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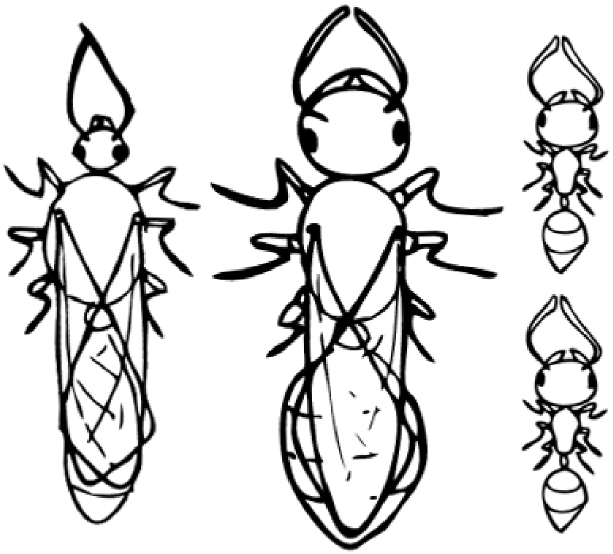
The Kindness of Strangers: Genetic Diversity Within Social Insect Colonies



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A Paper Presented to the

Faculty of Mount Holyoke College in

Partial Fulfillment of the Requirements for

the Degree of Bachelors of Arts with

Honor

Department of Biological Sciences

South Hadley, MA 01075

May 2011

This paper was prepared
under the direction of
Professor Stan Rachootin
for eight credits.

This work is dedicated
to the memory of the ants
whose lives were sacrificed in the
preparation of this thesis

Sum Quod Eris

A warm thank you to:

Advisor Stan Rachootin for his enthusiasm and motivational speeches

Committee members Renae Brodie and Sam Mitchell for their advice and participation in this process

Janice Gifford for her patience and expert advice on statistical method

Jennifer Fewell and Jon Harrison at Arizona State University for granting me the opportunity to do research in their labs

Members of the Fewell and Harrison Labs, especially Rick Overson and Tate Holbrook for kindly providing information via e-mail, and a special thank you to James Waters for being an excellent person, without whose support and instruction this thesis would not have been possible

The Scion Natural Science Association for the kind grant that made work at ASU possible

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ABSTRACT

It is generally believed that high relatedness between sisters, a characteristic of the hymenopteran method of sex determination, is what allows this insect order to build harmonious social organizations. However, at least in the most complex of the species, growing empirical evidence is showing that selection favors high levels of genetic diversity between colony members. Genetic diversity within a colony can be increased either through polyandry (mating with multiple males), polygyny (multiple females providing workers for a single colony) or genetic recombination. High within-colony genetic diversity has been shown to increase colony survival and foraging ability in ants, bees, and wasps. Higher diversity in response thresholds is assumed to be a major cause of these increases, and to lead to more resilient colonies. But, response thresholds are not the only intrinsically variable factors. I discuss studies of, and propose two hypotheses for, ways in which behavioral diversity due to genetic diversity can benefit a colony. I also outline techniques for testing these hypotheses, and present the Californian harvester ant, *Pogonomyrmex californicus,* as an ideal test subject.

INTRODUCTION

"Not all ants use violence to dominate their
world, some use more subtle methods..."
-- E.O. Wilson

Social insects, especially the ants, are wonderfully complex, incredibly efficient, and supremely charismatic creatures. They exhibit altruistic tendencies that humans both esteem and balk at. They show levels of integration, efficiency, and resiliency that we have only begun to unravel the workings of, and which have already provided numerous gains in areas such as computer sciences and robotics.

For many years, the mechanism that drove all this ingenuity was only attributed to high levels of genetic relatedness. It was thought that, without exception, their ability to cooperate and work efficiently was proportional to their genetic stake in the reproductive sisters they helped rear. Those who studied social insects grew to believe that diversity within an insect colony was a bad thing. Something that would corrupt social cohesion, and bring competition and detrimental selfish behavior into otherwise harmonious relationships. However, more and more empirical data are showing that natural selection is not necessarily working against diversity within the colony, but at times in favor of it. Recent studies are revealing that the most complex, and most derived of the Hymenoptera exhibit high levels of diversity between the individuals in their workforce.

BASIC BACKGROUND

Eusocial animals are characterized by the formation of colonies, containing overlapping generations of individuals who practice cooperative brood rearing and a division of reproductive labor. One or a few reproductive individuals produce tens, to hundreds, to thousands of sterile workers, which perform all of the tasks pertinent to survival except reproduction. In the eusocial hymenopterans, the reproductive individual at the head of the colony is the queen and the workers are most often her daughters. Males are only produced about once a year, at the same time as new queens. During the rest of the year, the queen makes only sterile workers who gradually amass resources within the colony. Genetically related reproductive males and queens are typically the only ways in which a worker can insure her fitness. Workers are able to produce offspring only on very rare occasions and in special circumstances such as at the death of the reigning queen.

HAPLODIPLOIDY AND KIN SELECTION

Biological altruism, as reviewed by Sober and Wilson (1998), involves increasing the fitness of another individual at the expense of your own. At first, this kind of behavior may seem unsustainable when natural selection is considered: how can a trait like altruism get passed on to the next generation, if the bearer of the trait sacrifices her welfare for others? The key to explaining altruism lies in remembering that natural selection favors actions that increase the net sum of your own genetic material that survives into the next generation. The offspring of an organism does not necessarily need to benefit, if the actions of the organism benefit a large number of kin, or generally make it more likely that its offspring will survive and produce many more generations of offspring. The roots of altruism lie within groups of individuals who concurrently gained the ability to act helpfully. Natural selection can act on groups with individuals that display helpful tendencies if that helpfulness allows the group to outcompete other groups who do not have helpful individuals (Levins, 1975). The groups could be families of closely-related individuals, or just groups of individuals competing in stressful environments. Once even basic helpful tendencies arise, more extreme forms of altruism can evolve.

The theory of kin selection, which was first proposed by Haldane in 1955 and formalized by Hamilton in 1964, formulaically links helpful tendencies and relatedness. This theory explains a form of natural selection whereby an individual gains indirect benefits while decreasing its chance of survival in order to increase that of its kin. It can be expressed by rearranging an equation known as Hamilton's Rule. This equation describes the conditions in which a genotype can evolve given relatedness (r), benefits (b), and costs (c):

$$rB\text{-}C > 0$$

When re-arranged, it provides the criteria for the amount of relatedness (R) necessary for kin selection to favor the evolution of altruistic behaviors:

$$R > c/b$$

Where R is the fraction of genes shared by altruist and recipient. It should be noted that while relatedness can be clearly defined and measured, "benefits" and "costs" are not generally not well defined or easy to experimentally manipulate or measure. The ease of calculating relatedness coefficients however, lead to the development of the "haplodiploid hypothesis", a theory based around the hymenopteran order's method of sex determination and one which is used to explain how eusocial hymenopterans (certain genera of bees, wasps, and almost all ants) could have come to exhibit the extreme altruism we see today – where individuals give up all personal reproductive rights for the benefit of their mother.

In hymenopterans, an unfertilized (haploid) egg will become a fertile male, and a fertilized (diploid) egg will typically become a female – either a queen or a worker. Because a female can only ever give one of her sets of chromosomes to an offspring, she will always be 50% related to her progeny (male or female). Because her male mate's haploid sperm is genetically identical, if a queen mates with a single male, all of her female offspring are guaranteed to have a copy of the same paternal chromosomes. Additionally, they have a 50% chance of sharing copies of the same maternal chromosomes. This adds up to a whopping 75% relatedness between sisters, more than the 50% relatedness seen between mothers and offspring. Consequently, a female will have a chance at putting more of her genetic material into the next generation if she insures the survival of a reproductive sister rather than of her own offspring.

There is a bit of trouble when males are considered. Brothers are only 25% related to a female sibling (since the paternal chromosomes are guaranteed to be missing, and again there's only a 50% chance of a brother having the same maternal chromosomes as the sister), while a male offspring would be 50% related to the mother. Therefore, a worker's fitness would increase if she was able to produce her own male offspring when possible. Because of this, social harmony between the workers must be maintained through methods such as reproductive control through queen pheromones, evolution of morphological constraints on worker reproduction (such as reduced or missing oviducts and spermatheca and reduction or loss of the ability to lay viable eggs: Khila & Abouheif, 2010), and worker policing (workers destroying the haploid eggs laid by sisters, or acting aggressively toward egg-laying sisters: Hölldobler & E. O. Wilson, 1990). This must happen because a worker in a social colony is practically never in a situation where she alone can raise her brood. The way tasks are split up, raising young is necessarily a group effort, and the fitness lost by all through the actions of a reproductive worker is more than that gained by the reproducing individual.

Haplodiploidy provides a working answer for how social systems developed in the hymenopterans, but it is neither a complete nor uncontested answer, and neither is kin selection (Nowak et al., 2010). Haplodiploidy is not necessary for kin selection to occur (Foster et al, 2006), and kin selection is not the only kind of selection that acts on social insects. Within the social insects of the Isopteran order – the termites – workers can be unrelated, yet still have a chance to gain direct fitness (can still have a chance to birth their own offspring) even when they are for the most part reproductively sterile (Johns et al., 2009). Termites have typical diplo-diploid sex determination, are highly social, and have very similar colony structures to ants. Termite colonies are composed of a multitude of developmentally inferior and sterile workers, who break up various tasks among themselves, can be morphologically specialized, and are produced and maintained by a king and queen that specialize in reproduction (E. O. Wilson, 1971). For them, their social structure cannot be explained simply through relatedness, but involves the slowing of development, formation of helper castes, and an ancestor that likely lived its entire life in a single cramped rotting log (Johns et al., 2009). Additionally, there are highly social aphids, shrimp, and mole rats.

