality, constrained by an inability to maintain a higher level of aggression.

While there are many limitations on behavior, one important limitation is energy. When supplemented with honey, *Polistes metricus* colonies produced 1st offspring earlier and produced offspring with a higher percentage of body fat, which suggests that colonies are energy limited (Rossi & Hunt 1988). We tested the possibility of an energetic limit on aggression by comparing the metabolic costs of interactive behaviors (including those involved in aggression) and non-interactive behaviors involved in foraging and other tasks. If a dominant foundress is energy limited, she must be expending at least as much energy as a subordinate. One way to test this hypothesis is to compare the energy use rate of interaction behaviors (which are mostly performed by the dominant) with foraging behaviors (which are mostly performed by the subordinate) (reviewed in Reeve 1991).

Interactions are keys to understanding social behavior, but we do not yet know how energetically costly these behaviors are to the animals. *Polistes* has often been used as a model genus for the development of models of helping behavior and hierarchy maintenance (Tibbets & Reeve 2000, reviewed in Reeve & Keller 2001). In order to better understand social behavior in this model genus, we examined the rates of energy use, as measured by CO_2 production, of various interactions between *P. dominulus* gynes.

We compared the ranking of these behaviors by energy use to the published rankings of these behaviors by level of aggression. We also compared the rates of energy use during interactions to the rates of energy use during other behavior.

Methods

Animals

All *P. dominulus* colonies were collected in summer 2005 from the Tufts Cummings School of Veterinary Medicine in Grafton, MA and maintained in cages (60 x 22 x 24 cm) on the Tufts campus in Medford, MA. The colonies were fed wax-worms and honey-water ad libitum, and the lighting schedule was matched to local sunrise and sunset. All test animals were females that emerged after colonies had begun producing an approximately equal ratio of males and females, and thus were assumed to be gynes. Individuals were taken from 19 colonies, and no individual was included in more than one test.

Behavioral observations

For each test, two *P. dominulus* gynes from different colonies were placed in a respirometry chamber, which consisted of a 250 cc clear plastic culture flask sealed with a rubber stopper. All trials were videotaped and all interactions were recorded by an observer, as were periods of walking, buzz-walking (defined below) and resting. The interactions we recorded were antennation (antenna-to-antenna or antenna-to-body contact, S1), darts (quick movements towards and away from the other wasp, S2), lunges (darts with contact), uncontested mounts (one animal climbs onto the other animal, the

bottom animal assumes a submissive posture), contested mounts (an attempted mount where the bottom animal attempted to prevent the other from mounting), bites (mandible-to-body contact, S3), and grapples (wrestling, S4) (e.g. Starks *et al.* 1998).

Only one lunge was recorded, and thus lunges were not included in the analysis. Although sustained free flight was not possible in the confinement of the chamber, there were periods during which individuals would fan their wings in repeated brief bouts, with locomotion that was a combination of rapid walking and very brief airborne intervals; it is these behaviors that we collectively term "buzz-walking" (S5). Buzz-walking is expected to be less costly than true flight, offering a very conservative comparison between interactions and foraging behavior. Video images of each test were used to confirm the observed behaviors. Each dyad remained in the respirometry chamber for 60 minutes and was observed continuously during that time.

Respirometry

Metabolic rate measurements were made at 27 ± 0.5°C. CO_2 production was measured continuously by differential open-flow respirometry using a Li-Cor 6262 CO_2/H_2O analyzer (Li-Cor, Lincoln NE, USA) calibrated with a gravimetric mixture (Scott Specialty Gases, Plumsteadville PA, USA) and re-zeroed between measurements (Woods *et al.* 2005). A flow rate of 500 cc min^{-1} of medical grade compressed air was maintained by Sierra Side-Trak mass flow meters (Sierra Instruments, Monterey NV, USA) and a Sable Systems flow controller (Sable Systems, Henderson NV, USA). Excurrent CO_2 concentration was sampled every 6 s; each recorded value represented the

mean excurrent CO_2 concentration for the previous 6 s as calculated by the Li-Cor unit (see Woods *et al.* 2005).

Respirometry data were collected and analyzed in Datacan V (Sable Systems). Behavioral observations were synchronized with respirometry data taking into account time lag and washout properties of the respirometry system as determined by bolus injection. Washout is simply the temporal sluggishness of the data arising from the "cushioning" of the raw signal in a chamber necessarily much larger than the subjects and the subsequent exponential decay of the signal as the chamber "washes out". We used the standard Z-transform implemented in Datacan V, which enhances temporal resolution by largely correcting for this effect (Bartholomew *et al.* 1981).

Behaviors were not used if they occurred less than 30 s apart. In our primary data set, all measurements were of pairs of animals. CO_2 production rate during each behavior was normalized to the resting rate, which was established separately for each trial during periods when both individuals were motionless. If only one animal was engaged in a particular behavior, the mean rate energy use associated with the behavior of the other animal was subtracted from the rate recorded. Our transformation reduced the energy use by half, so the remaining value was multiplied by 2. If both animals were engaged in the same behavior, the recorded rate was not transformed. Data were not used for bouts of behaviors that occurred less than 30 s apart. Each data point is the mean value over the duration of a bout of a particular behavior. The data were not normally distributed and so were analyzed for significance using a Kruskal-Wallis test followed by Dunn's multiple comparison test.

To enable comparison of resting CO_2 production with that of other animals, we measured a separate sample of 47 *P. dominulus* representing all castes. Methods were similar except that animals were placed individually in a 70 cc chamber with a flow-rate of 250 cc min^{-1}. The average of these data was then taken.

Results

The rates of energy use of all non-resting behaviors were at least double the resting rate, with buzz-walking having the highest rate (Figure 4.1). In order to compare the energy use rates of behaviors, we categorized the behavior as interactive or non-interactive. No interaction behavior differed significantly from any other interaction behavior (Kruskal-Wallis one-way ANOVA H = 53.44, df = 8, P<0.0001; Dunn's multiple comparison P<0.05 for all pairings, Figure 4.1). Buzz-walking had a significantly higher rate of energy use than darts, antennation and both contested and uncontested mounts (Kruskal-Wallis one-way ANOVA H = 53.44, df = 8, P<0.0001; Dunn's multiple comparison: Darts: P<0.05, Antennation: P<0.001, Uncontested Mounts: P<0.01, Contested Mounts: P<0.05, Figure 4.1), but did not have a significantly higher rate of energy use than bites or grapples (Kruskal-Wallis one-way ANOVA H = 53.44, df = 8, P<0.0001; Dunn's multiple comparison P>0.05 for both, Figure 4.1).

The rate of energy use of buzz-walking was significantly higher than the combined interaction behavior (Kruskal-Wallis one-way ANOVA *H* = 39.51, *df* = 3, *P* < 0.0001; Dunn's multiple comparison *P* < 0.001, Figure 4.2) and significantly higher than walking (Kruskal-Wallis one-way ANOVA *H* = 39.51, *df* = 3, *P* < 0.0001; Dunn's multiple comparison P<0.001, Figure 4.2). Walking and interaction behavior were not

significantly different (Kruskal-Wallis one-way ANOVA $H = 39.51$, $df = 3$, $P < 0.0001$; Dunn's multiple comparison $P > 0.05$, Figure 4.2). In general, the rate of energy use of a behavior correlated positively with how aggressive it has been considered (Figure 4.3).

For the separate sample of 47 *P. dominulus*, mean CO_2 production was 0.557 ± 0.199 ml g^{-1} h^{-1}. This value is consistent with values for arthropods of similar (0.106 ± 0.021 g) body mass (Lighton & Fielden 1995), especially those capable of flight (Reinhold 1999).

Discussion

Our results indicate that the rates of energy use of interactions in *P. dominulus* may be much lower than had been expected. Aggressive interactions are important in many species for developing dominance hierarchies. These hierarchies influence many factors, including food access (Scott & Lockard 2006, Brown *et al.* 2005), territory control (Brown *et al.* 2005), mate access (Mennil *et al.* 2004), and may even directly determine the proportion of reproduction an individual receives (Dewsbury 1982, but see Nonacs *et al.* 2004). However, the actual energetic costs of the behavior that appears to mediate these hierarchies are not known in many animals (but see *Acheta domesticus*: Hack 1997, *Pachycondyla obscuricornis*: Gobin *et al.* 2003, *Uca lactea perplexa*: Matsumasa & Murai 2005, *Oreochromis mossambicus*: Ros *et al.* 2006).

Our ranking by rate of energy use matched well with rankings of aggression that have been used by other researchers (Figure 4.3, e.g. see Pfennig *et al.* 1983 for *P. fuscatus*). This indicates that published assessments of the aggressiveness of interactive behaviors are well correlated with the energy use rate, and, hence, the energetic cost to

the animal, of the behaviors. This suggests that energy use could be a cost of interaction, with escalating aggressiveness being accompanied by escalating energetic costs, although we did find the rate of energy use to be low relative to the expected cost of foraging.

Our data show that interactions used less energy than buzz-walking (Figure 4.1). These data indicate that foraging is likely to be one of the most energetically costly behaviors expressed, since true flight is expected to be more costly than buzz walking. The rate of energy use in buzz-walking averaged only 7.1 times that of rest. In other insects with endothermic flight, such as sphingid and saturniid moths (Bartholemew & Casey 1978) and honeybees (Kammer & Heinrich 1978), rates of energy use during flight can be as high as 100 times resting rates. The lowest flight metabolic rate measured for an insect was 7.4 times rest in *Drosophila hydei* (Dickinson & Lighton 1995). Since *Polistes* wasps have elevated temperature during flight (unpublished data), their flight metabolic rate is expected to be closer to that of honeybees than that of *Drosophila*.

Also, on spring nests, trophallaxis is much more frequent than aggression (Dapporto *et al*. 2006), suggesting that foraging occupies a much larger portion of time than aggressive interaction. The subordinate generally does more foraging than the dominant (Pratte 1989), and is, therefore, probably expending more energy than the dominant. While the dominant may be physiologically constrained in other ways, preworker colonies seem to be energetically limited (Rossi & Hunt 1988), and so the energy use of foundresses is expected to be important to the colony's success. Increased energy expenditure will lead either to decreased brood production (Rossi & Hunt 1988) or increased foraging, which would increase the risk of foundress loss. The finding that the dominant likely uses less energy for non-reproductive actions than the subordinate is

important for understanding the costs and benefits of the two tactics. In future research, this difference could be clarified by the creation of energy budgets for the different tactics (as in Carefoot *et al.* 1998, Roberts & Harrison 1999, Woods *et al.* 2007).

P. dominulus is used as a model species for studying dominance hierarchies in eusocial societies. By examining the costs of interactions in *P. dominulus* this study brings a new understanding to these important and well-studied behaviors. Our data show that Pfennig and colleagues' (1983) ranking of aggressiveness correlates with energy use in *P. dominulus* and suggest that these behaviors have a lower rate of energy use than foraging. This study opens up the way for a more thorough understanding of these behaviors and, with this, the establishment and maintenance of dominance hierarchies in model species.
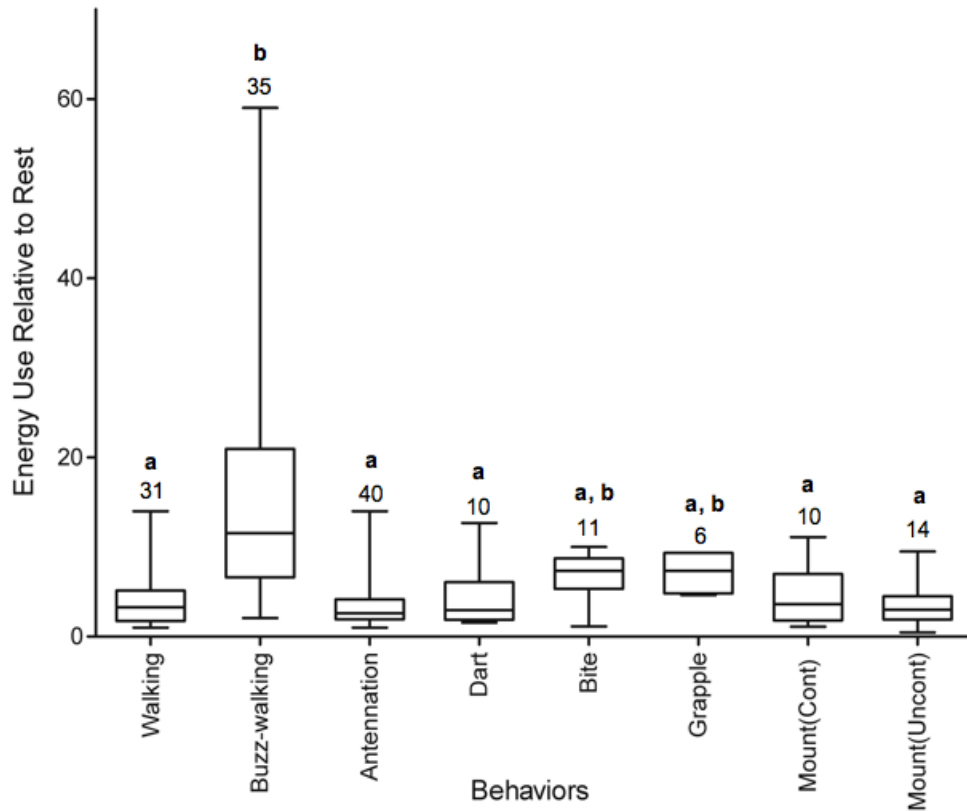
Figures