There is a deep rift forming between those who believe that haplodiploidy and kin selection are the explanation for the origins of sociality in the Hymentoptera, and those who do not (Neyfakh, 2011). The use of Hamilton's rule in general for studies in evolution has come into question, though some say the arguments presented by both sides are superficial (Gadagkar, 2010). Many argue that certain behaviors within the hymenopterans can only be explained through kin selection - such as worker policing, and reproductive skew (Strassmann et al., 2011). But questions of whether cooperation *leads* to levels of high relatedness (and behaviors that encourage it) or whether cooperation is *caused* by high relatedness remain unanswered (Nowak et al., 2010). There are some who believe helping behavior is something that can spontaneously develop several times in a population due to the innate nature of complex systems to randomly organize (Prigogine & Stengers, 1984), and afterwards can be improved upon through evolution (Nowak et al., 2010).

Haplodiploidy and kin selection may only provide an incomplete answer for the origins of sociality in the Hymenoptera, and it does not explain why most hymenopterans remain solitary - though it seems that, for the most part, there is strong selection to remain social once the ability has been gained, since no reversions from social to solitary living have been seen in the evolutionary history of the Hymenoptera (Chenoweth et al., 2007). Kin selection also does not explain a widespread evolutionary movement across the Hymenoptera towards the reduction and sometimes the elimination of relatedness between workers.

PREVALENCE OF GENETIC VARIATION WITHIN HYMENOPTERANS

The general trend in social evolution in the Hymenoptera seems to favor behaviors that increase genetic diversity in the workers within a colony. While most pre-social Hymenoptera exhibit monoandry - that is, females mate with a single male - the most derived eusocial hymenopterans exhibit extremely high levels of polyandry - females mate with multiple males (Oldroyd & Fewell, 2007). Even one additional mating (assuming that both sets of sperm come from unrelated males and are both used in the production of females) lessens the relatedness of workers to the point where kin selection no longer holds.

Furthermore, the hymenopterans exhibit many instances of polygyny – a condition where multiple unrelated reproducing females provide workers for a single colony. In this situation, despite having no shared genetic material, workers remain socially cohesive and cooperate as well as any monogynous colony.

General polyandry

Ever since the haplodiploid hypothesis was proposed, the frequent occurrences of polyandry and polygyny have been slightly annoying to myrmecologists whose field was built upon the premise of haplodiploidy and kin selection. Researchers for decades have been trying to get polyandry and polygyny to fit with the theory of kin selection with varying amounts of success.

Earlier studies of polyandry had researchers suggesting that multiply-mated females might circumvent low relatedness in their workers by mating with genetically similar or highly related males. Even then, this seemed unlikely, since hymenopterans typically mate in large chaotic swarms, none of the known polyandrous Hymenoptera mate with brothers within the hive, and even early electrophoretic studies supported random mating (Cole, 1983). Cole further reported that of the species in which mating frequency has been studied, polyandry is seen in about 69% of the ants and 41-47% of the social bees and wasps. More recent tests based on worker genotyping instead of mating observations are showing that the effective paternal representation (the number of patrilines actually expressed in the worker cohort) is fairly low for most polyandrous species. The average is around 1.16, and only one male contributes to paternity in about 1/3rd of polyandrous species (Boomsma & Ratnieks, 1996; similar results in *Proformica longiseta* by Fernández-Escudero et al., 2002). This means that though queens may be polyandrous (may be seen mating with more than one male) they are not effectively polyandrous (the workforce only represents the sperm of about one male). This seems to support the theory that natural selection would favor high relatedness between workers. After all, for kin selection to take effect, workers would be best served if they raised brood that was genetically related to them. Additionally, queens would benefit from reduced conflicts among workers because colony efficiency would be maximized, and a male would maximize his fitness by increasing his sperm's representation in the daughter queens produced by the mother queen. One weakness in this study was that only worker genotypes were taken into account as the study was attempting to explain why workers that seem to be genetically unrelated would nevertheless work together. The genotypes of the new queens produced by the colony were not examined, yet if the worker population mostly consists of one patriline, but the reproductive females they produce either are equally representative of mated males or representation is skewed towards patrilines not represented in the workforce, the support for kin selection once again is weakened.

Though it seems that initial reports of polyandry and the implications for genetic diversity within the workforce may have been exaggerated, several highly derived social genera with quite different natural histories consistently show high levels of paternal representation within the workers of a polyandrous queen's colony. The genera are the higher leaf-cutting ants (*Atta* and *Acromyrmex*), the seed harvester ants (*Pogonomyrmex*), the army ants (*Dorylus* and *Eciton*), the desert ant *Cataglyphi cursor*, the honey bees (*Apis*), and the yellow jacket wasps (*Vespula*) (Rheindt et al., 2005; Boomsma & Ratnieks, 1996; Fournier et al., 2008 and refs therein). Honey bees have the largest effective mating frequency, with an average of 12.48 paternal genotypes represented in workers at any one time. The genetic diversity within the European honey bee hive can be visually appreciated because different patrilines can be different shades of yellow. Of the ants, leaf cutter queens effectively mate with about 2.14 males and harvesters effectively mate with about 6.76, and the *Vespula* wasps effectively mate with about 3.68 males (Strassmann, 2001 and refs therein).

This suggests, as pointed out by Strassmann (2001), that true effective polyandry might be a rare occurrence among genera, but is widespread across the Hymenopteran order as it has arisen independently multiple times. This means that effective polyandry is not only real, but it hints that there may be some evolutionary benefit to being able to use the sperm of multiple males.

Sperm Bias

The striking differences in observed vs. effective paternity seen in most genera with multiply-mating species may suggest that there is some sort of sperm bias occurring – that is, a biased use of the sperm available from multiple matings. Sperm bias could be either effected by the queen to preserve worker harmony, or the male to promote use of his own sperm. Sperm clumping - when the sperm of a particular male stays associated rather than homogenizing in the spermatheca, causing a particular cohort of workers to have similar genetic relatedness – has been thought to be a method of preserving kin selection in multiply-mated queens. Sperm clumping, though, could still pose problems for kin selection, if as mentioned before, one male's sperm is overrepresented in the workers and underrepresented in the daughter queens.

Sperm bias does not seem to occur in most of the effectively polyandrous species that were previously mentioned (leaf cutter ants, harvester ants, honey bees, Vespula wasps). In the Vespinae wasps (the subfamily containing the highly polyandrous *Vespula* genus) sperm is mixed and used over time in equal proportions (Ross, 1986). In honey bees, though there is some sperm clumping effect as evidenced by related subsets of workers being produced at the same time, skew is never dramatic, and all males tend to be represented in significant proportions at all times during the course of the colony's lifetime (Laidlaw & Page, 1984). The high effective paternity seen in ants such as the leafcutters (*Atta* and *Acromyrmex*) and especially the seed harvesters (*Pogonomyrmex*) suggests a similar predilection for sperm mixing.

Furthermore, it has been suggested that for certain species, selective pressure acts on sperm longevity rather than semen's ability to eliminate or suppress other sperm. Queens of large social hymenopteran colonies can live for several decades, and often their reign is limited only by the exhaustion of their sperm stores. When they have no more sperm, they can only produce males (Cole, 1983). Furthermore, it often takes several years of making infertile female workers before a males' sperm is used for producing a queen. Therefore there may be selective pressure on queens to decrease sperm death (sperm competition may cause sperm death) and selection on males to focus on increasing the longevity of their sperm (den Boer et al., 2008).

Experiments conducted by den Boer et al. (2008; 2009) show that rather than sperm competition, sperm viability, transfer, and storage is increased by seminal secretions in the effectively polyandrous *Atta* leafcutter ants and in the European honey bee (*Apis mellifera*). Furthermore, in *A. mellifera*, secretions from the queen's spermatheca also increase sperm viability though with different proteins, showing that both female and male honey bees independently attempt to promote sperm viability.

Den Boer et al. (2008) point out that research has been focused primarily on finding ways in which sperm competes, and the scarcity of studies looking into whether sperm is selected upon for viability rather than competition is probably due to scientific oversight. For instance, mating plugs (torn-off male genetalia left after mating) have been interpreted as reproductive biasing strategies used by males, but mating plugs have only been observed and reported on in bees and fire ants (den Boer et al., 2008; 2009 and refs therein). Furthermore, mating plugs might simply function as a way to keep seminal fluid from leaking out of the queen, because it has been found that the mating plug does not prevent subsequent matings in honey bees, and in fact makes the queen more attractive to other drones. Male *A. mellifera* do not fight over queens, and do not remove the semen of other males (Boomsma & Ratnieks, 1996 and refs. therein).

One more interesting note about sperm, is that some reports suggest that even multiply mating queens of the effectively polyandrous leaf-cutter (*Atta* and *Acromyrmex*) and harvester (*Pogonomyrmex*) ants end up only storing the equivalent or less of one male‘s sperm in their spermatheca (Rheindt et al., 2005 and refs therein). This seems to disprove the hypothesis that increased sperm stores are the benefit of multiple mating, and hints that perhaps selection could be acting on an increase in genetic diversity of stored sperm rather than bulk.

Polygyny

Polygyny is another condition that can infuse a worker population with genetic diversity. In fact, if the reigning queens are completely unrelated, polygyny can bring about higher levels of genetic diversity within a colony than even polyandry could, since workers are not bound to relatedness through a shared mother. With polygyny, one often finds insects acting altruistically towards completely unrelated individuals.