Figure 4.1: Median observed average rate of energy use for each behavior as a multiple of resting rate. Bars show 1st and 3rd quartiles and lines show the extremes. Numbers refer to the number of times each behavior was observed. Different letters indicate significant differences.
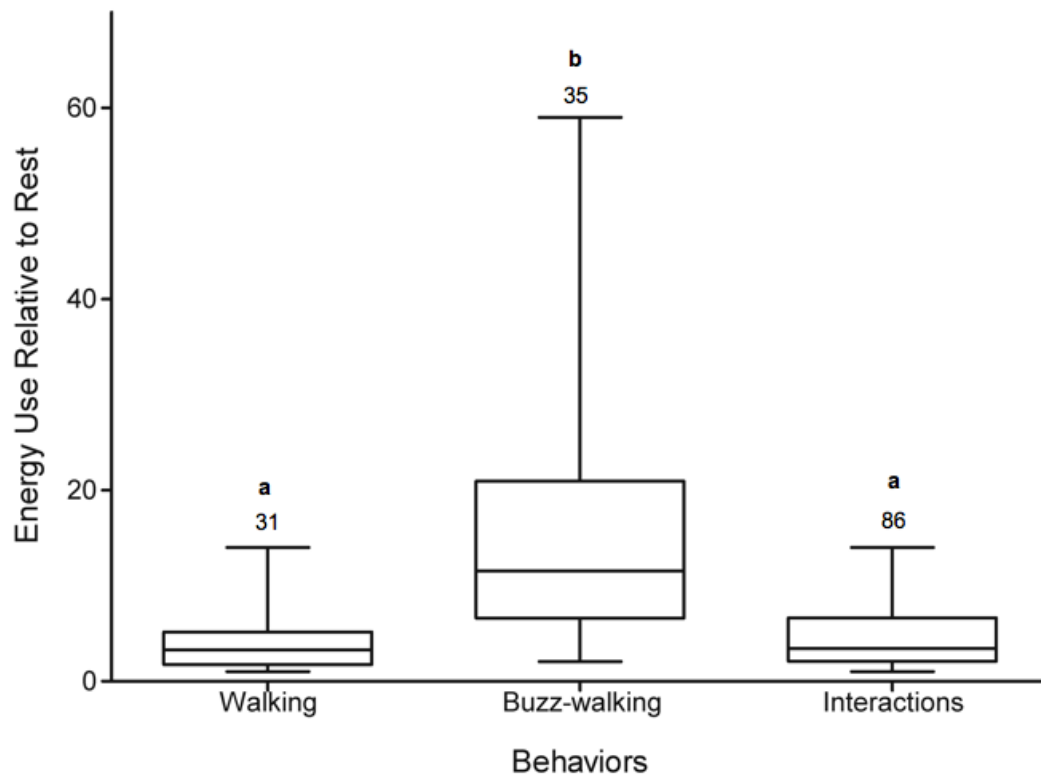
Figure 4.2: Median observed average rate of energy use for each behavior a multiple of resting rate.   The category of interactive behaviors includes bites, grapples, darts, antennations and mounts. Bars show 1st and 3rd quartiles and lines show the extremes. Numbers refer to the number of times each behavior was observed. Different letters indicate significant differences ($p < 0.001$).
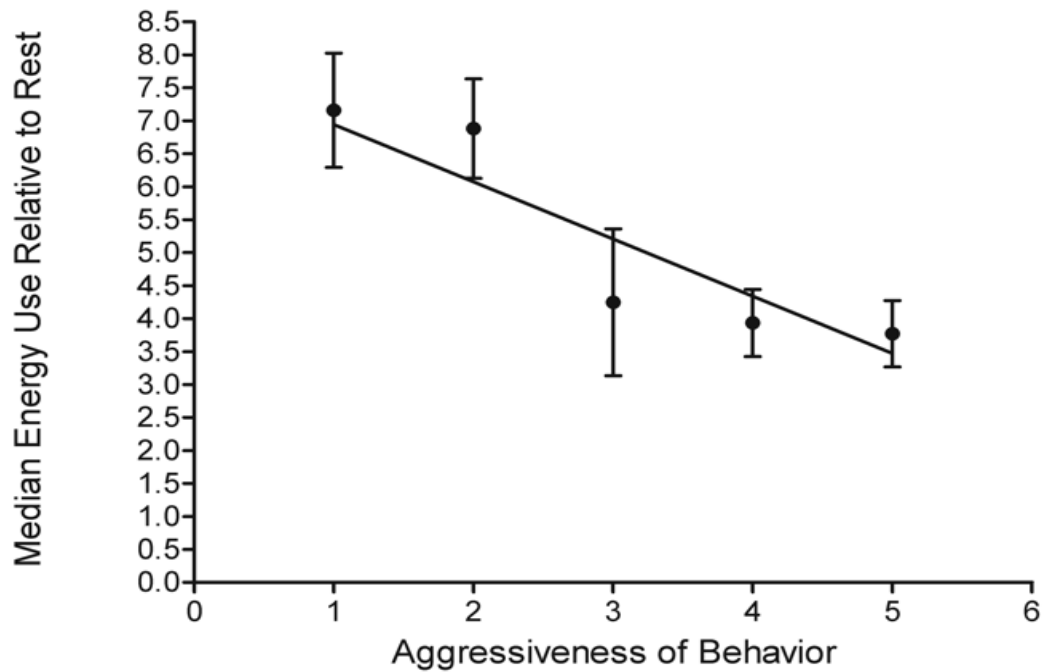
Figure 4.3: The correlation of the energetic cost of behaviors with the Pfennig and colleagues (1983) ranking of tolerance. The behaviors are ranked from least tolerant to most tolerant as follows: 1. Grapple (n = 6), 2. Bite (n = 11), 3. Dart (n = 10), 4. Avoid or detour (represented by walking) (n = 31), 5. Antennation (n = 40). Buzz-walking, at 11.57 would be off this graph. The significant regression is shown (P < 0.001, R^2 = 0.1143).

Chapter 5

Thermoregulation in the primitively eusocial paper wasp, *Polistes dominulus*

Abstract

Regulation of wing muscle temperature is important for sustaining flight in many insects, and has been well studied in honeybees. It has been much less well studied in wasps and has never been demonstrated in *Polistes* paper wasps. We measured thorax, head, and abdomen temperatures of inactive *P. dominulus* workers as they warmed after transfer from 8°C to ~25°C ambient temperature, after removal from hibernacula, and after periods of flight in a variable temperature room. Thorax temperature (T_{th}) of non-flying live wasps increased more rapidly than that of dead wasps, and T_{th} of some live wasps reached more than 2°C above ambient temperature (T_a), indicating endothermy. Wasps removed from hibernacula had body region temperatures significantly above ambient. The T_{th} of flying wasps was 2.5°C above ambient at $T_a = 21$°C, and at or even below ambient at $T_a = 40$°C. At 40°C head and abdomen temperatures were both more than 2°C below T_a, indicating evaporative cooling. We conclude that *P. dominulus* individuals demonstrate clear, albeit limited, thermoregulatory capacity.

Introduction

Polistes paper wasps are an important model system for the study of the evolution of eusociality and insect dominance hierarchies (West-Eberhard 2006). *Polistes* are considered primitively eusocial because they do not seem to have physiological caste differentiation (Wilson 1971; but see Hunt 2006). This lack of caste

differentiation, as well as general ease of study, has lead to the use of *Polistes* for testing theories about the evolutionary origins and maintenance of eusocial behavior (e.g. Field *et al*. 1998; Reeve *et al*. 2000; Hunt 2006; Nonacs *et al*. 2006). While much work has been done on the behavioral ecology of this system some very important basic physiology remains unknown.

While some work has been done in nest thermoregulation in *Polistes* species (Steiner 1930), our understanding of their thermal behavior remains limited. Many large insects (>50-200 mg) in several orders maintain high thorax temperatures (T_{th}) during flight (reviewed in Heinrich 1993), and some social insects maintain elevated temperatures in the nest (reviewed in Heinrich 1993). Thermoregulation is clearly important in some highly social insects, whether at the level of the nest (army ants: Franks 1989; termites: Korb 2003), the individual (yellow jackets: Kovac & Stabentheiner 1999), or both (bumblebees and honeybees: reviewed in Heinrich 1993). Elevated flight temperatures have also been shown in several wasps, including yellowjackets (Coelho & Ross 1996) and cicada killers (Coelho 2001). The latter has also been shown to warm prior to flight (Coelho 2001). To date, however, this work with wasps has been done only in large, strongly endothermic wasps (yellowjackets: Kovac & Stabentheiner 1999, Coelho & Ross 1996; cicada killers: Coelho 2001). *Polistes* are relatively small wasps, and so are expected to be, at most, only weakly endothermic.

Understanding individual thermoregulation in *Polistes* will provide background for researchers studying important questions about thermoregulatory behaviors, such as whether *Polistes* maintain elevated brood temperatures, as do bumblebees (Heinrich 1972) and honeybees (Heinrich 1985) and whether they use behavioral fever to combat

infection (Starks *et al.* 2000). Here, we investigated whether *P. dominulus* workers, both

flying and non-flying, have the capacity to elevate wing muscle temperatures above

ambient temperature, and, if so, whether they also display mechanisms for preventing

endothermic overheating during flight.

Methods

Animals

During the summer of 2007, 29 nests of *P. dominulus* were collected from field

sites in Waltham, Grafton, and Carlisle, MA. During the following summer (2008), 32

more nests were collected from the same sites. These nests were maintained in 0.037 m^3

cages on the Tufts campus in Medford, MA, and colonies were provided with wax worms

and 50% solutions of honey in water *ad libitum* (Sumana and Starks, 2004). All

individuals tested during the summers were females that emerged more than two weeks

prior to the first male and, thus, were assumed to be workers (Mead *et al.*, 1990). The

individuals used for hibernation experiments were gynes overwintering in wooden

hibernacula located inside mesh-walled enclosures in Medford, MA. These gynes were

produced by colonies from the previous summer and released into enclosures in October.

Enclosures were periodically provided with 50% solutions of honey in water. No

individual was used in more than one test, as the 24-hour mortality rate from the

procedure was high (greater than 30%) and puncturing may have altered the wasps'

ability to maintain temperature.

Temperature Measurements

Warm-up Measurements:

To determine whether *P. dominulus* workers elevate their temperatures after removal from a cold environment, we measured the body region temperatures of live and dead individuals over time. Dead wasps were used as comparisons to determine how warm-up differed from equilibration to ambient temperature (T_a) (e.g. Bishop & Armbruster 1999). Dead wasps were freeze killed at least 1 h, but less than 8 h before use to prevent desiccation. Live and dead wasps were constrained in a plastic bag and kept for 10 min at 8°C. The internal temperature of an empty plastic bag moved from 25°C to 8°C reached 8°C within 5 min.

After cooling, and while still restrained, wasps were moved to a temperature controlled room kept at 25.0±0.6°C. Wasps were maintained at this temperature for 0-300 s, before thorax, head and abdomen temperature (T_{th} T_h, and T_{ab} respectively) were measured by inserting into each body region a Physitemp MT 29/1B microprobe thermocouple (diameter 0.33mm, time constant 0.025s) connected to a Physitemp BAT-12 thermometer (Physitemp, Clifton, NJ, USA). Thorax was measured first, followed by head and then abdomen within 10 s. Each dead wasp was measured continuously in one body segment for 10 min and the temperature was recorded every 30 s. In order to determine whether the thermocouple probe provided a significant heat sink for the dead animals, one time course was done using a separate dead wasp for each elapsed time value for comparison. The results did not differ from the previous averages, so the data were combined. Each live wasp provided only one datum for each body region. Temperatures were reported as temperature excess relative to room temperature (wasp body region temperature – room temperature) in order to compensate for slight variations

in room temperature. An ANCOVA was done on the log of the temperature over time to compare the live wasps to the dead ones.

In order to test for evaporative cooling in dead wasps, wasps that had been freeze-killed were monitored for water vapor loss for two hours after removal from the freezer. H_2O production was measured continuously by differential open-flow respirometry using a Li-Cor 6262 CO_2/H_2O analyzer (Li-Cor, Lincoln NE, USA) calibrated with a gravimetric mixture (Scott Specialty Gases, Plumsteadville PA, USA) and re-zeroed between measurements (Woods *et al.* 2005). A flow rate of 250 cc min^{-1} of medical grade compressed air was maintained by Sierra Side-Trak mass flow meters (Sierra Instruments, Monterey NV, USA) and a Sable Systems flow controller (Sable Systems, Henderson NV, USA). Excurrent H_2O concentration was sampled every 6 s; each recorded value represented the mean excurrent H_2O concentration for the previous 6 s as calculated by the Li-Cor unit (see Woods *et al.* 2005). Respirometry data were collected and analyzed in Datacan V (Sable Systems). The same procedure was repeated with live wasps.

Hibernation measurements:

The internal temperatures of hibernating females were recorded in February and March 2009 in order to determine wasps' ability to warm up from hibernation in early spring. Wasps were removed from hibernacula and placed in plastic bags that were maintained at ambient temperature (7-9°C). T_{th}, T_h, and T_{ab} were measured by inserting into each body region a Physitemp MT 29/1B microprobe thermocouple (diameter 0.33mm, time constant 0.025s) connected to a Physitemp BAT-12 thermometer

(Physitemp, Clifton, NJ, USA).   After T_{ab} was measured, T_{th} was remeasured to check

for order effects. Some individuals had their internal temperatures measured immediately

(within 10 s of removal); the remaining wasps were kept in the plastic bags for periods of

up to 5 min. During the period prior to measurement, the bags were rotated constantly to

maintain activity. An ANCOVA was used to compare the body region temperatures. A

repeated measures ANOVA was used to compare the initial temperature measurements

with the post-disturbance measurements.

Flight Measurements:

　　　To determine the temperatures of flying wasps, the body region temperatures of

individuals were recorded immediately following capture from flight. For each test, the

wasp was released into a variable temperature room and allowed to fly for 30-60 s. No

differences in body region temperature were found between flights of 30 s and flights of

60 s (2-tailed t-test, $T=0.139$, $p=0.893$), so these trials were combined. If a wasp

attempted to land, the area where she tried to land was tapped several times with a stick.

If she persisted in landing, the flight was not used. After a wasp had flown for 30-60 s,

she was captured in a net and immediately restrained against a piece of soft foam. Within

10 s of capture, T_{th}, T_h, and T_{ab} were measured by inserting into each body region a

Physitemp MT 29/1B microprobe thermocouple (diameter 0.33mm, time constant 0.025s)

connected to a Physitemp BAT-12 thermometer (Physitemp, Clifton, NJ, USA). A

regression line was fitted for the internal temperature of each region relative to T_a and the

slopes were compared to a slope of 1 (representing region temperature $= T_a$) using an

ANCOVA.