Polygyny has been observed in ants, wasps, and rarely in bees (only occurring in a few stingless species and typically involving biased reproductive capabilities - that is, not all queens get a fair share at reproduction: Velthuis et al., 2001). It is interesting to note that polygyny is fairly common in wasps and occurs in 20 of the 26 known social wasp species (Hölldobler & E. O. Wilson, 1990). In neotropical wasps, colonies can be highly polygynous, and workers within the colonies can be decidedly unrelated (Strassmann et al., 1991). Additionally, even many primitively social wasps (that is, those which only recently became social) are highly polygynous, yet produce worker casts that freely work together leading West-Eberhard (1978) to suggest that monogyny may not be completely necessary for social evolution in the wasps.

Polygyny can happen in one of two ways: queen adoption by existing colonies, and pleometrosis leading to polygyny.

Queen adoption and supercolonies

Queen adoption can occur in small colonies, where small mother colonies absorbs returning inseminated queens (Hölldobler & E. O. Wilson, 1990), and in giant supercolonies, where unrelated queens are adopted into one of the colony's several nests. Supercolonies are formed by "unicolonial" species – species that form a single colony composed of many separate nests, each nest containing at least one (but often more than one) genetically unrelated queen. Workers of different nests freely cooperate with each other to monopolize the resources of whatever habitat they live in. The traditional theory of how unicolonial species are formed holds that low aggression between colonies causes colony boundaries to dissipate, yielding a supercolony. The high occurrence of polygyny in supercolonial nests lead Hölldobler & E. O. Wilson (1990) to suggest that the formation of a supercolony might be attributed to the homogeneity of worker genotypes created when queens become polygynous (as nests become genetically diverse due to polygyny, workers are less able to distinguish between home and foreign nests). Although supercolonies do have incredibly large amounts of genetic diversity within them (Fjerdingstad & Crozier, 2006), genetic diversity does not necessarily lead to the formation of supercolonies. The argentine ant, *Linepithema humile*, is highly polygynous, and both introduced and native species form supercolonies. Populations of the introduced species tend to form single, gigantic supercolonies while native populations form many smaller supercolonies (Tsutsui & Case, 2001; Vogel et al., 2009). However, in the case of *Acromyrmex versicolor*, and *Pogonomyrmex californicus*, a single colony contains one nest with multiple queens, never grows to the size of a supercolony, and still fiercely maintains its colony borders (Cahan & Julian, 1999; Johnson, 2004).

One study of *Linepithema humile* supercolonies found that many of the nest queens are killed off shortly before the annual period of sexual (queen and male) production. This means that while all queens may contribute to the workforce, not all queens are able to reproduce. While first observers suggested that this might be explained by kin selection (workers were killing unrelated queens to maximize their own fitness: Keller et al., 1988) this explanation proved false when tested. Workers did not specifically target unrelated queens for elimination, showing that regicide was not an attempt by workers to promote their own genetic fitness but probably had other fitness benefits for the entire colony. This is supported by the observation that workers in a nest were similarly likely to be related to any queen in the colony (Reuter et al., 2001).

Pleometrosis and polygyny

Pleometrosis is a method of colony foundation, prevalent in both ants and wasps, where newly-mated, relatively unrelated and workerless queens found a colony together (the opposite – a single queen founding a colony herself – is termed haplometrosis). The colonies produced by these queens, if all founding queens remain and contribute to the worker population, will be highly genetically diverse yet still fight neighboring colonies for territory.

There has been considerable speculation in the past as to whether pleometrotic associations did not eventually end up becoming functionally monogynous, either through the elimination of all but one queen, or suppression of reproduction in all but one queen. Observations of workers killing extra queens after fierce territory wars in the fire ant (*Solenopsis invicta*) was originally cited as a hypothetical model for all pleometrotic associations (Hölldobler & E. O. Wilson, 1990). Recently, pleometrosis has been studied more carefully and extensively, and researchers are becoming receptive to the idea that this kind of association can smoothly lead to polygyny.

Pleometrosis in itself is an interesting phenomenon that has evolved a number of times in different genera. Pleometrotic colony foundresses are all usually fertile and unrelated to one another (Cahan & Julian, 1999 and refs therein). Unlike unrelated workers who are born into the same nest and tricked into cooperation, queens cooperate because more heads (or gasters) can be better than one. Cooperation during colony founding between unrelated queens can produces more initial workers per colony (Cahan & Julian, 1999) and greatly lessens the probability that a queen will perish or lose her brood during the risky foundation phase (Pfennig, 1995; Cahan & Julian, 1999).

In ants, two different nesting strategies are observed in both single and cooperating colony foundresses, and both strategies can be enhanced by pleometrosis. Since ant queens typically only use their wings for a single mating flight, wing muscles are metabolized post-copulation to provide nutrition for their first set of brood. Claustral founding depends on a queen being provisioned with enough stored nutrition in her wing muscles and body reserves that she may seal herself off from predators and use only her reserves (bodily stores are turned into food through the laying of trophic - non-viable - eggs). Semi-claustral founding, on the other hand is a condition where inadequate nutritional reserves make it necessary for the queen to forage as well as metabolize wing muscles. During claustral founding, multiple queens working together means multiple food stores, which translated to the production of more initial workers. During semi-claustral founding, not only do multiple cooperating queens bring multiple food stores, but some queens can guard the nest while other queens forage. This brings a real advantage to semi-claustral queens, as it increases brood number, decreases time spent excavating, and enhances brood safety.

Pleometrosis has been found to proceed into polygyny in several genera. The seed harvester *Pogonomyrmex californicus* and the leafcutter *Acromyrmex versicolor* are two ants that seem to have developed very similar founding techniques despite distant relatedness. Both have been found to practice pleometrosis with unrelated queens in the field (all *A. versicolor* are typically pleometrotic, though only one known population of *P. californicus* is pleometrotic). Both are also effectively polygynous and polyandrous, and both are semi-claustral. In laboratory tests, grouped *A. versicolor* queens were more likely to grow successful fungus gardens and produce workers than solitary queens (only 3 out of 19 solitary colonies survived and produce brood, while 14 out of 19 grouped colonies survived and produced brood: Cahan & Julian, 1999). Similar patterns were observed for a population of typically pleometrotic *P. californicus* – solitary queens from the pleometrtic population were much less likely to survive and produce brood than grouped queens from the pleometrotic population, although interestingly haplometrotic queens from a nearby population were as good as grouped queens from the pleometrotic population in surviving and producing brood (Bespalova, 2011). It is also notable that pleometrosis in both species is characterized by a division of labor between co-founding queens: some specialize in nest protection, while others specialize in foraging or nest digging. Whether each queen persistently specializes in a task, or they trade off, varies (*A. versicolor*: Cahan & Julian, 1999; *P. californicus*: Cahan & Fewell, 2004).

Polygyny arising from pleometrosis has been shown to increase colony growth rate in many species (*Pogonomyrmex californicus*: Johnson, 2004; *Atta texana:* Mintzer, 1987; *Linepithema humile*: Ingram, 2002; *Formica selysi* had higher initial growth rate though later came to be of equal size: Reber et al. 2010; *Formica truncorum* had larger colonies but fewer reproductives per colony: Sundstrom, 1995; although no correlation in *Acromyrmex versicolor*: Cahan & Julian, 1999). Faster growth rate can greatly increase colony survival and shorten the amount of time before the first reproductives (queens and males) are produced (Cole & Wiernasz, 1999).

Pleometrosis takes on a similar pattern in wasps. Multiple queens allow for division of labor between those who guard the open nest and those who scout for food. Wasps that are haplometrotic typically produce several daughters in quick succession to reduce predation on brood, rather than several at the same time as seen in pleometrotic wasp queens of the same species (Clouse, 2001).

Questions about equal genetic representation

Currently, much more research has been done on effective polyandry than on effective polygyny (Oldroyd and Fewell, 2007). Studies of polygyny report a range of results - from equal maternal contribution to workers and reproductives (Heinz et al., 2001), to reproductive skew (Fournier et al., 2004), to functional monogyny (Hölldobler & E. O. Wilson, 1990). Because of kin selection, a trend toward functional monogyny is always suggested. It is also difficult to correlate genetic diversity in the workers to genetic representation in the reproductives in studies of polygyny. A queen can be easily eliminated before reproductives are produced, as seen in *Linepithema humile* (Reuter et al. 2001). Pleometrotic queens, even when contributing equally, are likely to contribute fewer offspring than haplometrotic queens of the same species (Hölldobler & E. O. Wilson, 1990). The understanding is that pleometrotic queens can at best lay just as many eggs in a group as they would if alone (eggs of both workers and reproductives). As of yet there is no evidence of more workers per queen being produced in pleometrotic association versus haplometrotic associations of the same species, so a similar trend might hold for reproductives. Some reports are showing that multiple matrilines can be represented at least in the worker population (*Acromyrmex versicolor*: Julian & Fewell, 2004; *Pachycondyla inversa* and *P. villosa:* Kellner et al., 2007).

BENEFITS OF GENETIC VARIATION BETWEEN WORKERS

Current studies are revealing that researchers should be cautious about defaulting to kin selection to explain their observations. There is support for kin selection in many studies of social insect colonies, but there also many instances where the expectations of what should be going on given kin selection are often unsupported when observational data is collected – expectations such as that queens and workers should be maintaining genetic relatedness in the workforce, or that workers should get rid of unrelated queens. This suggests that although kin selection may play a part in more primitive ant and bee societies, at least in the more derived hymenopteran societies, genetic variation in the worker population within a nest is not as severely selected against as kin selection would suggest (Hölldobler & E. O. Wilson, 1990). In fact, recent research is putting aside kin selection to exploring the ways in which genetic variation in the workforce might actually be selected for.

Growth rate

There are several reports of increases in growth rate in colonies where genetic diversity is due to polyandry rather than polygyny (that is, growth rate is correlated with genetic diversity rather than simply more queens producing more offspring: Cole, 1983). In *Pogonomyrmex occidentalis,* a monogynous species in which queens mate with varying numbers of males, studies over several years in the field concluded that colonies with more genetically diverse workers (due to the female mating with more males) had faster growth rates than colonies with more related workers (Cole & Wiernasz, 1999). Faster growing colonies are more likely to survive, and produce reproductives sooner. Therefore, greater growth rate is a benefit, though what it is about genetic variation that causes an increased growth rate could not be pinpointed in these studies.

Pathogen resistance

Artificially-induced multiple mating has been shown to increase pathogen resistance in bumble bees (Baer & Schmid-Hempel, 1999) although in nature, most bumble bees are actually or effectively monoandrous in part due to proteins found in their mating plugs (Duvoisin et al.,1999; Strassmann, 2001). Patrilines within honey bee colonies vary in their resistance to the American foulbrood pathogen (Palmer & Oldroyd, 2003), and an experimental study of the ant *Acromyrmex echinatior* found that different patrilines within a colony were variously resistant to a general pathogen at low doses and showed some highly resistant patrilines at high doses (Hughes & Boomsma, 2004).

Diploid males

Sex determination in Hymenoptera depends on a few sex-determining alleles. A diploid male with streak gonads will develop from a fertilized egg if a female mates with a male that is homozygous for the sex-determining allele. It is therefore theorized that polyandry may have arisen to prevent the energetically taxing occurrence of diploid males, since they take up valuable resources as they mature because they often go undetected until in they are in the larval stage (Cole, 1983). However, some experiments suggest that this benefit is perhaps just a bonus to other benefits (Cole & Wiernasz, 1999). There have also been some interesting though very rare occurrences of reproductive diploid males that sire sterile triploid females (Cournault & Aron, 2009).

Shape of workers

Diversification of worker size and shape is generally thought to be a benefit to a colony, because it can lead to a better division of labor (and therefore more efficient performance of tasks). That is why workers within a colony can vary in shape, size, and form. A colony with polymorphic workers can have a simple gradient of small to large workers, who can generally perform all tasks but perform some tasks better due to their size. A diversity of sizes can be very effective in some cases. For instance, colonies of fire ants (*Solenopsis invicta*) have workers that cover a wide range of sizes, and tests preformed to see whether major or minor workers dug more efficiently showed that a mix of majors and minors actually dug best – smaller workers were better at navigating tunnels, while larger workers could move heavier loads (Gravish et al., 2011).

Polymorphic workers can also have unique specialized morphological structures that are used for a particular task. Examples of this would be the soldier caste of the turtle ant Cephalotes, which has a giant flat, disk-shaped head used to seal off entrances to the nest, or the soldier caste of the army ant *Eciton*, which has incredibly large jaws used for capturing prey. In a study comparing different taxa, polymorphism in workers has been found to be most diverse in species whose workers are more genetically diverse (Fjerdingstad & Crozier, 2006). But, questions of whether genetic diversity can reliably translate into morphological diversity within a species are unresolved. In the ant *Cataglyphis cursor*, workers do not show a correlation between genetic diversity and diversity in size (Fournier et al., 2008). But, for the ant *Pogonomyrmex badius*, workers containing the same paternal chromosomes had a propensity to turn into a particular morphological caste (a major worker or a minor worker: Rheindt et al., 2005).

Genetic diversity leads to more efficient task performance

The theory of Genetic Task Specialization postulates that genetic diversity may create a more resilient system of task performance (Oldroyd

& Fewell, 2007). Mounting research is bringing support to the idea that slight differences between the behaviors of individuals due to minute differences in genetic set-up may be a large factor governing the efficiency of task division, and the amazingly plastic responses exhibited by social Hymenoptera.

The ability of social insect colonies composed of thousands of individuals to respond quickly to an ever-changing environment is a feature of their lack of centralized control. A stimulus affects individuals directly; it does not have to travel through a central processing center that analyses the best response. Each worker responds according to her own experience of the stimulus, and the colony's response is the additive response of all individuals. The external stimulus which acts on a worker can be an environmental one. Something like, food reserves in the nest are low and a worker begins to forage, or it gets hot in the hive and a bee begins to fan, or an ant sees a larva out of the nest so she picks it up and carries it home. An external stimulus can also be the actions of other workers. Something like a receiver bee refuses to take nectar from a returning forager (because honey comb is full) and so signals the forager to stop collecting, or one worker antennates another or performs a waggle-dance and signals the other to follow her to food. But, with every external signal, a worker must internally come to respond (Beshers & Fewell, 2001). That means that before performing a task, a worker must "decide" (or more accurately, “be or not be triggered to”) to perform it. There are generally two things considered to be the cause of variation between individual internal “decisions.” One is age-related fluctuations in hormones, another is the genetic makeup of the individual (Beshers & Fewell, 2001).

Differences in task performance may be attributable to response thresholds

How do internal differences get translated into differences in response? One model, the response thresholds model proposed by Robinson and Page (1989), postulates that internal differences (genetic or hormonal) translate into response thresholds that effect how long it takes an individual to respond to a stimulus. The most intuitive way to explain this idea is with Fewell’s dishwashing metaphor (Oldroyd & Fewell, 2007). Say two people live in one house with one sink. One of these people, due to some genetic, environmental, or hormonal predisposition for type-A behavior, has an extremely low threshold for washing dishes. The stimulus (dishes in the sink) can be very low, yet this person will be bothered enough to wash them. The other individual whose threshold is slightly higher, never has to wash the dishes because they are never in the sink long enough to cross her dishwashing threshold. She is free to do other tasks, hopefully something like the laundry, while the other is washing the dishes.

Translated into the habits of colony workers, this means that workers with the lowest threshold for a task (such as putting debris into piles) will perform that task before others can get to it, removing the stimulus. An individual that performs a task has an increased chance of performing that task again (Beshers & Fewell, 2001), thereby continually removing the stimulus. This allows other workers with low thresholds for other tasks to focus on performing whatever task it is they are most inclined to do, and usually prevents the stimulus from piling up enough to cause the others to switch tasks.

The response threshold model requires that there be some difference between individuals in their response to a stimulus, or at least some variation in the amount of stimulus felt by individuals. For a monogynous colony, headed by a monoandrous queen, this difference in individuals could have two causes. The first could be differences in hormone levels due to differences in age. There are studies suggesting that there may be changes in response thresholds as individuals age due to differing levels of Juvenile Hormone (Beshers & Fewell, 2001 and refs therein; differing levels of JH also correlate with division of tasks during colony foundation in pleometrotic *Pogonomyrmex californius* queens: Dolezal et al., 2009). The second cause, suggested by Oldroyd & Fewell, (2007), could be genetic variation due to the extremely high rate of recombination seen in social hymenoperans. A recent comprehensive study of the frequency of genetic recombination across all animals (from invertebrates to vertebrates) produced the surprising result that social hymenopterans have the highest rate of genetic recombination of any animal. Social hymenopterans had a rate of recombination three times higher than parasitic hymenopterans, six times higher than other insects, and about 12 times higher than vertebrates (Wilfert et al., 2007). Honey bees happen to have the highest rate of recombination within the social Hymenoptera, and *Pogonomyrmex rugosus* has the second highest, which is interesting as their genera also hold the record for the highest and second highest (respectively) effective number of matings represented in their workforce (Strassmann, 2001). Because of the possibility for a worker's matrilineal genes to be a random mix of the mother's two sets of chromosomes, there is still a possibility for some genetic variation between workers, even when overall relatedness is high.

This model assumes that the default state of an individual is "resting" and it will only do a task if otherwise triggered. This is used as an explanation for "lazy ants." In a laboratory setting where there are no problems getting food, and no need to travel long distances or fight enemies, colonies may have "lazy ants" – ants not doing anything, perhaps because they have no stimuli to get them going. If you take these lazy ants and put them together, they start performing tasks again. However, the possibility of resting itself being a beneficial task should not be dismissed (See ant sleep: Cassill et al. 2009).

Also, some organization can arise simply from the fluctuation of signals in the environment. For instance, the total amount of signal for a task can be affected if other workers are doing the task. If an undertaker ant finds a dead worker, it will put it into the colony's "graveyard", thereby removing the signal. In the same way, the first worker born to a queen would find an overwhelmingly high stimulus for foraging, since there is no food in the nests and the larvae are undoubtedly hungry. If that worker continues to perform the task, the next worker born will experience less stimulus, and thus eventually a worker will be born for whom the foraging stimulus is so weak, that she will stay in the nest and care for brood. Therefore, even without taking genetic differences into account, one can get some division of labor.

Something else to note, which is not mentioned but perhaps implied, is that there can be actions that are set to occur when signals dip below a threshold. A classic is the queen pheromone response: as long as the queen is in the hive, most workers will not produce eggs (in accordance with the idea of varying thresholds, sometimes a worker will still produce eggs, despite the queen's presence). Also, whether a worker is already doing a task or not has an influence on whether it will respond to a signal (Beshers & Fewell, 2001). A waggle dance signal is more likely to attract a forager bee that is looking for work rather than bee that has already made a few trips to a nice patch of flowers.