Results

Warm-up Measurements

After being kept at a T_a 8°C for 10 min, all body regions of dead wasps had reached a temperature of 8°C, while many live wasps maintained slightly elevated temperatures in all body regions (Fig. 5.1). After 4 min at room temperature (25.0±0.6°C), all body regions of most of the live wasps were at or above T_a (Fig. 5.1), and T_{th} of all but one of the live wasps was above T_a after 5 min (Fig. 5.1A). Dead wasps did not reach room temperature until they had been at room temperature for about 7 min. After 20 min, the T_{th} of live wasps had returned to T_a. These data demonstrate that the live wasps were endothermic, since they elevated T_{th} more quickly than the dead wasps (ANCOVA on log transformed data, F=1301.187, p < 0.0001, Difference Contrast, p < 0.0001 for all regions) and reached T_{th} and T_h above T_a. Dead wasps did not appear to be subject to significant evaporative cooling, as the water loss in dead wasps did not differ significantly from 0 (One sample T-test, t = -1.380, p = 0.240) and was significantly lower than the water loss in live animals (Independent sample T-test, t = -5.592, p = 0.001).

Hibernation Measurements

Animals removed from hibernacula had internal temperatures above ambient in all body regions (Fig. 5.2 Repeated measures ANOVA, T_{th}: p < 0.05, T_h: p < 0.05, T_{ab}: p < 0.01, Repeated T_{th}: p < 0.01). After remaining out for 30-300 s, animals warmed up slightly, but not significantly (Repeated measures ANOVA, p > 0.05 for all regions).

Time after removal did not correlate significantly with body region temperature (Linear regression, p > 0.05 for all body regions). This indicates that, at least in the early spring, many individuals maintained a slightly elevated body temperature in the hibernacula, and that, in addition, they warmed up further upon removal.

Flight Measurements

Slopes of the internal temperatures of all regions against T_a were significantly different from 1 (ANCOVA, F = 3201.766, Simple contrast p < 0.0001 for all body regions). At the highest T_a, both head and abdomen (T_{ab}) temperatures were 2-3°C below T_a, and two animals had T_{th} below T_a (Fig. 5.3). This suggests that *P. dominulus* maintains thermal stability in flight at high temperatures by evaporative cooling.

Discussion

In our experiments, we found that *P. dominulus* maintained elevated body region temperature in an 8°C environment and that the T_{th} of live animals rose more rapidly than the T_{th} of dead individuals after being moved to an approximately 25°C environment. After 5 min in the 25°C environment, live wasps had T_{th} values above T_a, indicating endothermy (Fig. 5.1). This was confirmed by the finding that individuals removed from hibernacula during the spring had elevated body region temperatures (Fig. 5.2). In individuals removed from hibernation, T_{th} was the lowest body region temperature, a surprising result, considering that heat in hymenoptera is generally produced by the shivering of wing muscles (Heinrich 1993). However, when T_{th} was remeasured, it was higher than the other body segments. This suggests that warming occurs following (and

66

perhaps in response to) the measurement procedure. We also found that flying *P. dominulus* workers maintained a restricted T_{th} range relative to the range of T_a. In particular, T_{th} was more than 2.5°C above ambient where $T_a = 21$°C, and at or below ambient where $T_a = 40$°C (Fig. 5.3).

Honeybees can fly when T_{th} is between 30-45°C (reviewed in Woods *et al.* 2005) and can maintain these temperatures when T_a is between 15-46°C. *P. dominulus* individuals are smaller than honeybees, and thus are unlikely to be able to maintain such tight control over internal temperatures (Porter & Gates 1969; Bartholemew & Epting 1975). Indeed, the slope of T_{th} on T_a in flying honeybees has been reported at between 0.18 and 0.52 (Heinrich 1979, 1980a, b; Harrison *et al.* 1996; Roberts & Harrison 1999; Woods *et al.* 2005), while in *P. dominulus* it was 0.85. However, at the highest T_a values, both T_h and T_{ab} were below T_a, as found in honeybees (Roberts & Harrison 1999; Woods *et al.* 2005) and, to a more limited extent, in yellow jackets (Coelho & Ross 1996). This could be the case only if *P. dominulus* individuals underwent evaporative cooling, whether passive (through open spiracles) or active (through regurgitating droplets of fluid). The abdominal cooling could be a passive effect of water evaporation through spiracles opened for increased gas exchange (Hadley 1994). However, since T_h also drops below ambient temperature, the wasps are most likely regurgitating fluid and allowing it to evaporate in order to actively cool the head (Esch 1976; Heinrich 1979, 1980a). In direct sunlight, *Polistes* wasps do sometimes regurgitate droplets of fluid (personal observation).

The temperature increase in non-flying *P. dominulus* could be due to shivering of the wing muscles, as in honeybees (Heinrich 1993), but the maintenance of elevated

temperatures in hibernation clusters is unlikely to be the result of shivering, since temperature elevation occurred in all regions. Also, elevated temperatures in hibernation clusters appear to be maintained over extended periods. However, the hibernation clusters generally contained between 4 and 10 individuals clustered very tightly together; as such, small amounts of heat produced by individuals could cause slight increases in the temperature of all individuals in the cluster. Temperature elevation occurred in all regions in the warm-up experiment as well. In this experiment, wasps were moved from a cool environment to a warmer environment; thus, some of the rapid warming observed may have been due to the inspiration of warmer ambient air, although this cannot explain those wasps that had body region temperatures above ambient. Our results raise the question of whether some or all of this temperature increase could be due to a futile cycle as has been observed in bumblebees (Surholt *et al.* 1991; Staples *et al*. 2004). While the futile cycle does not appear to be a large part of warming in bumblebees (Staples *et al.* 2004), the weak temperature elevation observed at low temperatures in *Polistes* could potentially be caused by a futile cycle. Further testing is required to address this possibility.

The findings that non-flying *Polistes* increase their body region temperature and that flying *Polistes* stabilize thorax temperature in response to changes in air temperature are important first steps toward a broader understanding of *Polistes* temperature relations, autecology, and social structure. For instance, in our measurements of wasps allowed to warm from 8 to 25° C, body region temperature in non-flying animals eventually returned to ambient temperature, suggesting that elevation above ambient temperature was a response to chilling.

Maintaining an elevated body temperature is costly (Heinrich 1993), and must be figured into the costs and benefits of many *Polistes* behaviors. For example, *Dolichovespula maculata* and *Vespula vulgaris* elevate temperatures prior to aggressive actions against intruders (Heinrich 1984). If temperatures are raised prior to aggressive interactions with conspecifics or potential nest predators, these interactions may be more costly than had been previously thought (Weiner *et al.* 2009).

The data described here provide important background for addressing other questions about thermoregulation in *Polistes*. For example, it is unknown whether any primitively eusocial insect maintains elevated brood temperatures, as do bumblebees (Heinrich 1972), or engage in behavioral fever, as do honeybees (Starks *et al.* 2000). In addition, thermoregulatory abilities can affect geographic distribution. *P. dominulus* is a very successful invasive species in North America, having spread throughout most of the Eastern USA as well as parts of California and Canada since their first sighting in Massachusetts in 1978 (reviewed in Liebert *et al.* 2006). The importance of energetic costs and responses to temperature in the geographic distribution of African honeybees has been well documented (Harrison & Hall 1993, Harrison 2006). Similarly, the impact of temperature on the range of *P. dominulus* may be important for predicting the full extent and effect of its invasion.

Acknowledgements
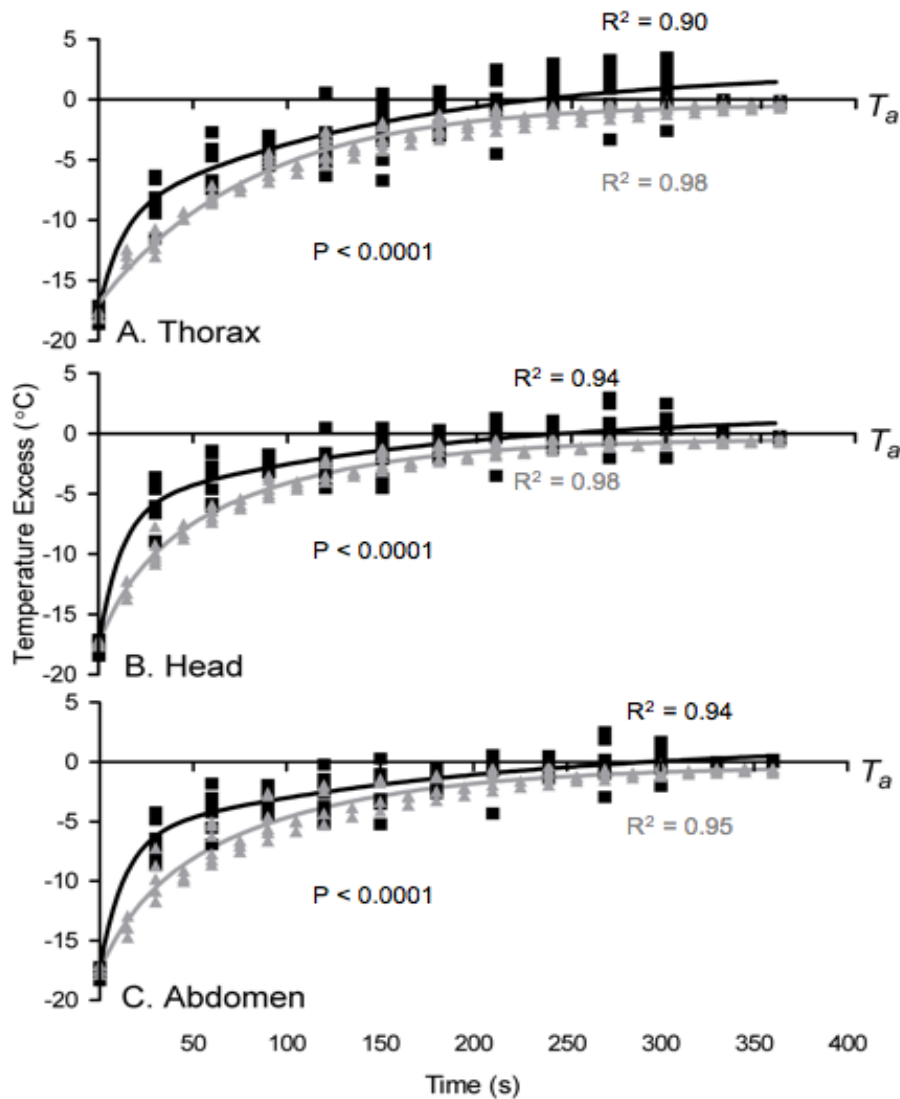
Figures

**Figure 5.1. Time course of body region temperatures of live and dead *P. dominulus*

after transfer from 8 °C to 25 °C.**   Temperature values are relative to ambient

temperature (25° C). Each black square represents the temperature of a single body region of a single wasp each of whose regions were measured only once. The points have been fitted with two phase exponential decay curves. Thorax (Panel A), then head (Panel B), then abdomen (Panel C) temperatures were measured for each wasp. Grey triangles represent 1 dead wasp monitored continuously (n=6). P values indicate the difference between live and dead wasps.
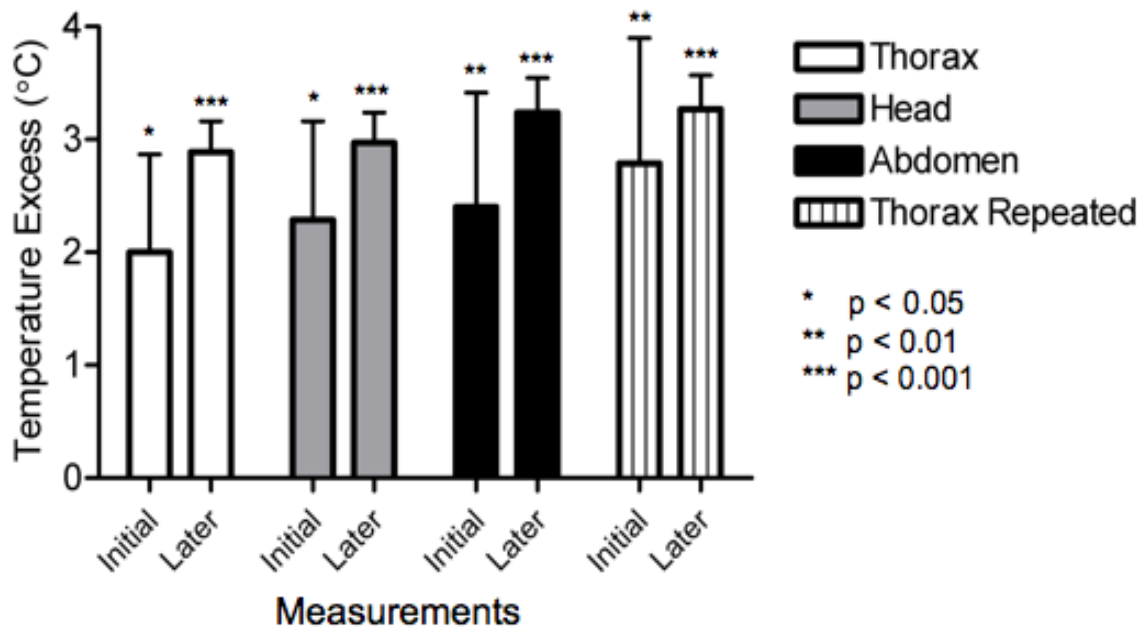
Figure 5.2. Temperature excess of body regions of hibernating wasps. The bars show the mean and standard error of the recorded temperature. The initial measurements were taken within 10 s of removal from the hibernacula; the post-disturbance measurements were taken after at least 30 s of disturbance. The thorax repeated bars indicate a second measurement of thorax temperature taken after the other measurements. Asterisks indicate a significant difference from ambient temperature.
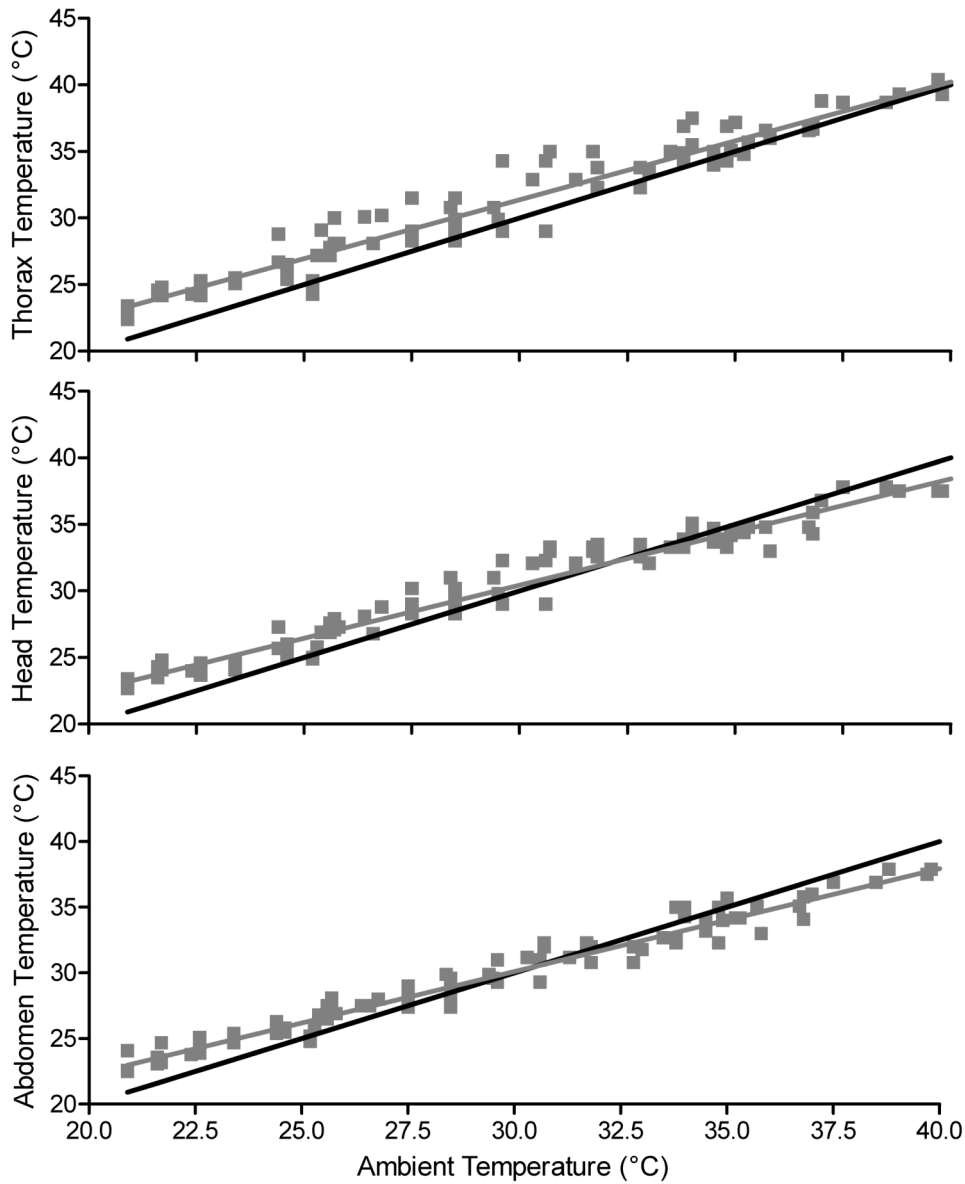
Figure 5.3. Body region temperatures of *P. dominulus* workers captured in flight.

Each point represents the temperature of one region of one wasp. Thorax, then head, then abdomen temperatures were measured for each wasp. Data for each region were fitted with a linear regression. The slope of each line differed significantly from the slope of $T_{region} = T_a$ (shown as a black line) (ANCOVA, $p < 0.0001$).

Chapter 6

The role of thermoregulation in the Polistes dominulus invasion: A comparison of the thermoregulatory abilities of P. dominulus and P. fuscatus

Abstract

Social insects are excellent invaders that have had negative impacts on native species and humans. Many invasive species move from warmer to cooler climates. For these species, thermal adaptations may both be important for their ability to invade and to limit their invasion range. The invasion of *Polistes dominulus* to North America provides an example of a primitively eusocial invader from a warmer climate. We studied the differences in thermoregulation between *P. dominulus* and the native *P. fuscatus.* We found that, during flight, thorax temperature in *P. fuscatus* was less affected by ambient temperature than the thorax temperature of *P. dominulus*. We also found that *P. dominulus* and *P. fuscatus* showed different patterns of warming after removal from a cold environment. Unlike *P. dominulus*, live *P. fuscatus* never fully cooled down in a cold environment. *P. fuscatus* also reached their relative minimum flight temperatures earlier than *P. dominulus*, but *P. dominulus* maintained higher elevated temperatures for longer. These differences in thermoregulatory ability suggest that the lower winter survival of *P. dominulus* could be offset by a greater thermal tolerance during flight, while the lower thermal tolerance of *P. fuscatus* in flight is offset somewhat by better thermoregulatory ability.

Introduction

Since the process of globalization began, many exotic species have found their way to new habitats around the world, including over 50,000 species in the USA alone (Pimentel *et al.*2004). Of all invaders, social insects are particularly effective because a single female can bring the sperm of one or more males (Moller 1996). This means that one social insect invasion event can have the genetic impact of several invasion events in other species. In some cases, social factors such as unicolonialism (the mutual tolerance of interconnected neighboring colonies) following a genetic bottleneck can also help invasive eusocial insects out-compete natives (e.g. Starks 2003).

Eusocial insects are group living animals with overlapping generations, reproductive division of labor, and communal brood care (Wilson 1971). Although eusociality has played an important role in many biological invasions, the invasion of *Polistes dominulus* to North America is the first time that the invasion of a primitively eusocial insect has been studied in detail (see Liebert *et al.* 2006). Workers in primitively eusocial insects are totipotent, meaning that all females can mate and produce offspring (Wilson 1971). Therefore, in primitively eusocial species, an isolated worker could potentially form a new colony, which could have a significant effect on the spread of an invasive species. *P. dominulus* was first found in the USA in Massachusetts in 1978, and has since spread rapidly throughout the USA and Canada (Johnson & Starks 2004, Liebert *et al.* 2006). *P. dominulus* has been a very successful invader, and this may be due in large part to its high production of reproductives (Gamboa *et al.* 2002).

There are many features of *P. dominulus* biology that may facilitate production of more reproductives than native congeners, but an important one is that *P. dominulus* colonies produce workers earlier in the season (Pickett & Wenzel 2000, Gamboa *et al.*

2002). After temperate *Polistes* gynes emerge from hibernation, they found nests alone or in associations of a few foundresses (see Reeve 1991) or wait and adopt abandoned nests (Starks 1998, 2001). In the early founding period, prior to worker emergence, foundresses must forage for themselves and for the developing brood. This period has a high risk of nest loss, and the sooner that workers emerge, the better the colony's chance of surviving (see Reeve 1991). Finally, the earlier production of workers in *P. dominulus* provides those workers with more time to forage for and rear a large work force, thus producing a large brood of reproductives.

       P. dominulus are particularly successful in cities (see Liebert *et al.* 2006), which has allowed them to spread throughout New England, in regions previously dominated by *P. fuscatus*. As befits the name, nests of *P. dominulus* are commonly found on the eaves of buildings and other human habitations. Both urban environments and proximity to heated buildings provide a slightly warmer environment than the surrounding climate (Landsberg 1982). While *P. dominulus* is found in much of central and southern Europe, it is of highest abundance in Mediterranean Europe and Northern Africa (see Cervo *et al.* 2000), which is warmer and has milder winters than many areas of North America where *P. dominulus* is now found (The Weather Channel 2010). *P. dominulus* gynes do not appear to survive hibernation as well as *P. fuscatus* gynes (Gamboa *et al.* 2004), which may limit their invasion success in areas with particularly harsh winters. Indeed, many gynes are lost during this overwintering period (Starks 2001, Gamboa *et al.* 2004).

       The higher susceptibility of *P. dominulus* to death during winter suggests that thermoregulation may be important to the invasion of *P. dominulus* to North America. One the one hand, *P. dominulus* is less successful than the native congener at

overwintering in New England, which should serve to decrease population size. On the other hand, *P. dominulus* begins foraging earlier in the day than *P. fuscatus* and rears its first brood more quickly (Gamboa *et al.* 2004). If *P. dominulus* are able to fly at lower air temperatures, they may be able to forage more effectively during nest initiation, which would allow them to more effectively provision their early brood, possibly shortening their development time. *P. dominulus* could potentially fly at lower temperatures either by more effectively maintaining an elevated thorax temperature or by flying at a wider range of thorax temperatures, therefore needing to spend less energy warming at cold temperatures. In this study we explored the differences in thermoregulatory abilities between *P. dominulus* and *P. fuscatus*. These differences could help both to explain the success of the *P. dominulus* invasion and to predict its limits.

Methods

Animals

In summer of 2007, combs and associated adults were collected from 29 nests of *Polistes dominulus* and 12 nests of *P. fuscatus* found in Waltham, Grafton, and Carlisle, MA. In the summer of 2008, an additional 32 *P. dominulus* and 28 *P. fuscatus* nests were collected from the same sites. The nests were maintained in 0.037 m³ cages on the Tufts campus in Medford, MA. Colonies were kept on a summer light cycle (17L:7D) at ~26 °C and were provided with wax worms and a 50% sugar water solution *ad libitum* (Sumana & Starks 2004). In 2008, colonies were provided with construction paper for nest construction. All individuals tested were females that emerged more than two weeks prior to the first male and, thus, were assumed to be workers (Mead *et al.* 1990). No live

individual was used in more than one test, as the 24-hr mortality rate from measurement of internal temperature was high (greater than 30%) and the procedure may alter the wasps' ability to maintain temperature.

Temperature Measurements

Warm-up Measurements:

To compare the ability of *P. dominulus* and *P. fuscatus* workers to defend their body temperature during exposure to cold air (~8 °C) and to elevate it when returned to normal temperature (~26 °C), the body region temperatures of live and dead individuals were measured during the 10 min following their return to laboratory air temperature. Dead wasps were measured as they equilibrated from 8 °C to ambient temperature (T_a) in order to establish a baseline to which living wasps could be compared (e.g. Bishop & Armbruster 1999). Dead wasps were freeze-killed at least 1 h, but less than 8 h before use, to prevent desiccation. Live and dead wasps were placed in a plastic bag and kept for 10 min at 8°C. The validation of this procedure was presented by Weiner and colleagues (2010a). After cooling, wasps were moved to a temperature controlled room kept at 26±0.6°C, while still restrained. Body region temperature was measured by inserting a Physitemp MT 29/1B microprobe thermocouple (diameter 0.33mm, time constant 0.025s) connected to a Physitemp BAT-12 thermometer (Physitemp, Clifton, NJ, USA). Thorax was measured first, followed by head and then abdomen. All these measurements were taken within 10 s.

Each dead wasp was measured continuously for 10 min and the temperature was recorded every 30 s (Weiner *et al.* 2010a). Each live wasp was used for only one data

point. Temperatures are reported as temperature excess relative to room temperature in order to compensate for variation in room temperature. An ANCOVA was done on the log of the temperature over time to compare the live wasps to the dead ones. The average of the dead wasp body-region temperature for each time point for the appropriate species was subtracted from each live wasp time point. A non-linear regression was used on the cumulative difference between live and dead animals over time to compare the two species. Most of the warming data on *P. dominulus* found in this paper has been reported by Weiner and colleagues (2010a).

Flight Measurements:

To compare the temperatures of flying *P. dominulus* and *P. fuscatus*, the body region temperatures of individuals were recorded immediately following capture from flight. For each test, the wasp was released in a temperature-controlled room and allowed to fly for 30-60 s (as in Weiner *et al.* 2010a). After a wasp had flown for 30-60 s, she was captured in a net and immediately restrained in the net against a piece of soft foam. Thorax temperature (T_{th}), then head temperature (T_h), and then abdomen temperature (T_{ab}) were measured within 10 s using the Physitemp thermometer and probe. Body region temperatures were regressed upon the corresponding T_a values, and the slopes were compared between species and to a slope of 1 (representing region temperature = T_a) using an ANCOVA. The *P. dominulus* portion of this data set has been reported by Weiner and colleagues (2010a).

Results

Warm-up measurements: Live *P. dominulus* and *P. fuscatus* individuals differed significantly from dead conspecifics in their warming patterns (ANCOVA on log-transformed data, *P. fuscatus*: F = 71.264, *P. dominulus*: F = 83.998, p < 0.0005 for both species). Both species eventually reached temperatures above ambient. The warming patterns of the two species (relative to dead conspecifics) differed from each other (non-linear regression, Thorax: p = 0.008, Head: p = 0.097, Abdomen: p < 0.001, Fig. 6.1). These results indicate that both species show endothermy. Since the patterns of warming relative to dead conspecifics differed between the species, our data suggest that the rates of warming differ in ways that are not fully explained by body mass. If the differences were due only to body mass, the patterns of warming for live animals relative to dead conspecifics would be similar between the species. This difference suggests that active warming patterns differ between the species.

Flight measurements: *P. dominulus* did not initiate flight below ~22 °C, while *P. fuscatus* initiated flight at temperatures below 20 °C, although these flights did not last for the full 30 s. The slopes of regressions for all body regions of both species were significantly different from 1 (95% confidence intervals of slopes from regression for *P. dominulus*: Thorax = 0.88 ± 0.04, Head = 0.79 ± 0.03, Abdomen = 0.78 ± 0.03; *P. fuscatus*: Thorax = 0.75 ± 0.08, Head = 0.74 ± 0.06, Abdomen = 0.78 ± 0.07, Fig. 6.2). The slopes of body region temperature on T_a during flight differed significantly between species (Fig. 6.2, ANCOVA, Thorax: P = 0.002; Head: P = 0.001; Abdomen: slope P = 0.936, intercept P = 0.021). In particular, the slope of T_{th} on T_a was significantly less steep in *P. fuscatus* than in *P. dominulus* (slope confidence interval *P. dominulus*: Thorax = 0.88 ± 0.04, *P. fuscatus*: Thorax = 0.75 ± 0.08). This indicates *P. fuscatus* defends a

narrower T_{th} range than *P. dominulus*. Weight did not have a significant effect on T_{th} relative to T_a in flight (multiple linear regression, n = 58, t = 0.76, p = 0.45).