This model can account for the dynamic response exhibited by a colony in dealing with a varying environment. When a colony is at equilibrium (the temperature is nice, food is abundant, the brood have sufficient workers tending them), tasks will be performed by those who have the lowest thresholds for performing that task. However, if a major change occurs (an especially nutritious patch of food is found, temperatures get dangerously high, pollen stores are depleted by external factors), the added stimulus will cause workers with higher thresholds to gradually be recruited and provide extra help as needed, as every individual in a colony is generally able to do every task.

Signals that cannot be dealt with instantaneously, like a waggle dance, a temperature response, or a perception of low food reserves, cause a time lapse between the perception of a task and removal of the signal. This means that if the task is noticed by several individuals with the same response threshold, before those individuals can diminish the stimulus, others may also start responding. Too large of a group with the same threshold may overshoot the ideal response because of their added contributions. This is why Oldroyd and Fewell (2007) propose that in a colony containing mostly genetically similar individuals, responses to a crisis may oscillate before equilibrium is reached again. A good example given is that of a hive full of bees who have the same threshold for cooling the hive. If the temperature goes above a certain degree, all workers will be triggered to fan the hive at the same time, causing a rapid fall in temperature. This could potentially have adverse effects on their brood, as temperatures must remain between 32 and 36 °C (with 35°C being optimal) for normal development (Seeley, 1995).

This is where the high genetic variation brought in by polyandry and polygyny can be a benefit. More variation in the workforce means a more gradual response to a stimulus. Taking the fanning bee example again, if the temperature goes up just a little, the bees with the lowest response thresholds will start fanning far before anyone else notices, and therefore deal with the heat without recruiting excess workers to the task. Moreover, in a colony with low genetic diversity, it may so happen that all workers will have the same low threshold for a task, and consistently be sidetracked from doing other tasks. There may be a lot of bees always foraging, but few bees building comb for the nectar brought back. This kind of colony would be considered a "poor" colony in the wild. However, incorporated into a colony with other genetic lines, this group of individuals would be considered "specialized". In a genetically diverse colony, having a group with an especially low threshold for foraging will actually be a benefit, because there will be a constant group of workers who prefer to perform that task, leaving others to focus on their preferred tasks, and allowing for more tasks to have an adequate number of workers devoted to them.

Empirical evidence for the benefits of genetic diversity which can be explained by the response thresholds model

Jones et al. (2004) found that honey bee colonies produced by multiply inseminated queens were able to regulate brood nest temperatures at both high and low external temperatures better than colonies produced by singly inseminated queens, as would be expected with the variable response thresholds model. Fewell & Bertram (1999) found that when pollen stores were gradually removed from a honey bee colony, pollen foragers increased in a step-wise manner, and the increase in foragers was accompanied by an increase in genetic diversity. When pollen stores were high (so signal for pollen foraging was low), one genetic lineage dominated pollen collection. Because growths in foraging stimulus brought in more recruits with different genetic backgrounds, it was hypothesized that as foraging stimulus increased, worker patrilines with higher genetically set thresholds were recruited.

Cahan & Fewell (2004) placed newly mated queens from solitary and pleometrotic populations of the ant *Pogonomyrmex californicus* into groups. Though haplometrotic queens typically fought each other, in situations where they did not fight but cooperated on nest building, division of labor was observed. In both experimental groups, one queen typically performed a disproportionate amount of the digging, while the other one tended to the brood. Support for a genetic threshold model in this study comes not only from the observation of a division of labor, but also because the queen who became the dominant digger often displayed a propensity for digging before she was paired.

Wiernasz et al. (2004) Performed several field studies of colonies of *Pogonomyrmex occidentalis*. The queens of this species tend to naturally mate with a variable number of males. This study reported that colonies with more genetic diversity had a faster growth rate, and later studies (Wiernasz et al., 2008) found that more genetically diverse colonies began foraging earlier and foraged for longer than those with low genetic diversity. Moreover, the ants that foraged earlier were genetically similar compared to the rest of the colony. Later they showed that earlier activity meant more foraging trips and more food retrieved for the colony (Cole et al., 2008). Studies over seasons and a wide variety of thermal ranges still showed that more genetically diverse colonies foraged for longer lengths of time than colonies with more genetically related workers, suggesting that benefits of genetic diversity were not specific to a time period, nor compensated for later with decreases in foraging (Cole et al., 2010). The researchers proposed that the benefits were due to a larger diversity of temperature tolerances and behavioral types.

Shared thresholds in genetic lines may lead to specialization by genetic lines

Passive division of labor and further selection on thresholds could lead to genetic lines that specialize in one task and neglect to perform other tasks - I discussed this briefly earlier, when I mentioned genetic lines could make for “poor” colonies in the wild, but be considered “specialized” genetic lines in a genetically diverse colony. There is empirical evidence of such distinctions occurring. Note that the term “specialization” used henceforth only refers to the tendency of a worker or patriline to perform certain tasks more often than others, and nothing about how well they perform the task (though specialization can effect performance, as will be discussed later).

First of all, there is proof that you can select for specialization (specialization is not just a passive outcome of division of labor). Honey bee lines with foragers that prefer pollen foraging over nectar foraging and vice versa have been artificially selected for (Hellmich et al., 1985). In the high pollen foraging line, workers can become specialized to the point where numbers of brood are negatively affected due to decreases in nectar foraging (Page & Fondrk, 1995). Gene loci that differ between bees that specialize in pollen collection and those that specialize in nectar collection, and which affect the amount of pollen collected, have been identified (Hunt et al., 1995).

Robinson & Page (1995) found that a genetically distinct group of "undertaker bees" - worker bees who removed the dead from the nest - responded to both low and high numbers of dead bees in the hive. When this distinct group of undertakers was removed, bee bodies piled up for several days before removal began again. This points to specializations in certain genetic lines that make them more apt to become undertakers, and a loss or reduction of the ability to perform undertaking behavior in other lines. Similarly, in the ant *Acromyrmex versicolor,* one small subgroup of ants specialized in removing dead, and were better at it than nonspecialists, though genetic factors were not examined (Cahan & Julian, 1999).

Julian & Fewell (2004) further found that in *A. versicolor,* a species that seems to be obligatorily polygynous, workers of certain matrilines were more inclined to specialize in tending fungus while others were more inclined to specialize in foraging. Different matrilines also varied in the age at which workers transitioned from taking care of the nest (typically the job of younger workers) to foraging or fungus-tending.

Mattila & Seeley (2010) inseminated queen bees with either the sperm of a single male or sperm that had been combined from multiple males. They found that colonies with more genetically diverse workers showed far more activity related to foraging than colonies from singly-inseminated queens. The genetically diverse workers foraged more often, and preformed more waggle dances. Single patriline colonies had a significantly higher percentage of foragers that never danced. Genotyping showed that there was an over representation of three distinct patrilines in the foragers and dancers of all colonies. In a subsequent study (Mattila & Seeley, 2011), single and multi-patrilineal colonies were tested for rate of discovery of new food sources through the activity of specialized foraging scout bees. It was found that scouts from colonies of multiply-mated queens performed longer, more information-rich waggle dances, and returned to colonies at significantly faster rates, though rates were not different between the groups if all foragers (both scouts and regular foragers) were taken into consideration. Genotyping identified specific patrilines that were over-represented in the bees that became scouts, and also patrilines that were disproportionately underrepresented (termed "scout rich" and "scout poor" lines).

Page et al. (1995) inseminated four groups of queens with a single male's sperm (males from colony A, B, C, or D) and a fifth group of queens with the combined sperm from males of all four colonies (a mix of sperm A, B, C and D) to create four groups representing different single patrilines, and a fifth representing colonies composed of all patrilines. They looked at 19 colony traits (including the sex of the brood, comb production, foraging and defense) and found significant differences between the singly-mated groups of queens in eight of those traits (that is, there was variability in eight traits across patrilines). There was only a significant difference in two of the 19 traits when traits were average across singly-mated queen's colonies and compared to the multiply-mated queen’s colonies. What this means is that the patrilines showed a lot of variation in performance across different traits, but when multiple patrilines were represented in a single colony, the variation in traits exhibited by the colony decreased. What should be remembered is that task performance was measured on a colony-wide level, so the performance was the additive contribution of all individuals. If a particular patriline made a large amount of comb in a single patriline colony, it was because there were a great number of individuals with the same comb-building ability, and the effect of the same patriline would be less in a mixed patriline colony because there are fewer representatives from the high-comb colony. This paper also notes that care should be taken when talking about whether one patriline is “better” at a task than another. For instance, high comb production is not necessarily better, especially if it takes away from other activities such as tending to the nest. Colony fitness, measured in numbers of successful reproductive queens and males produced, is the only true assessment of a better colony.

Thresholds don't answer: who does it better?

The empirical studies available show that threshold level may be just one way in which an individual can differ from other individuals. Performing a task first or most often does not mean that a worker is the best at it. Learning can be a part of specialization - bumblebee workers that are new to foraging do worse than more experienced workers (Cartar, 1992) - but there can also be differences in the speed of learning - bumble bees can vary between colonies in how fast they learn a foraging task (Raine and Chittka, 2008).

Dornhaus (2008) looked at individual task specialization (whether a worker performed one task more often than other tasks), compared to the efficiency with which the individual carried out the task (measured as work completed in a set time period). The species used was *Temnothorax albipennis*, a monogynous, monoandrous ant whose workers do not show any specialization associated with size. Four tasks were observed - honey and protein foraging, brood transport, and sand transport. First of all, the study found clear behavioral differences between the highly related individuals of the colony, supporting the idea that there can be differences in behavior even when overall genetic diversity is low. Secondly, she found that the efficiency of task performance was not based on who did a task the most, who came to respond to a stimulus first, or how hard they worked on other tasks. In fact, most of the work was performed by specialized but not necessarily the most efficient workers, and for sand grain collection specialized workers actually performed worse. Furthermore, she found that if workers were efficient at one task, they were not necessarily efficient at other tasks.