Discussion

      Our data show that *P. fuscatus* and *P. dominulus* differ in their thermoregulatory abilities in ways that could affect invasion success. Differences in warming behavior following removal from a cold environment may indicate thermoregulatory differences that affect the ability to start a colony early in the season. Live *P. fuscatus* maintained an elevated T_{th} during 10 min at 8 °C, unlike live *P. dominulus* and the dead individuals of both species. This ability to maintain an elevated temperature in cold environments could help *P. fuscatus* (whose minimum flight T_{th} is higher *P. dominulus'*) avoid being trapped by cool patches in early spring. However, after approximately 3 min in the ~26°C environment, live *P. dominulus* individuals had actually warmed up more than *P. fuscatus*. This ability to warm up relatively quickly and maintain elevated temperatures in response to favorable conditions could allow *P. dominulus* to make more flights in cooler temperatures, such as those that occur during the founding period.

      Despite the willingness and ability of *P. dominulus* to maintain elevated temperatures, *P. fuscatus* reached its minimum observed flight temperatures 30 s earlier. This ability to warm up quickly also may help *P. fuscatus* gynes survive the winter by helping them evade predators in early spring or late fall. In flight, T_{th} in *P. fuscatus* was less affected by T_a than the T_{th} of *P. dominulus*, especially at higher temperatures, thus expanding the range of air temperature within which *P. fuscatus* can fly. While the larger size of *P. fuscatus* workers may be important to their thermoregulatory abilities at rest,

we found no effect of weight on T_{th} in flight, suggesting that other characteristics are important for allowing them to defend a narrow range of flight temperatures.

As has been demonstrated in the example of the Africanized honeybee, thermoregulation can be important in both allowing and limiting social insect invasions (Harrison & Fewell 2002, Harrison *et al.* 2006). The *P. dominulus* invasion resembles the invasion of the Africanized honeybee in several ways: both are social insect invaders from a region with milder winters, both invaders are highly productive during the summers, and both invaders have lower survival than their native or naturalized congener during the winter.

Our data suggest that thermoregulation may be important to explaining the range of the *P. dominulus* invasion. If *P. fuscatus* needs to warm up to a higher T_{th} before sustaining flight, despite their ability and willingness to undertake brief bouts of flight at slightly lower ambient temperatures, it may partially explain why they do less foraging in the spring (see Liebert *et al.* 2006). Making more foraging trips in spring could facilitate the earlier rearing of workers by *P. dominulus* (Gamboa *et al.* 2004), which shortens the risky preworker period.

P. dominulus, being smaller and from warmer climates may spend more energy during the winter elevating their temperature to prevent freezing, decreasing their overwinter survival. While *P. fuscatus* maintained slightly elevated temperatures in a short-term cold environment, *P. dominulus* has been observed to maintain elevated temperatures in hibernacula (Weiner *et al.* 2010a). *P. fuscatus* gynes are both better able to maintain these elevated temperatures and also are well-prepared for winter with cryoprotectants (Gibo 1972, Strassman *et al.* 1984). Resting metabolic heat may play a

role in elevating hibernacula temperatures, although to facultative endothermy would appear to exceed a reasonable energy budget (Masters *et al.* 1988a,b). While our work was done on workers, caste differences in *Polistes* are small, and there is overlap between workers and reproductives in many traits including size. Therefore, we expect that this work should apply to gynes and foundresses as well; however, future work should look at reproductives to confirm this.

In addition, the maintenance of elevated temperatures in *P. dominulus* upon warming to more moderate temperatures could be costly in certain environments (Rau 1930). For example, in New England, temperatures during the winter can vary widely, leaving a gyne stranded in cold environments or causing her to use up her energy before resources are available (Table 6.1). The ability of *P. fuscatus* to maintain elevated temperatures in cold environments could be very beneficial if a foundress encounters spatially or temporally limited cold zones. Indeed, *P. dominulus* foundresses on warm days in early spring have been observed to land on patches of remaining snow and be unable to take off due to the thermal mass (personal observation).

P. dominulus has been a very successful invader in North America (see Liebert *et al.* 2006). The success of this invasion seems to be due, at least in part, to the fact that *P. dominulus* colonies are more productive than *P. fuscatus* colonies (Gamboa *et al.* 2002). This increased productivity is likely at least partially because they produce workers earlier (see Liebert *et al.* 2006). *P. dominulus* may be able to produce workers earlier because *P. dominulus* foundresses make more foraging trips (Gamboa *et al.* 2002). *P. dominulus* foundresses also make shorter foraging trips, suggesting that their foraging may be more efficient (Gamboa *et al.* 2002). If *P. dominulus* does not need to

thermoregulate as closely and is able to fly at higher T_{th}, the differences in their thermoregulatory behavior may help explain the differences in their foraging patterns. Coupled with the slightly shorter development time of *P. dominulus*, the differences in thermoregulation between *P. dominulus* and *P. fuscatus* may help explain the larger colony sizes of *P. dominulus*, especially in areas with short summers, like New England.

Our data show that *P. dominulus* and *P. fuscatus* differ significantly in their ability to warm up after removal from cool environments and in their ability to defend an optimal thorax temperature in flight. These differences may partially explain the high overwintering mortality and the high early season productivity of *P. dominulus*. As such, thermoregulatory behavior is likely to influence invasion patterns in this system.

Figures

Table 6.1 Average High and low winter and summer temperatures for locations in Eastern North America and Mediterranean Europe. Highlighted boxes indicate the lower temperatures to which *P. dominulus* is exposed in New England winters.

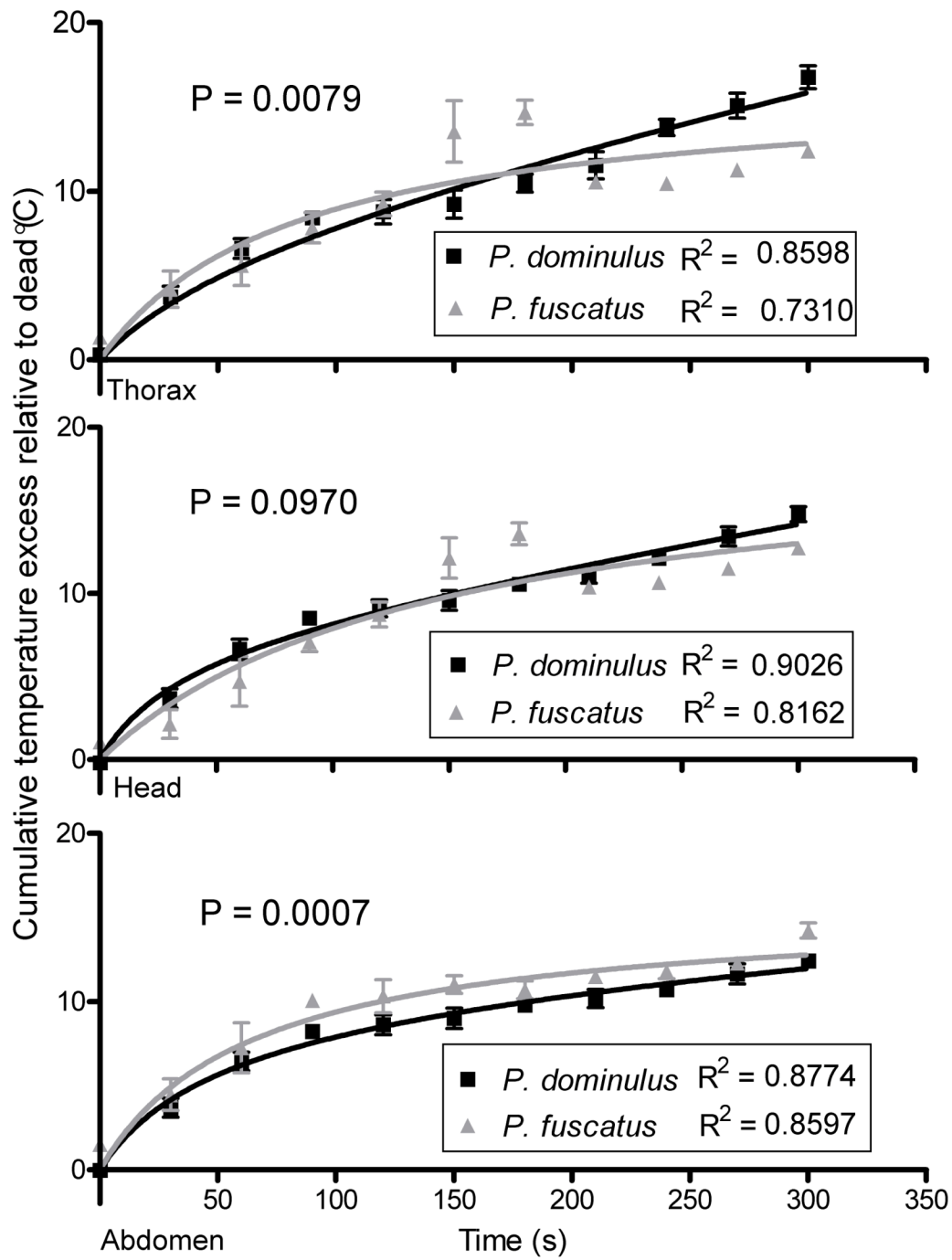|	Summer high	Summer low	Winter high	Winter low
Hartford, CT	27.6	15.7	3.3	-7.2
Boston, MA	26.5	17.0	3.9	-4.1
Portland, ME	24.6	13.5	0.9	-9.1
Burlington, VT	25.9	14.3	-1.5	-10.9
Mobile, AL	32.7	21.7	17.0	11.0
Rome, Italy	29.6	15.7	12.6	2.4
Madrid, Spain	30.7	15.2	11.3	1.1
Athens, Greece	31.3	6.9	13.5	7.0

Figure 6.1. Time course of cumulative body region temperature excess of *P. dominulus* and *P. fuscatus* after transfer from 8 °C to 26 °C. Temperature values are relative to the average temperature of a dead animal of the same species under the same

conditions. Each point is cumulative, representing the total excess temperature since removal. Points indicate means and error bars show standard deviation. Each time point, represents 8 *P. dominulus* and 6 *P. fuscatus*.
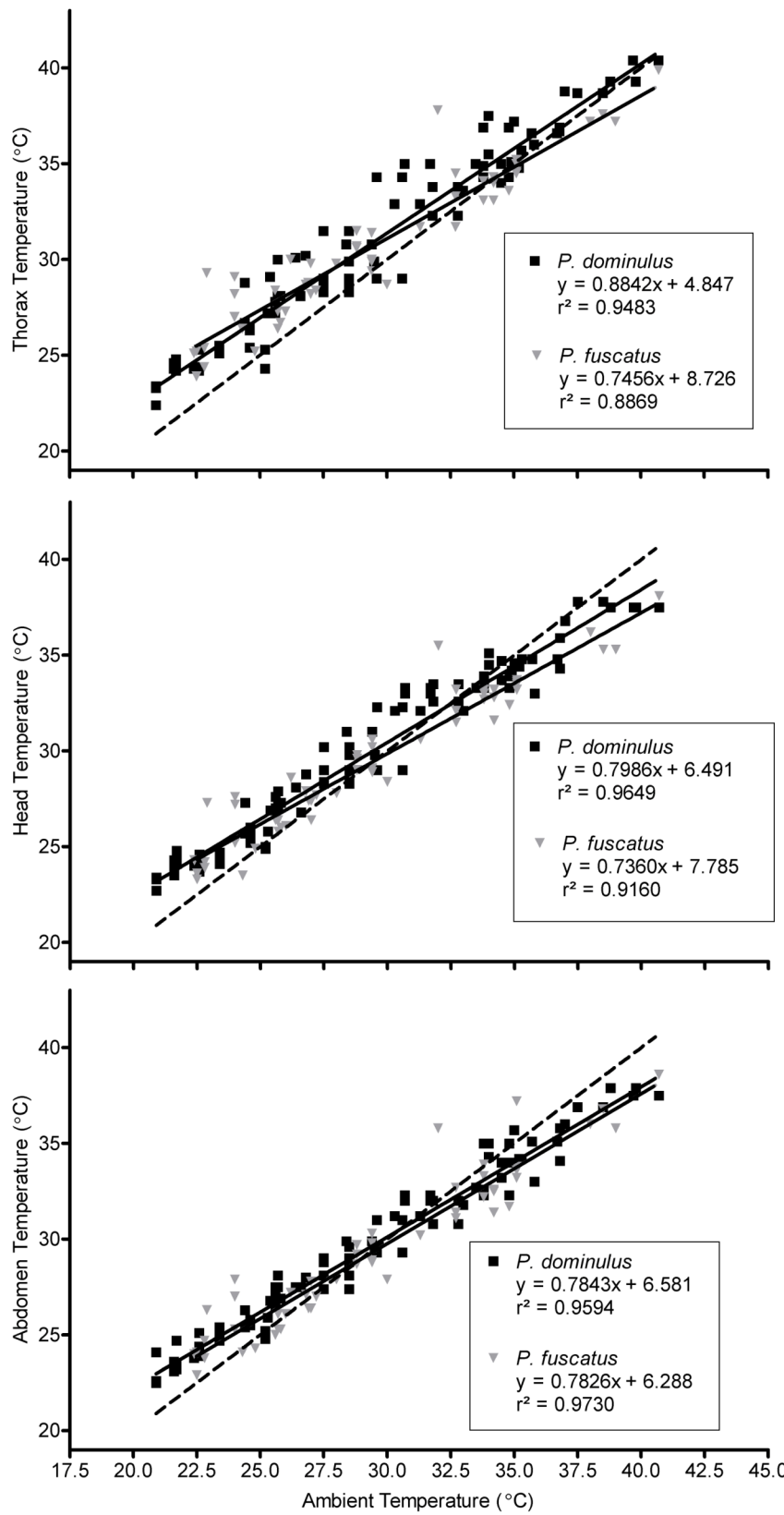
Figure showing three scatter plots of Thorax, Head, and Abdomen Temperature (°C) versus Ambient Temperature (°C) for *P. dominulus* and *P. fuscatus*.

Thorax Temperature (°C)
- ■ *P. dominulus*
  y = 0.8842x + 4.847
  r² = 0.9483
- ▼ *P. fuscatus*
  y = 0.7456x + 8.726
  r² = 0.8869

Head Temperature (°C)
- ■ *P. dominulus*
  y = 0.7986x + 6.491
  r² = 0.9649
- ▼ *P. fuscatus*
  y = 0.7360x + 7.785
  r² = 0.9160

Abdomen Temperature (°C)
- ■ *P. dominulus*
  y = 0.7843x + 6.581
  r² = 0.9594
- ▼ *P. fuscatus*
  y = 0.7826x + 6.288
  r² = 0.9730

Ambient Temperature (°C)

Figure 6.2. Body region temperatures of *P. dominulus* and *P. fuscatus* workers captured in flight. Each point represents the temperature of one region of one wasp. Thorax (Panel A), then head (Panel B), then abdomen (Panel C) temperatures were measured for each wasp. Data for each region were fitted with a linear regression. The slope of each line differed significantly from the slope of $T_{region} = T_a$ (shown as a black dotted line) (ANCOVA, n = 156 *P. dominulus*, 50 *P. fuscatus*, $p < 0.0001$).

Chapter 7

The energetic costs of flight in *P. dominulus* and *P. fuscatus* and the effect of that cost on the *P. dominulus* invasion

Abstract

Polistes dominulus is a primitively eusocial paper wasp from Mediterranean Europe that is invasive to North America. In Eastern North America, *P. dominulus* is in competition with *P. fuscatus*. A major reason for the success of *P. dominulus* is that their nests produce more reproductive offspring than *P. fuscatus* nests. One partial explanation for this difference is that *P. dominulus* foundresses make more foraging trips in the pre-worker period, which likely helps them to rear workers more quickly. We found that *P. dominulus* had a lower "overall" flight metabolic rate, but that *P. fuscatus* had a lower mass specific flight metabolic rate. In addition, in *P. fuscatus*, wing loading correlated with flight metabolic rate, but that this was not the case in *P. dominulus*. This suggests that *P. fuscatus* is not able to transport large loads inexpensively. Therefore, the lower overall cost of transport of *P. dominulus* may provide an advantage, allowing the foundresses to make more relatively efficient foraging trips. In addition, we compared time in flight by *P. dominulus* and *P. fuscatus* over a range of temperatures and found that, while *P. fuscatus* flew well over a broad range of temperatures, *P. dominulus* had a relatively narrow range of optimal temperatures for flight (30-33° C). This suggests that *P. dominulus* is a relative "temperature specialist" on the temperatures that are found in summers in both Mediterranean Europe and New England.

Introduction

From monkeys stealing fruit in Costa Rica (Engeman *et al.* 2010) to the western corn rootworm in Europe (Carrasco *et al.* 2010), invasive species have become a major economic and ecological problem in our modern global society. As our global economy continues to spread species around the world, and changing land use and global climate change alter habitats in ways that do not match the conditions under which native species evolved, invasions are expected to become an even bigger issue. Social insects are particularly adept invaders, due both to the sperm storage capabilities of hymenopteran females and the ability of workers to greatly multiply the reproductive output of an individual foundress (Moller 1996).

Polistes dominulus is a primitively eusocial paper wasp native to Mediterranean Europe that has become a very successful invader in North America (reviewed in Liebert *et al*. 2006). In much of Eastern North America, *P. dominulus* is found alongside a native congener, *P. fuscatus* (reviewed in Liebert *et al*. 2006). *P. dominulus* has been a highly successful invader, in large part because *P. dominulus* nests produce more reproductives than *P. fuscatus* nests (Gamboa *et al.* 2002). In order to address how *P. dominulus* is able to out-produce *P. fuscatus*, first it is helpful to understand the colony cycle in *Polistes*, and how the colony cycles of the two species differ.

In temperate *Polistes* species, including both *P. dominulus* and *P. fuscatus*, new reproductives emerge in the fall, then they mate and the females overwinter (reviewed in Reeve 1991). In the spring, reproductive females found nests either alone or in small groups and begin laying (primarily) worker-destined eggs. Until that first brood emerges, the foundresses do all the foraging and nest building (reviewed in Reeve 1991). When

the first brood emerges, they mostly stay on the nest as workers, and take over foraging and other tasks. These workers then help the queen raise more workers, and eventually a new brood of reproductives (reviewed in Reeve 1991).

Until the first workers emerge, the foundress or foundresses do all the foraging. Foraging is a risky and energetically expensive job, as it involves flight, and, for single foundress nests, requires the foundress to leave the nest undefended. The sooner that the first workers emerge, the less likely a nest is to fail (Reeve 1991). In addition, the sooner workers emerge, the more workers a nest can rear to help provide for reproductives at the end of the summer. *P. dominulus* workers emerge earlier in the summer than *P. fuscatus* workers (Gamboa *et al.* 2004). This could partially explain how *P. dominulus* nests are able to produce more reproductives.

However, this opens the question of how *P. dominulus* produces workers earlier in the season. One possible partial explanation of this is the fact that *P. dominulus* foundresses make more foraging trips during the pre-worker period (Gamboa *et al.* 2002). While foraging can be hazardous, early colonies are energy limited (Rossi & Hunt 1988), and additional foraging trips could, potentially, increase the growth rate of the brood (Mead & Pratte 2002). One way that *P. dominulus* foundresses might be able to benefit from making more foraging trips is if flight is less energetically costly for them than for *P. fuscatus*. This could allow them to spend less energy to bring back the same amount of food, making foraging more energetically efficient.

In this study, we compare the energetic costs of flight in *P. dominulus* and *P. fuscatus* at a range of temperatures. This allows us to test both whether flight metabolic

rate is generally lower for *P. dominulus* and whether *P. dominulus* has a lower cost of transport at the low spring temperatures that foundresses would experience.

Methods

Animal Care

In the summer of 2009, 19 *P. fuscatus* nests and 22 *P. dominulus* nests were gathered from field sites in Waltham, Carlisle and Grafton, MA. The nests were maintained in 0.037 m^3 cages on the Tufts campus in Medford, MA. Colonies were provided with wax worms and a 50% solution of sugar in water, as well as construction paper for nest-building. All individuals tested emerged during the summer from these nests. No individual was used in more than one test.

Flight measurements

For each measurement, one individual was placed in a respirometry chamber, inside a transparent temperature control chamber set up outside, in the shade (Woods *et al.* 2005). Temperature was raised by means of a warm air blower whose output was ducted by 3.2 cm PVC tubing through a fitting in the floor of the cabinet and directed away from the respirometry chamber; a 5 cm aperture in the top Plexiglas panel of the cabinet served as an exhaust (Woods *et al.* 2005). The respirometry chamber was a 1L Pyrex Erlenmeyer flask lined with fluon to discourage landing. Flight was encouraged by tapping or moving the flight chamber (Woods *et al.* 2005). Animals that tried to land were discouraged by tapping the flight chamber, but those that persisted in doing so were

not prevented. All periods of flight were recorded, and only the longest flight was used for each animal.

CO$_2$ production was measured continuously by differential open-flow respirometry using a Li-Cor 6262 CO$_2$/H$_2$O analyzer (Li-Cor, Lincoln NE, USA) calibrated with a gravimetric mixture (Scott Specialty Gases, Plumsteadville PA, USA) and re-zeroed between measurements. Air scrubbed of water and carbon dioxide by soda lime-Drierite-soda lime columns was flowed at 860·cc·min–1 through the respirometry chamber. Excurrent CO$_2$ concentration was sampled every 6 s; each recorded value represented the mean excurrent CO$_2$ concentration for the previous 6 s as calculated by the Li-Cor unit (see Woods et al 2005). Air temperature (T_a) inside the chamber was monitored to ±0.1°C using a Physitemp BAT 12 field-thermometer (Physitemp Instruments, Clifton, NJ, USA) with its sensor inserted through the chamber stopper.

Respirometry data were collected and analyzed in Datacan V (Sable Systems International, Las Vegas, NV). Behavioral observations were synchronized with respirometry data taking into account time lag and washout properties of the respirometry system as determined by bolus injection (Bartholomew et al. 1981). We used the standard Z-transform implemented in Datacan V.

Flight Speed

The flight speed of *P. dominulus* was measured by releasing foragers and timing their return to the area of their nest. Six nests were founded naturally in wasp boxes arrayed behind a fence. Returning foragers were captured in a net and carried 10 m away from the fence while stored in a 50 mL Falcon tube. They were released and allowed to

exit the tube without additional disturbance. The time it took each wasp to reach the fence was recorded. Only wasps that took a relatively straight path back to the fence were included in the data.

Wing loading

The right wings were removed from the wasps, and photographed under magnification. Images were converted to binary files in ImageJ. If necessary, "unsharp mask" in Adobe Photoshop was first used to clarify the distinction between the wings and the background. Then, the wings were measured using "analyze particles" in ImageJ. The animal's weight divided by the recorded wing area was reported as wing loading (Dudley 2000).

Results

In both *P. dominulus* and *P. fuscatus*, ambient temperature (T_a) was significantly associated with time in flight (Figure 7.1). In *P. dominulus*, the flights of longest duration occurred at a T_a of 30-33°C, while in *P. fuscatus*, flight time increased with T_a up through our highest temperature measurements at 37°C (nonlinear regression comparison, F = 2.679, p = 0.033 Figure 7.1). Despite this correlation of time in flight with T_a, no correlation was found between T_a and CO_2 production in either species (linear regression, *P. dominulus*: $r^2 = 0.008$, p = 0.498; *P. fuscatus*: $r^2 = 0.030$, p = 0.309, Figure 7.2). The average flight speed of *P. dominulus* was 3.84±0.89 m/s. Our observed flight speed for *P. dominulus* matched predicted flight speeds based on previous work in other animals (Predicted maximum flight speed = 4.3 m/s, or about 12% greater than our

measurement, McNeill 2002). We were not able to get direct flight speed measurements for *P. fuscatus.* However, these calculations predict that *P. fuscatus* would have a flight speed of 3.2% higher, meaning 4.4 m/s for the calculated speed, or 4.0 m/s using our measured speed.

When the total rate of CO_2 production of both species in flight was compared, *P. fuscatus* produced more CO_2 than *P. dominulus* for the same period of flight (Mann-Whitney U = 994, p = 0.001, Figure 7.3A). However, when mass-specific CO_2 production was considered, *P. dominulus* produced more CO_2 than *P. fuscatus* per gram of body mass (Mann-Whitney U = 386, p = 0.0001, Figure 7.3B). To address how this might apply in foraging, we looked at CO_2 production relative to wing loading. We found that, in *P. fuscatus*, CO_2 production correlated strongly with wing loading, but there was no significant correlation between CO_2 production and wing loading in *P. dominulus* (Species comparison ANCOVA, df = 41, F = 3.843, p = 0.295, Figure 7.4).

Discussion

In this study we found that, while *P. dominulus* had a lower overall flight metabolic rate (FMR), *P. fuscatus* had a lower mass-specific FMR. We used the measured speed for *P. dominulus* and 3.96 m/s for *P. fuscatus* (calculated based on McNeill 2002), and calculated the cost of transport to be $2.5 \times 10^{-3} \pm 7.1 \times 10^{-4}$ ml $g^{-1} m^{-1}$ for *P. dominulus* and $2.1 \times 10^{-3} \pm 5.6 \times 10^{-4}$ ml $g^{-1} m^{-1}$ for *P. fuscatus*. However, the total cost of flying a given distance would be $1.9 \times 10^{-4} \pm 5.5 \times 10^{-5}$ ml m^{-1} for *P. dominulus* and $2.3 \times 10^{-4} \pm 6.1 \times 10^{-5}$ ml m^{-1} for *P. fuscatus*. The lower total flight energy use could be an

advantage for *P. dominulus*, allowing the foundresses to use less energy on each foraging trip.

However, since *P. fuscatus* is larger and has a lower mass-specific FMR, if *P. fuscatus* foundresses are able to cheaply carry larger foraging loads, they could actually be more efficient foragers. In order to test this, we measured the effect of wing loading on FMR in both species, and found that FMR correlates much more strongly with wing loading in *P. fuscatus*. This suggests that *P. fuscatus* foundresses would not be able to take advantage of their lower mass-specific FMR in order to efficiently carry larger foraging loads, as higher loads would increase their wing loading, presumably increasing their FMR as well. Instead, *P. dominulus* may be able to forage at lower energetic cost. During the pre-worker period, this lower energetic cost could allow foundresses to put more energy into rearing offspring, which may partially explain the earlier emergence of workers in *P dominulus*.

While species and wing loading (in *P. fuscatus*) did affect FMR in *Polistes*, temperature did not have an effect. This stands in contrast to what has been observed in honeybees (Woods *et al.* 2005), and surprising, since *P. dominulus* has been shown to thermoregulate in flight (Weiner *et al.* 2010a). Thermoregulation in flight suggests that there is a range of optimal thorax temperatures, which the organism tries to defend during flight. This suggests that there would be a disadvantage to flying outside the optimal range, and a change in FMR could have explained this disadvantage. However, while FMR was not affected by air temperature, time in flight was. *P. dominulus* flew for the longest periods within a temperature range of approximately 30-33°C, while *P. fuscatus* flew longer at higher temperatures, and did not appear to reach a maximum temperature

within the temperature range that we tested. These results suggest that, despite the lack of correlation between temperature and FMR, *P. dominulus* individuals are more inclined to fly at specific temperatures. In particular, extended flights (of 30 s or more) did not occur at all at the lowest temperatures for either species or at the highest temperatures for *P. dominulus*. These extended flights would be expected to be necessary for foraging.