In the bee foraging study mentioned previously, Mattila & Seeley (2010) found that patrilines which specialized in foraging neither visited the feeder more often than other patrilines, nor had specifically low or high thresholds for waggle dancing (thresholds were determined as the concentration of sucrose needed to elicit a waggle dance). Also, the two studies differed in that the multiple food source study showed no significant correlation between *number* of dances preformed, just length of the dances (Mattila & Seeley (2010, 2011). Therefore it seems some behaviors, like the stimulus to waggle dance, are quite nuanced and may follow rules contrary to those proposed by the threshold model.

No proof of thresholds

The response threshold model can be invoked to explain a lot of the organization seen in social hymenopteran colonies, but independent proof of varying response thresholds among individuals has not yet been provided. In fact, as previously mentioned, Mattila & Seeley (2010) found no genetic distinction between waggle dance thresholds. The presence of response thresholds have been inferred from looking at how tasks are partitioned among genetic lines in within normally functioning colonies, but there are very few studies looking for thresholds across different tasks in individuals (Dornhaus, 2008). Phenomena attributed to differences in response thresholds can also be explained with more general "differences in ability." As one example, the variability in task performance seen in Page et al. (1995) bee study could be due to different patrilines having lower thresholds for certain tasks and therefore having more bees perform one task and consequently having fewer bees available for other tasks. It seems equally plausible that workers from one patriline could have been gifted with especially efficient individuals for a certain task, or ones that had special ability in regard to a particular task (for instance, they could produce more wax from their wax glands). Similarly, the aforementioned high pollen-foraging bee strains selected for by Hellmich et al. (1985) had two to 13 times more pollen in their hives than low-pollen foraging strains – a seemingly highly variable outcome. While the two strains differ the most in the propensity of a worker to forage for pollen rather than nectar (Page and Fondrk, 1995) the amount of variability even in the high-pollen strain colonies might not be due to numbers alone, but possibly to better pollen-gathering technique, higher efficiency, or better recruitment of other foragers to pollen foraging.

Genetic variation in task thresholds may adequately explain division of labor (who specializes in what and how often workers switch), and differences in threshold may account for differences in specialization seen in colonies comprised of multiple genetic lines, but diversity in thresholds does not seem to be the only kind of diverse behavior exhibited by individuals in a colony.

Specialist workers versus generalist workers?

One question that seems to be missing in discussions of thresholds is how disparities between threshold levels in themselves could be different among individuals. Different genotypes could create, and selection could conceivably act on thresholds that make both specialists and generalists. Having both specialists and generalists may be important to a colony (Dornhaus, 2008).

Specialists may be those individuals who can remain focused on one task due to low thresholds for a particular tasks but high thresholds for other tasks - ones that continue taking care of brood while others are called to deal with a crisis. Specialists may be selected for if there is some benefit to always having someone working on a particular task, no matter what the stimulus level (tasks like brood tending, or scouting, or foraging come to mind). But a colony of individuals who are too involved in their task and unable to switch easily when a crisis emerges, may be detrimental - as was highlighted in the study on undertaker bees (Robinson & Page, 1995).

Generalists may be those that are equally likely to help in any task, and may benefit the colony as "fillers" during minor fluctuations in food intake, or brood care. Dornhaus (2008) notes that specialists and generalists are a real phenomenon - her experiment showed that not all ants specialized in one task. Furthermore, Julian & Fewell's (2004) study of *Acrimyrmex versicolor* also noted that some individuals specialized in fungus tending and never left to forage, while those who foraged were likely to come back to tend the fungus garden when needed.

It might also be interesting to look into how division of labor via thresholds works when the colony is not stressed (food intake is continuous, temperature is optimal) compared with times of crisis (when food stores are lowered, or temperature becomes unfavorable). Are specialists more favorable in one case over another, and how does the efficiency of workers performing the task change in the two scenarios?

The tradeoffs in benefits and expenses for one task over another may be infinitely complex. It is likely that no "best" scenario for most efficient task performance could be decided upon, unless variables such as the environment, colony size, and the relationships, strengths and weaknesses of all colony members are considered. That, in the end, may be why a mix of skills created through increased genetic diversity in the workforce may be more favorable than even a combination of "the best" skills. As nature constantly changes which response is the “best” fit for an environment, a much broader array of random scenarios could be dealt with. Remember again the study of digging efficiency in *Solenopsis invicta*. Researchers wanted to test whether the ability of larger ants to move larger sand grains, or smaller ants to navigate tunnels, was a better attribute for efficient nest digging. Results, however, showed that a mix of large and small workers actually dug the best (Gravish et al., 2011). The one thing that empirical evidence seems to be clearly supporting is that genetic diversity improves overall efficiency and stability in social hymenopteran colonies.

PROPOSAL OF A REVISED MODEL

Empirical data show that genetic diversity increases colony fitness. But the reasons for this effect are poorly understood and likely to be quite complex. The genetic response threshold model proposes a way in which a more fine tuned division of labor and a colony response that preserves homeostasis may arise in a genetically diverse colony. But as discussed in the previous section, it does not address other differences in behavior – ones that can be attributed to differences in genotype but not necessarily to thresholds.

A more general model with a slightly updated metaphor may be needed to encompass all of the benefits attributed to increased genetic variation. Instead of a genetic response threshold model, it might be more aptly called a complementary genetic attribute model. Here I will try to explain how such a model would work, and how it could increase the understating of the variety of benefits available to a genetically diverse social insect colony.

An intuitive way to explain this model is perhaps with another metaphor – the relationship between two parents (let us assume these parents have adopted a child, to eliminate any vested interest in passing on their genes). Research has shown that it is usually easier for two parents rather than one to meet the needs of their family without outside help (Biblarz & Stacey, 2010). This is because two parents can more easily take care of the house and children, work, and finish errands. Sometimes one parent can perform a special task which the other parent cannot, and which the rest of the family benefits from. Other tasks, both parents are equally good at, or one parent is better at than the other. Sometimes both share in tasks that both can do, other times one specializes in the task because the other is busy with something else, other times one parent specializes just because they are more bothered by the task being incomplete, but not necessarily because they are better at it (an example would be, parent A might be a math wizard and finish the taxes extremely fast, but parent B is the one who does them because they are more bothered by having them unfinished). Finally, deficiencies in one parent may be made up for by attributes of the other parent.

This scenario labels the workers produced with a particular matriline or patriline’s genes the “parents.” I may be in danger of anthropomorphizing with this metaphor, but no benefits have been presented within it that have not been demonstrated in empirical studies. There are sometimes workers with special beneficial physical attributes that other workers do not have (such as specialized mandibles or heads). Most workers can perform most tasks, at varying degrees of efficiency. Some workers can perform certain tasks more efficiently than others, but this does not necessarily mean they are the ones specializing in the task. Some workers are similarly good at tasks, and may share in the tasks, or one may specialize because the other is busy doing a different task, possibly because they have a lower task threshold for it. This metaphor also takes into account that an individual (or perhaps a patriline or a matriline) may specialize in a task simply because the other individual (or genetic line) is typically busy with another task (this is not a new proposal: see Beshers & Fewell, 2001).

Explanation for specialization

This way of modeling benefits may also help elucidate how specialization of genetic lines benefits genetically diverse colonies; specialization has a twofold benefit. Not only does it allow for individuals that are always performing certain important tasks, but it also could allow for a rapid increase of genetic variation within the population by lessening a genetic bottleneck.

Specialization and complementary genetic attributes may increase ingenuity

As described before, where genetic lines with a diversity of task performance ability could be genetically "poor" or unfit when alone, once integrated into a colony with multiple genetic lines, they become selected for, as they can became genetically "specialized" lines. Because qualities that would have lead to a line's elimination are no longer detrimental, since other lines can compensate for weaknesses, the genetic bottleneck is lessened. Therefore, populations with queens that practice polygyny or polyandry would not only show a variety of thresholds because specialization is selected for - they may just show a variety of genes all together, and perhaps have more individuals doing unusual and different things. Different thresholds for performing tasks can lead to a more resilient response, but different ways of performing different tasks can lead to increased ingenuity in task performance.

Hypotheses

In effect, I have proposed two hypotheses I would like to test. The first is that differences in ability, not just thresholds, could be a benefit of genetic diversity within a colony. My second hypothesis is that cooperation can compensate for weaknesses in genetic lines (and in fact, task repertoires that were "weak" when a genetic line had to survive alone turn into "specialized" repertoires when cooperating with other genetic lines) leading to a lessening of a genetic bottleneck, causing a rapid increase in diversity and the preservation of unique abilities. This can in turn generate a more ingenious workforce.

How to test these hypotheses:

To test both hypotheses, first a species of social hymenopteran is needed that can adequately model the effects of genetic diversity in a population, without adding conflating factors. The ideal species would be one that exhibits different propensities for polyandry or polygyny in different, geographically isolated populations (where there is no gene flow between populations). In one population, queens would ideally be monogynous and monoadrous. In the second population, they would ideally be either effectively polygynous or polyandrous.

To test the first hypothesis, a series of experiments need to be devised which can examine individual ability and threshold in a controlled environment - that is, outside of the colony where other individuals have a confounding effect, and in an environment where only the stimulus for which you want to test a response is available. This way, more detailed observations of differences between individuals can be collected. Secondly, it is important to observe how those abilities and thresholds play out in the colony environment, to get a better picture of how actual behavioral difference translate into colony differences and not just how different genotypes happen to be divided in a colony setting. Thirdly, individuals would need to be genotyped to see what, if any, effect association to a particular genetic line had on behavior.