The more limited optimal range in *P. dominulus* suggests that it might be a relative "temperature specialist" while *P. fuscatus* is more of a generalist. *P. dominulus* is native to Mediterranean Europe, which has relatively little temperature variation (Table 6.1). *P. fuscatus*, however, is spread throughout Eastern North America, throughout a wide range of climates, including some with high temperature variation within and between seasons (Table 6.1). However, summer temperatures in New England are relatively similar to summer temperatures in Mediterranean Europe (Table 6.1). The highest temperatures that we measured were substantially above the average highs for Mediterranean Europe, but closer to the average highs for the some parts of the southern United States (Mobile, AL, Table 6.1).

At these high temperatures, *P. fuscatus* flew for longer periods than *P. dominulus*, and *P. dominulus* flew for shorter periods than they did at lower temperatures, suggesting that *P. dominulus* may have reached thorax temperatures that were too high to maintain extended flight. This is supported by previous work showing that *P. fuscatus* has lower thorax temperatures during high temperature flight than *P. dominulus*, and that thorax temperature in flying *P. fuscatus* drop below ambient temperature above 35°C, while thorax temperatures in *P. dominulus* do not drop below ambient temperature in flight (Weiner *et al.* 2101a, b). This enhanced thermoregulation could allow *P. fuscatus* to act

as a "temperature generalist." However, if *P. dominulus* is a temperature specialist on the temperatures commonly found in Mediterranean summers, the summer temperatures in New England would generally fall within this range. While the winters of New England are much harsher and may lead to higher mortality for *P. dominulus* (Gamboa *et al.* 2004, Starks 2001), hibernating in or on heated buildings may protect *P. dominulus* from some of the cold related mortality. This specialization might allow *P. dominulus* to spend be less reliant on thermoregulation while maximizing their flight power in a specific temperature range.

These results suggest that the likely lower flight costs of *P. dominulus* relative to *P. fuscatus* could allow more efficient foraging by *P. dominulus* foundresses. This could help explain the early emergence of workers on *P. dominulus* nests relative to *P. fuscatus* nests. In addition, the differences in the flight behavior of the two species at different temperatures suggests that *P. dominulus* could be temperature specialized in ways that might help to explain its success in New England summers. These results could be a step towards better understanding the invasion of this eusocial animal, and could, potentially, provide avenues of exploration for better understanding other insect invasions.
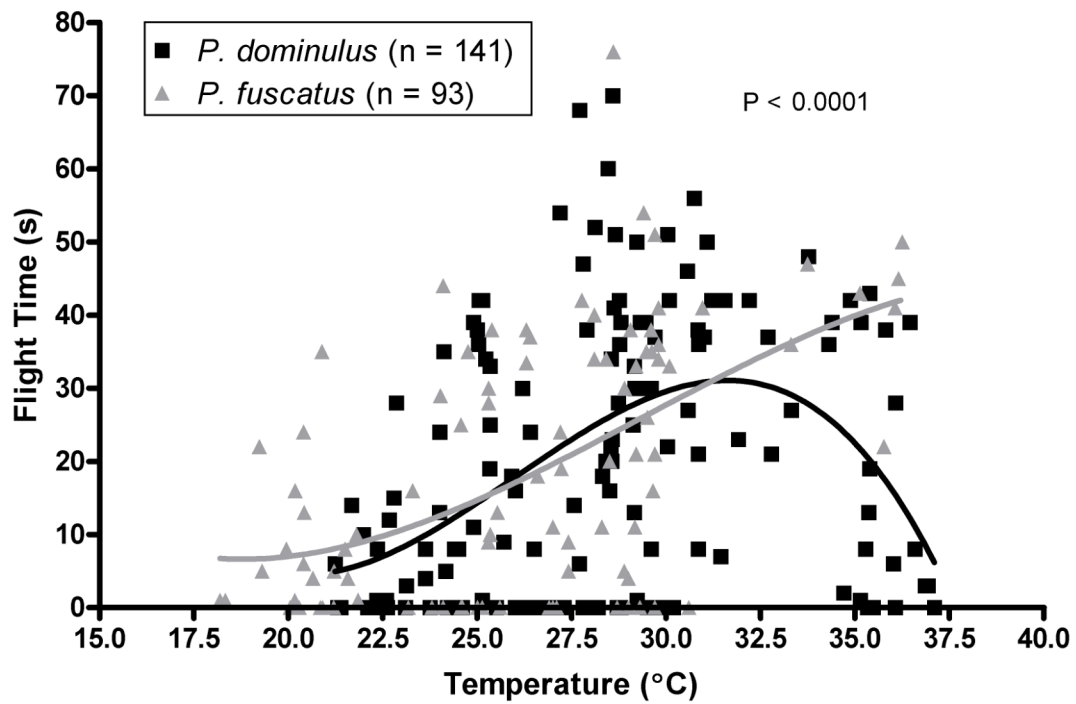
Figure 7.1 Longest time flown by T_a for *P. dominulus* and *P. fuscatus*. Each point represents the longest flight recorded for one individual. The curves are 3rd order polynomials.
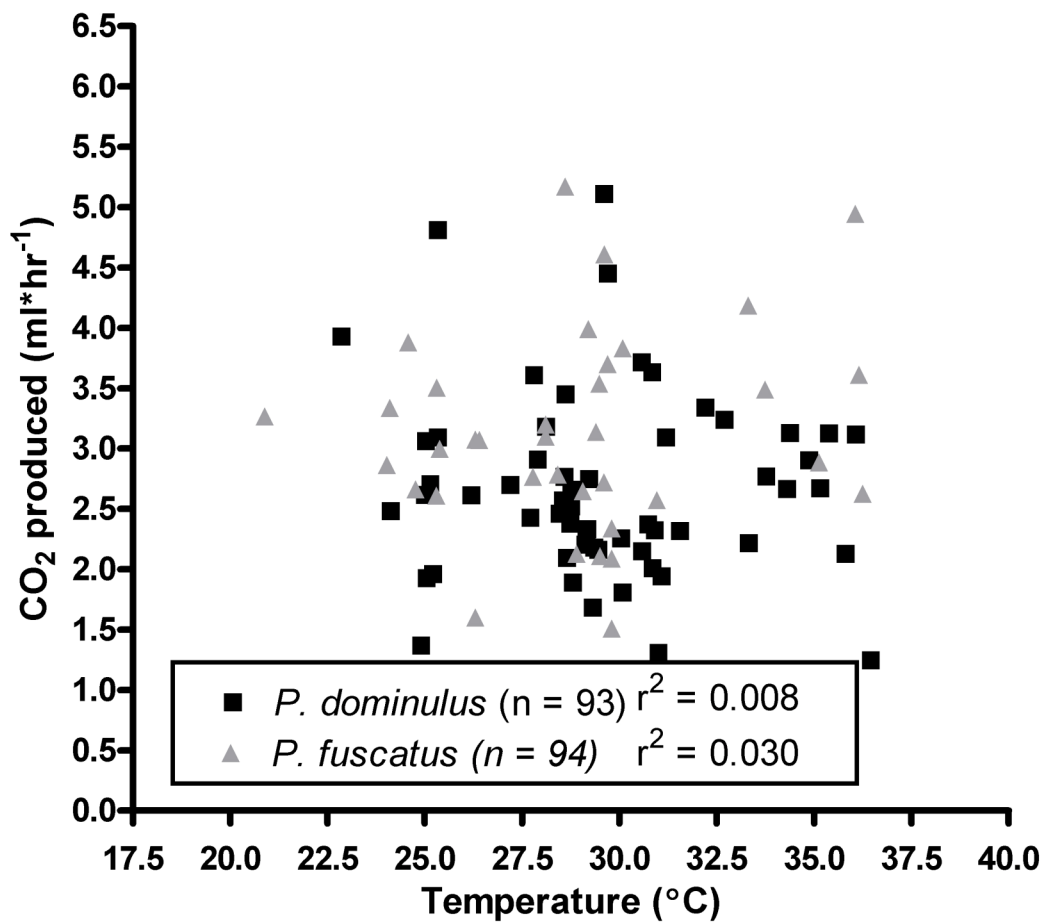
Figure 7.2 Rate of energy use in *P. dominulus* and *P. fuscatus* relative to temperature. Each point represents the energy use rate of the longest flight recorded for one individual. Only flights over 25 s are shown. A linear regression was performed on the data, but no correlation was found (*P. dominulus* $r^2 = 0.008$, $p = 0.50$; *P. fuscatus* $r^2 = 0.030$, $p = 0.31$).
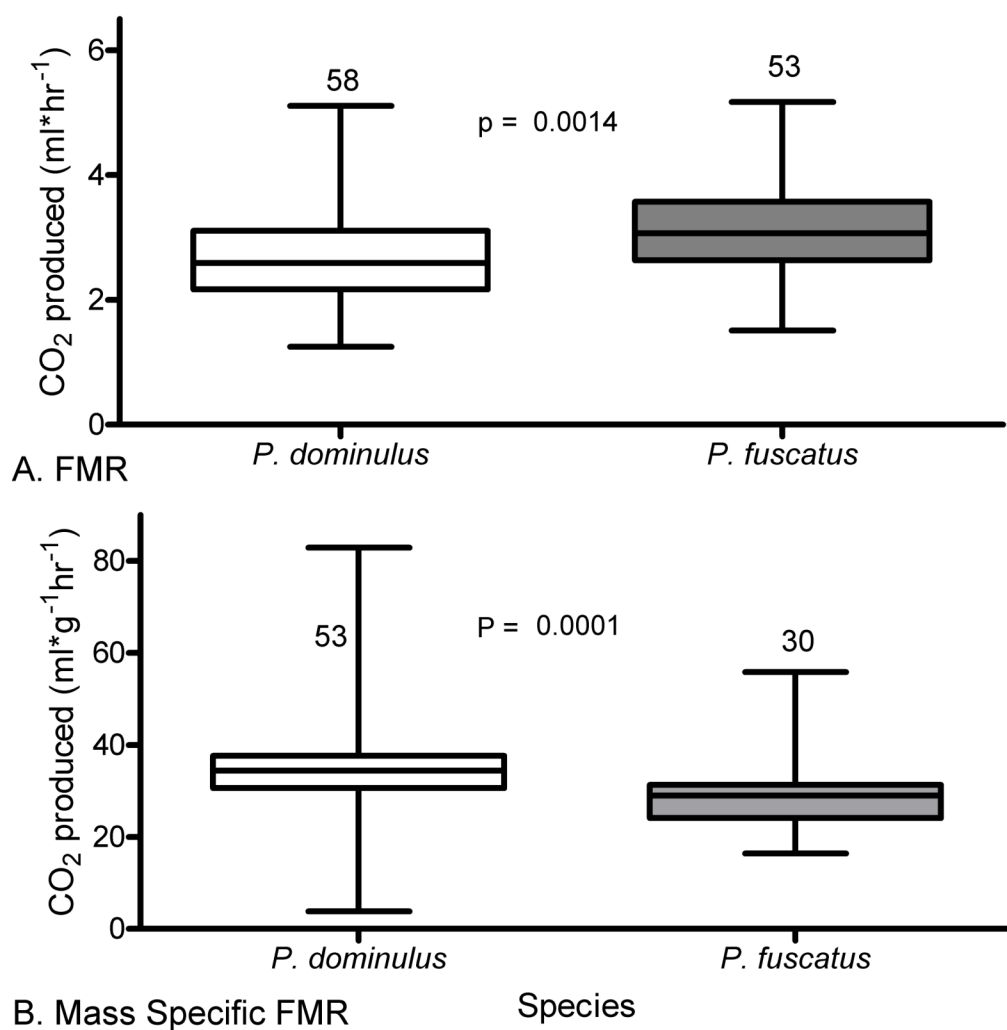
Figure 7.3 Flight metabolic rate for *P. dominulus* and *P. fuscatus*. Absolute flight metabolic rate is shown in panel A and mass-specific flight metabolic rate is shown in panel B. Only the longest flight was used for each individual. Only flights over 25 s are included. (Mann-Whitney U Test, FMR: U = 994, Mass-specific FMR: U = 386).
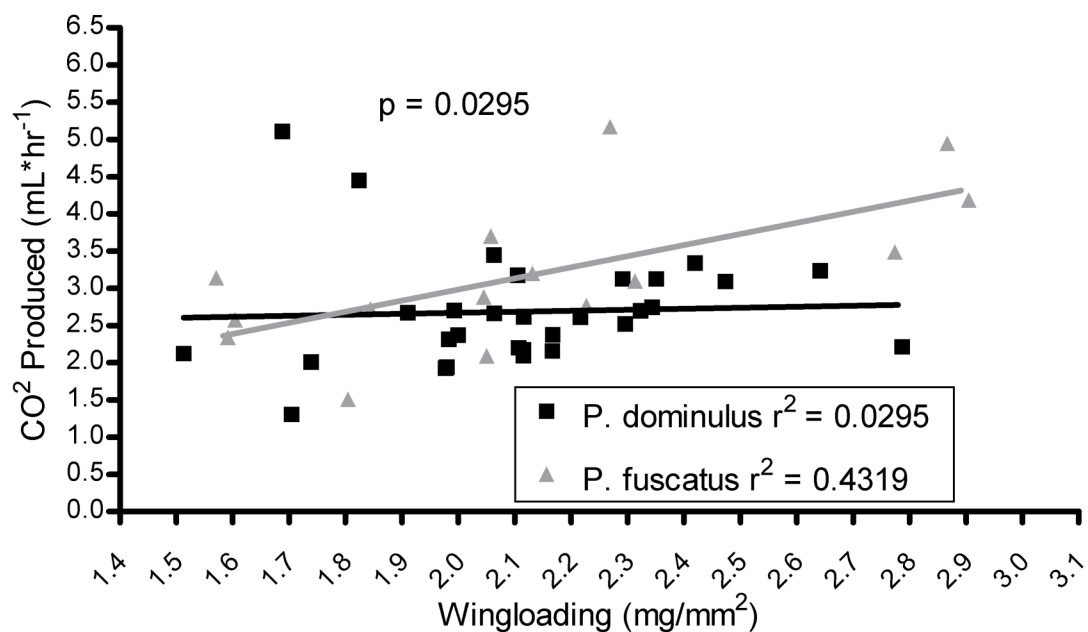
Figure 7.4 Flight metabolic rate for *P. dominulus* and *P. fuscatus* relative to wing loading. Each point represents the energy use rate of the longest flight recorded for one individual. Only flights over 25 s are shown. An ANCOVA was used to compare the results.