To test the second hypothesis, one would need to demonstrate that there is a higher diversity of alleles in the population with polygynous or polyandrous queens as compared to the population of monogynous and monoandrous queens. Secondly, relative ages of the two populations would need to be established through comparisons of mitochondrial DNA between individuals of a population, in order to correct for differences in allele diversity simply due to one population being older than another. Additionally, observations of abilities and responses to stimuli from tests of the first hypothesis can be useful in noting whether the polygynous and polyandrous populations are showing novel abilities or novel morphological attributes.

What can I test?

The testing of these hypotheses is quite elaborate and necessitates the development of novel testing techniques that measure the behavioral responses of individuals outside of the colony environment. Additionally, a good candidate species needs to be identified, and tests need to be calibrated to the species. In the following sections, I will propose a candidate species as well as outline testing techniques for measuring aggression and temperature tolerance in individuals. These techniques measure the level of response by an individual to a single level of stimulus, and can later be used to test a variety of relationships. Ones such as the relationship between an individual’s level of response to a stimulus and the level of signal needed to illicit the response, as well as how behaviors exhibited by an individual outside of the influence of the colony environment change when tested within the colony environment. I will then present results from preliminary trials, and discuss what new information they add in regard to further development of the procedure.

BACKGROUND FOR METHODS

Profile of Pogonomyrmex californicus

One very promising candidate species is the Californian seed-harvester ant *Pogonomyrmex californicus*. *P. californicus*, like all others in its genus, is typically haplometrotic (i.e. founds colonies alone). Recently, a population of *P. californicus* was identified in Pine Valley, California that is pleometrotic (i.e. founds colonies in groups) and polygynous. Both pleometrotic and haplometrotic *P. californicus* are semi-claustral – as previously defined, this means newly mated queens cannot survive on body reserves and must forage before their first brood emerges. It is suspected that the added benefit provided by the division of labor between queens is the main reason pleometrosis has been selected for in that particular population. But, while newly mated pleometrotic queens are tolerant of other queens and cooperate in colony foundation, established colonies will fight with each other for territory and will not tolerate the addition of new queens. Why there is only one known population of its kind is a mystery, and whether polygyny developed due to environment or due to chance is not yet understood. Polygyny often arises in stressful environments, such as places with little food or little water, but this particular pleometrotic population exists in an area with higher precipitation and more plant biomass than surrounding areas containing haplometrotic populations (Johnson, 2004).

This species is not the most ideal, as all members of the species also have a high effective mating frequency (R. Overson, personal communication, July, 2010). This is a characteristic trait of the *Pogonomyrmex* genus, which counts among its members the species with the highest mating frequencies known in ants (Rheindt et al., 2005); typical effective mating frequencies for this genus are about 6.76 males

(Strassmann, 2001 and refs therein). However, with genotyping, genes connected to behaviors associated only with matrilines can be determined.

Genetic tests show that associating queens are unrelated, and form associations with other queens randomly during the colony founding stage. The pleometrotic population shows diversity in nuclear DNA, but also a higher-than-normal level of similarity in mitochondrial DNA compared to nearby haplometrotic populations. Similarity in mitochondrial DNA hints that the population may be fairly young. There are considerable barriers to gene flow between the pleometrotic and neighboring haplometrotic populations, though some genetic intermixing occasionally occurs (R. Overson, personal communication, November 15, 2010). Also, there is a draft genome available for *Pogonomyrmex barbatus* (Smith et al., 2010) which can be used to find comparable genotypic markers for *P. californicus*.

Though this population may be young, it seems that it has already become obligatorily pleometrotic. Personal observations revealed that pleometrotic queens did very poorly in solitude. Eggs that were laid were not made into clutches but most likely eaten, and queens were so prone to fungal infections that most died at the end of a month. The solitary queen used for experimentation in this thesis was unique in that she started off in a group of three queens (which I had assembled), of which she was the only queen that survived. She was still able to lay eggs after all her compatriots had died. Because she did not start to assemble clutches until well after the other queens were deceased, I am certain that none in her brood were workers from other queens. Single haplometrotic queens, as expected, survived as just as well as grouped pleometrotic queens in this study (Bespalova, 2011).

I did not have the finances to perform genetic testing at Mount Holyoke College. I was lucky enough, though, to have that single queen from the pleometrotic population produce a substantial number of workers. Tests could therefore effectively be performed on this single matriline from the pleometrotic population, and compared to a single queen of the haplometrotic population.

Worker mass

Reports about whether genetics have an influence on size are mixed (Fournier et al., 2008; Rheindt et al., 2005). Because weight is an easy measurement to take, it was added to this study as an additional potential measure of worker variability. The typical trend for *P. californicus* as well as other ant species is that worker size increased with colony size (T. Holbrook, personal communication, March 27, 2011), so care needs to be taken not to compare individuals in colonies of variable size. Bigger colonies will naturally have a larger variety of worker sizes as workers can live for many months.

In species where queens are found in both monogynous and polygynous colonies, workers are often much smaller in the polygynous colonies compared to monogynous ones. This phenomenon is sometimes termed "polygyny syndrome." Theories as to why this occurs include excess queen pheromones in polygynous colonies, and scarcity of resources in colonies with faster egg production due to multiple queens (Schwander et al., 2005 and refs therein). Poor nutrition often has an adverse effect on worker body size (Johnson, 2004). Queens in monogynous colonies are sometimes also heavier and bigger than queens of polygynous colonies (Bourke & Franks, 1995; Goodisman & Ross, 1996).

Temperature tolerance

A study looking at how different variables affected foraging in different species of *Pogonomyrmex* (Morehead & Feener, 1998) found that workers run faster as temperature gets hotter. Typically, the species' studied forage between the range of 20 and 53°C, and one of the species examined in the paper is the closest relative of *P. californicus* (*P. occidentalis*). They also found that differences in body mass did not affect running speed. *P. occidentalis* was also the subject of the tests by Wiernasz and Cole (see section entitled "Empirical evidence for the benefits of genetic diversity…" for explanation of experiments) who determined that genetic diversity greatly added to the fitness of *P. occidentalis* colonies, partly, they speculate, due to a diverse array of temperature tolerances. Because of this, perhaps measuring running speed at a particular temperature would be an informative test of differences in behavioral response - those who do not run faster at this temperature may need a higher stimulus to be affected. However, separate tests of whether fast running speeds at high temperatures – or slow running speeds at high temperatures (and perhaps therefore better tolerance) – are more beneficial to a colony need to be conducted before any value can be attributed to the behavior.

Aggression

Ants will occasionally fight territorial wars in the field, but they will always fight intruders found in their nest. A comparative study by Roulston et al. (2003) of several different methods for aggression assays determined one-on-one ant battles were a reliable way to measure aggression, and Newey et al. (2010) determined that individual workers respond fairly consistently in their aggression response. However, the reaction of an individual who is shown aggression is governed by different mechanisms than the reaction of an individual who wishes to show aggression (Newey et al., 2010). Therefore care needs to be taken to differentiate between aggressive responses and reactions to an aggressive response. Callow workers – newly eclosed workers that are still light-colored in appearance – are typically non-aggressive. Care also needs to be taken in choice of enemy if one wishes to test aggressive response –a docile, or restrained (tethering techniques: Fénéron, 1996) enemy is suggested, as it will allow for the most number of trials. Aggressive unrestrained enemies will kill test subjects. In honey bees, colony defense behavior is heritable (Page et al., 1995), and in polygynous colonies, high tolerance of other queens is also typically thought to be heritable.

MATERIALS AND METHODS

Colony Set-up

Haplometrotic and pleometrotic queens were collected following their annual mating flights on July 1st and 2nd, 2010. Haplometrotic queens were collected at a site near Lake Henshaw, CA and pleometrotic queens were collected in Pine Valley, CA. Colonies were set up in the laboratory with one, two or three randomly associated pleometrotic queens, or single haplometrotic queens. Colonies were maintained in an incubator at 29-32°C, first in 10cm x 1cm diameter test tubes. Tubes were filled halfway with water and plugged with cotton at the water line, and at the mouth of the tube. Water was mostly provided for humidity, but individuals occasionally drank from the cotton. Later, colonies were transferred into 12.7 x 12.7 x 2.5 cm plastic boxes. Small test tubes were filled with water, plugged with cotton and placed inside the boxes to add humidity. Colonies were started at Arizona State University and later shipped to Mount Holyoke College. Ants were fed to excess with a constant diet of

Kentucky bluegrass seeds, fruit flies, and occasional sunflower seeds, oats, rye seeds, and finch mix.

Three vigorous colonies manage to withstand brood loss during shipment, relocation stress, and the unnatural environment provided by laboratory equipment. Experiments were conducted on these three successful colonies: a colony headed by two queens from the pleometrotic population, a colony headed by one queen from the pleometrotic population, and colony headed by a queen from the haplometrotic population. They had 38, 20, and 27 workers respectively at the start of experiments.

Weighing and individualization

Ants were weighed using a Mettler AE 163 analytical balance. Workers were weighed up until the colony contained 38 non-callow individuals, and subsequently produced workers were not included in analysis in order to measure across the same colony size. After weighing, every non-callow worker was individually marked with a pattern of three colored dots. Sharpie paint markers (Sanford L.P) in four colors were used for painting.