Chapter 8

Are subordinate roles a conditional strategy?

An energy budget of the female roles of *P. fuscatus*

Abstract

Polistine paper wasps have long been a model system for studying the evolutionary origins and maintenance of eusociality because they are primitively eusocial and relatively easy to study. Many different explanations have been proposed for the reproductive division of labor in eusocial organisms, but one of the common types of explanations is reproductive skew models. These models have been largely developed and tested in *Polistes*, but recent tests have found them to be poor predictors of reproductive division of labor in that system. The tests of reproductive skew models assume that, in order for an individual to benefit from taking a helper role (and reproducing less), she must have as much success as an average individual founding alone. However, our recent research found that subordinate foundresses use more energy than solitary foundresses in order to maintain the same level of ovarian development. In order to understand the importance of this difference to the success of a nest, I created an energy budget for each role, and found that being a solitary foundress is very energetically costly. For a subordinate foundress to take on the role of a solitary foundress, she would need to expend even more energy than a solitary foundress. This could substantially decrease her success founding alone. If a subordinate foundress would have lower success founding alone, she may not need a high level of success as a subordinate in order to benefit from adopting that role. This could offer a way to resurrect

skew models by taking into consideration the variability among individuals, and, therefore, help explain the maintenance of eusociality.

Introduction

The evolution of eusocial organisms has long puzzled biologists (Darwin 1859). Natural selection selects for genes that allow each organism to maximize its genetic representation in the next generation. Eusocial organisms, however, have reproductive division of labor, meaning that one or a few individuals do most or all of the reproduction (Wilson 1971). This means that many (or even the large majority) of the individuals are doing little or no reproduction. This opens the question of why an individual would surrender reproduction in favor of helping another individual reproduce.

Polistes colonies provide an ideal model system for studying the evolutionary origins and maintenance of eusociality (West-Eberhard 1996, 2006). *Polistes* are primitively eusocial, meaning that all individuals are physiologically capable of reproducing and founding a colony (Wilson 1971). Also, *Polistes* has two types of non-reproductive (or less reproductive) helpers: workers and subordinate foundresses. In temperate species, workers are individuals that emerge during the early to mid summer and stay on the maternal nest to help rear sisters (Reeve 1991). Subordinate foundresses, however, emerge in the fall with the other reproductive females. They mate and overwinter like solitary and dominant foundresses, but, in the spring, instead of founding their own colony, they join and help on the nest of another foundress, usually a sister (Reeve 1991, Field *et al.* 1998, Nonacs *et al.* 2006).

Unlike workers, subordinate foundresses are mated females that have the opportunity to found a nest at the same time as the other nests are being founded. In addition, they are (at best) rearing nieces, not sisters, and, therefore, getting less indirect fitness benefit (Hamilton 1964). These multiple foundress associations have become a standard model system for studying the evolution of cooperation and reproductive division of labor in insects (Reeve & Keller 2001, Nonacs 2006, West-Eberhard 2006). In particular, skew models, one of the major models attempting to explain the maintenance of eusociality, have been largely developed and tested in *Polistes* (Reeve *et al.* 2000, Reeve & Keller 2001, Nonacs 2006).

Skew models use the success of multiple foundress nests and solitary foundress nests to predict when joining should happen, and what the distribution of reproduction should be among foundresses on a nest (Reeve *et al.* 2000, Reeve & Keller 2001). In recent tests, however, skew models have frequently failed to predict these behaviors in *Polistes* (Nonacs *et al.* 2004, Liebert & Starks 2006, Nonacs 2006, Nonacs *et al.* 2006).

Tests of reproductive skew models generally assume that, were a subordinate to found alone, she would have the same reproductive success as the average solitary foundress (Reeve & Keller 2001, Nonacs 2006). However, if subordinate foundresses are less able to successfully found nests and rear offspring, they may not need the same level of success as a solitary foundress in order to benefit from accepting a subordinate role. In our previous work (Chapter 3) we found that subordinate foundresses used more energy to maintain ovarian development than did solitary foundresses. This suggests that there are metabolic differences between subordinate and solitary foundresses that might make a

subordinate foundress less likely to successfully rear a nest of brood, particularly if being

a solitary foundress is very energetically expensive.

If being a solitary foundress is very energetically costly, and subordinate

foundresses need to spend more energy to maintain ovaries, it is possible that these

individuals might benefit from taking a subordinate role, even at a relatively low fitness

benefit. These subordinate foundresses could still benefit from this role because they

would have low success as solitary foundresses. Even if a subordinate role is not less

energetically costly, an subordinate that dies due to exhaustion or too many risky

foraging trips has still benefited the dominant and will have some success (Shreeves *et al*.

2003). However, if a solitary foundress dies before workers pupate, all her investment is

lost and her fitness is 0. To test the possible differences in energetic costs between

different roles, I created an energy budget for each female role in *P. fuscatus*: workers

and dominant, subordinate and solitary foundresses. This will allow the calculation of

the energy use of being a solitary foundress, and, therefore, address the possible benefits

of accepting a subordinate role. This could allow for the resurrection of skew models by

taking into account individual differences and the effects of these differences on

reproductive success.

Methods

Time budget

Video transcriptions from Nonacs (personal communication) of *P. fuscatus* nests

in the summers of 1994-1997 were used to determine the time spent by individuals on

different tasks. The behaviors recorded were absence from the nest, resting, grooming,

walking, inspecting, constructing, fanning, mounting, laying eggs, darting, lunging,

biting, soliciting food, and abdominal wagging. All times were calculated as a percentage of the total time to compensate for different periods of video-taping.

Individuals were recorded as workers or solitary, subordinate or dominant foundresses based on their time of emergence and their behavior. Individuals that emerged from a nest were considered workers, as no tapes were recorded after males had begun to emerge. The dominant foundress was identified by one of: being the sole individual observed to lay eggs, never leaving the nest while all other individuals did or initiating over half of the total on-nest interactions. If no individual met one of these criteria, or two individuals met different criteria, the nest was excluded from the analysis. Individuals were grouped by role (worker, dominant, subordinate or solitary) and whether or not workers had emerged on the nest. For this work, dominants and subordinates were only included if workers had not yet emerged. Solitaries all had at least one worker present, as tapes for lone solitaries were not available.

Energy costs of behaviors

Previously measured energy use rates were available for *P. fuscatus* for flight and rest and for *P. dominulus* for resting, grooming, walking, fanning, mounting, darting, biting and abdominal pumping (Weiner *et al* 2009, Chapters 3 & 7, personal observation). For *P. fuscatus*, the standard metabolic rate (SMR) for workers was 0.198 ml*$g^{-1}$$hr^{-1}$, while the SMR for *P. dominulus* workers was 0.358 ml*$g^{-1}$$hr^{-1}$. The energy use rate of flight relative to rest for *P. dominulus* was 107.9, and for *P. fuscatus* the relative rate was 147.1. This fits with our previous findings that flight was somewhat more energetically expensive for *P. fuscatus*, and allowed us to estimate the energetic

costs of other behaviors relative to rest based on *P. dominulus* data. Cell inspection was considered equivalent to walking, as much of the time spent in inspections is spent walking from cell to cell. Construction and solicitation or trophylaxis were considered equivalent to grooming, as many of the movements are similar. Egg laying and abdominal wagging were considered to be equivalent to abdominal pumping (Table 8.1).

Off nest periods were assumed to be primarily foraging bouts. In order to assess the cost of foraging, first a median foraging bout of 1194s was considered. The average foraging trip for *P. fuscatus* is 48.1m (Dew & Michener 1978). Based on our calculated flight speed for *P. fuscatus* (Chapter 7), the trip out and back would take 22.4s, if the individual flew directly. We added some search time, as *Polistes* foragers are observed to spend some time looking for food sources from the air, bring our estimated average flight time to 30s. Of the remaining time, about half was assumed to be used for butchering or wood removal, which were assumed to be approximately equivalent to biting. One quarter was assumed to be used for walking, either searching for resources or moving between them. The remaining quarter was split between resting and consuming nectar or grooming (Table 8.2). This is based on previous work in the lab observing attacks on wax worms, and on work assessing the frequency of different types of foraging (Reeve & Gamboa 1987). While the time distribution on foraging is very approximate, we consider this relatively conservative, given the low time allocated to flight.

Calculation of energy budget

From the average time budget for each role, the energetic cost of each behavior was used to calculate an energy budget (as in Gremillet *et al.* 2003, Woods *et al.* 2007).

This indicated how much energy was spent on each behavior, and how much energy each role spent relative to the others.

Results

Workers used the most energy overall and dominant foundresses used the least (Figure 8.1, 2-way ANOVA, $p < 0.0001$, Bonferroni post-test: dominant vs. worker $p < 0.01$, solitary vs. worker $p < .01$, subordinate vs. worker $p < 0.05$, others $p > 0.05$). The only individual behavior that differed among the roles was foraging. Dominants spent significantly less energy on foraging than subordinates or workers, and workers spent more energy on foraging than did any other role (Table 8.3, 2-way ANOVA, df = 23, $p < 0.0001$, Bonferroni post-test: dominant vs. subordinate $p < 0.001$, dominant vs. worker $p < 0.001$, solitary vs. worker $p < .001$, subordinate vs. worker $p < 0.05$, others $p > 0.05$).

Discussion

From these energy budgets, I found that being a solitary foundress is very energetically costly. In this budget, solitaries and subordinates spent about the same amount of total energy. However, the solitaries that were used in this energy budget all had at least one worker, and on all but one solitary foundress nest at least one worker was observed foraging. It is expected that before worker emergence, solitaries are doing more of their own foraging, and, therefore, the energy use of lone solitaries would be higher.

A solitary foundress must forage for the nest, as well as doing all the nest defense and brood care, and maintaining developed ovaries (Reeve 1991). I also found that foraging is a very energetically costly behavior, and that solitaries, workers and

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subordinates all spend more than half of their energy budget during the day on foraging behavior. This was true even though solitaries were only recorded after worker emergence. A resting subordinate uses 16.6% more energy than a resting solitary foundress (Chapter 3), which would cause a subordinate that founded as a solitary foundress to need even more energy than a standard solitary foundress.

Energy use is particularly important in pre-worker nests, and productivity in early nests can be increased by honey supplementation, indicating that energy is already limiting (Rossi & Hunt 1988). Every time a solitary foundress goes out to forage, she not only risks predation and weather while foraging, but also leaves her brood undefended (Shreeves *et al*. 2003). A subordinate foundress that needed more energy to do the same tasks would either need to forage more often (putting herself and her brood at risk) or rear a smaller brood. This would make her less successful overall as a solitary foundress. In addition to the added risks of foraging and leaving the colony alone, a solitary foundress that dies loses all fitness success. However, a subordinate foundress that dies has still helped the dominant foundress, and, as long as she is related to that dominant, may still benefit (Shreeves *et al*. 2003).

These results help explain the failures of skew models in *Polistes*. If subordinate foundresses would need even more energy to create and maintain a nest alone, and being a solitary is already energetically costly, maintaining a colony alone might be energetically prohibitive for a subordinate foundress. She might simply not have the fat stores for it to be viable, or even if she does, she would need to do more foraging, which is both energetically costly, and risky.

Since subordinates require more energy to maintain a colony, a subordinate may have substantially lower success founding along than the average solitary foundress. This would mean that accepting a subordinate position might be the best choice, given the situation, even if her success as a subordinate foundress is lower than the average success of a solitary foundress. These new data could allow skew models to be revised and resurrected by taking into account individual variation among foundresses. If some individuals are less able to found alone due to maternal manipulation, developmental conditions and competition with other individuals (Chapter 3), these individuals may benefit by taking helper roles. This could make the subordinate position a condition dependent tactic that does not need to be as successful as solitary founding is on average in order to benefit certain individuals. This could help us understand the evolution and maintenance of eusociality, both in *Polsites*, and, potentially, in other primitively eusocial species.

Acknowledgements

Table 8.1 Behaviors recorded and their categorization. All recorded behaviors are listed below, with the form in which they were measured and how they were categorized in table 8.3. Energy costs of behaviors marked with an *a* were reported by Weiner and colleagues (2009).

Behavior recorded	Measured as	Categorized as
Absent	(Table 8.2)	Foraging
Resting	Resting[a]	Resting
Grooming	Grooming	Grooming
Walking	Walking[a]	Walking
Inspecting	Walking[a]	Nest care
Fanning	Buzz-walking[a]	Nest care
Mounting	Mounting[a]	Interacting
Constructing	Grooming	Nest care
Egg laying	Abdominal pumping	Egg laying
Darting	Darting[a]	Interacting
Lunging	Darting[a]	Interacting
Biting	Biting[a]	Interacting
Solicitation	Grooming	Interacting
Abdominal wagging	Abdominal pumping	Nest care

Table 8.2 Percentage of time assigned to each behavior during foraging. Behaviors marked with *a* were reported in Weiner *et al*. 2009, behaviors marked with a *b* were reported in Chapter 7.

Behavior	Percentage of time
Flight[b]	2.5%
Walk[a]	24.4%
Bite[a]	48.7%
Groom[a]	12.2%
Rest[a]	12.2%

Table 8.3 Energy spent on activities as a percentage of total daytime energy budget by each role.

	Dominant	Solitary	Subordinate	Worker
Foraging	13.3%	21.3%	24.6%	22.1%
Resting	16.1%	5.9%	6.4%	3.3%
Grooming	2.2%	1.0%	0.8%	0.5%
Walking	7.6%	3.9%	1.8%	1.4%
Egg Laying	4.1%	0.4%	0.0%	0.0%
Interacting	1.4%	1.1%	0.4%	0.3%
Nest Care	20.7%	17.7%	10.2%	7.7%

Figure 8.1 Energy budget for *Polistes dominulus* females by role. Each bar shows the average energy used by individuals in that role in total during a given active period, and by activity. Error bars show standard errors. Energy was calculated relative to spending the entire time resting. Different letters indicate p <0.05 for the total energy use.

Appendix

The data for Figure 4.3 were retested for correlation using ordinal regression. The correlation remained significant (chi-square 16.02, df $= 4$, pseudo $r^2 = 0.16$, p $= 0.003$).

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