Temperature response

Individual workers within a colony were placed in a narrow test tube with a cap and stand for the base made out of Play-doh modeling clay (Hasbro) (Fig.1). Each worker was given her own labeled test tube, to prevent any possible effect caused by foreign pheromones deposited by other individuals. Test tubes were positioned horizontally on a Fisher Slide Warmer (model no. 12-594-5), the clay raising the tube 2 mm off of the heating plate. Temperature was monitored with an Omega HH306A data logger with a TP-K01 bead probe thermal testing tip which was placed inside a similarly prepared test tube positioned in the middle of the heating plate. Test tubes containing workers were placed on the heating plate when the temperature inside the dummy tube was at 32°C, and were recorded with a digital camera from above. Recordings lasted from 35°C until temperature reached between 42-44°C. 8-10 successful trials per colony were completed with at least a 24 hour rest between trials. A centimeter ruler taped to the heating plate and a stop watch recorded on the video were used to determine centimeters run per second between 40-40.9°C using Image J (NIH).

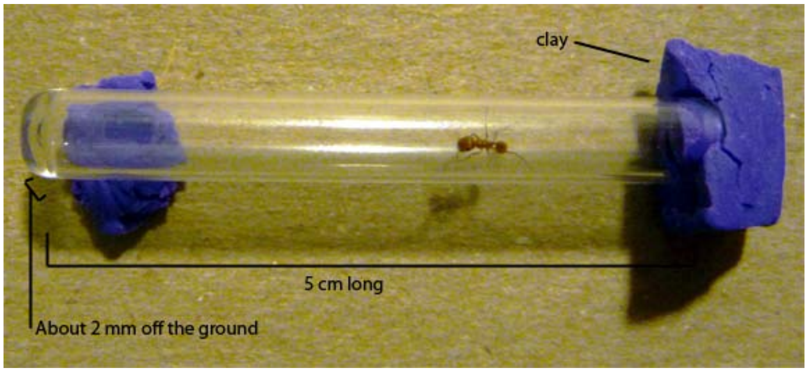

Figure 1: Temperature response testing chamber. *P. californicus* workers from each of three test colonies were sealed in their own 6 x 50 mm tubes to test running speed at high temperatures. For temperature to increase more evenly, the tube was raised about 2 mm off the surface of the heated plate.

Aggression testing

A fighting arena was constructed out of a 20ml beaker with inner sides painted with INSECT-a-SLIP (BioQuip Products, Inc.). INSECT-a-SLIP is a solution containing Ethylene tetrafluoroethylene, a plastic that is slippery when dry and often used to prevent insects from climbing on surfaces. The bottom was lined with filter paper that had been rubbed on the sides of the colony box and kept in the nest area of the colony so as to pick up the colony scent. Seeds from the colony box were also added during the trials in an attempt to boost colony scent in the arena (Fig. 2).

Enemy ants were *Monomorium minimum* found locally (South Hadley, MA). One *M. minimum* workers measured about half the length of one *P. californicus* worker. *M. minimum* were only very rarely aggressive towards *P. californicus* workers, and most often tried to hide under seeds. Two *M. minimum* put in the test colony boxes the night prior to aggression test initiation may have helped induce *P. californicus* to act aggressively towards *M. minimum*, as *P. californicus* workers were comparatively docile when the two were previously placed together (though the filter paper doused in colony scent had not previously been employed either).

Individual workers were allowed to acclimate for 2 min after being placed in the fighting arena. Then, an *M. minimum* individual was dropped from above as far away from the *P. californicus* worker as possible so as not to cause shock. One to three encounters were observed per trial (depending on my confidence in the encounter – fights were easy to score, intermediate aggression harder). The aggression assay was based on scoring intervals from Carlin & Hölldobler (1986) with my own observations replacing behaviors that did not match up with those observed in *P. californicus* (Carlin & Hölldobler's scale is widely used but based on carpenter ants, who spray formic acid as a defense – a strategy that is not employed by harvester ants. Also, mandible-to-mandible grappling was not witnessed in fights between *P. californicus* and *M. minimum*). Aggression ranges used were similar to those presented by

Tsutsui et al. (2000) (ignore behavior was scored lowest, aggression highest, and avoidance in between). This scale is also widely used in aggression assays, but inconvenient for this experiment due to the narrow range of the scale. Table 1 lists the scores and corresponding behaviors used for this analysis. The final aggression assay was established half-way through aggression testing, but notes on previous scores allowed first trials to be re-scored. 8-10 successful trials per colony were completed with at least a 24 hour rest between trials.

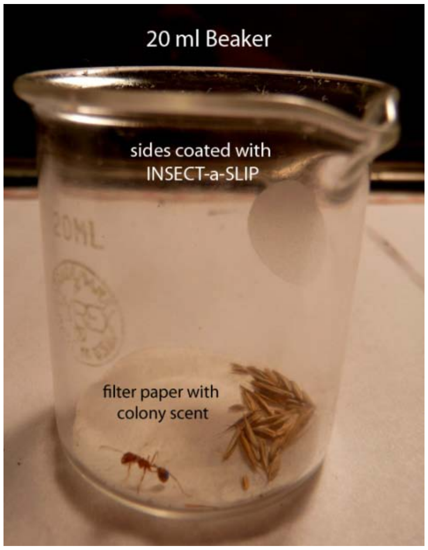

Figure 2: Fighting arena. The arena was lined with filter paper, and seeds from the colony box were added in order to trick *P. californicus* workers into thinking they were defending their colony. Workers were allowed to acclimate to the fighting arena before enemy *M. minimum* were dropped in.

Table 1: Aggression Assay

SCORE	BEHAVIOR
0	**Ignore** Ignore or antennate in friendly way.
1	**Violent avoid** Touch and run away.
1.5	Touch and jerk back with antenna down. Touch, pull antenna back and freeze.
2	**Partial avoid** Touch and change walking direction. Touch and pull antenna back, but keep walking.
2.5	Weak open mandible threat (mandibles only open a tiny bit, or for a brief moment).
3	**Open mandible threat** Open mandibles wide and stand still. Lunge forward with antenna back without baring mandibles.
3.5	Creep up to enemy with mandibles open. Jump with mandibles open and antennae back.
4	**Lunge and mandible threats** Charge then freeze with mandibles open. Lunging once or twice without trying to catch (mandibles open).
4.5	Hover over enemy with jaws open Multiple small and jerky charges with mandibles open.
5	**Multiple lunges and mandible threats** Lunging multiple times without trying to catch (mandibles open). Lunge and lightly bite the enemy but no attempt to grapple.
5.5	Actual unsuccessful bite (lunge past and bite a seed).
6	**Grapple**

This aggression assay was used to test the level of aggressive response induced in individual *P. californicus* workers by “intruding” *M. minimum*.

Statistical analysis

Statistical analysis was carried out with SPSS 18.0.1 for Windows (SPSS Inc., 2009). Histograms were constructed to visualize the distribution. Due to small sample sizes, the Shapiro-Wilk test for normality was used to test the normality of the distributions. Pairwise independent-sample t-tests were use to assess differences between the means of the three variables (Weight, running speed, aggression score) across colony types, Pearson's correlation was used to test for correlations between variables within colony types.

RESULTS

The colony types in this section are identified as follows:

1) HAPLO workers: workers from the colony founded by a single queen from the haplometrotic population.
2) PLEO 1 workers: workers from the colony founded by a single queen from the pleometrotic population.
3) PLEO 2 workers: workers from the colony founded by two cooperating queens from the pleometrotic population.

Because of the large number of statistical tests conducted, more stringent values of p=.01 were used to determine true significance.

Weight

The wet weight (in mg) of 38 workers was used to construct histograms for weight analysis (Fig. 3).

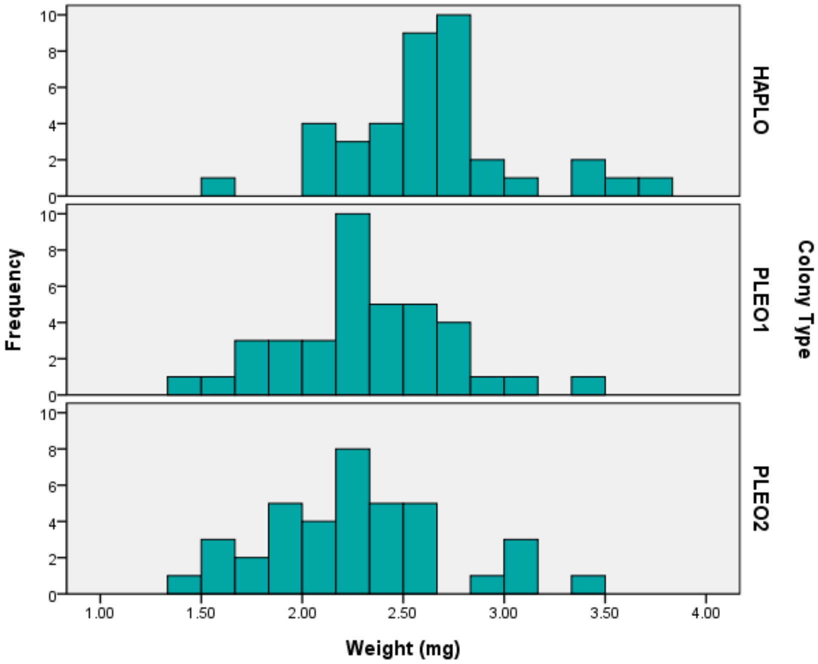

Figure 3: Histograms of weight distribution in three colony types. Individual *P. californicus* workers from three colony types were weighed in milligrams. Weight distribution is close to normal for all colony types, and the mean weight is higher in workers of the haplometrotic queen compared to workers of the pleometrotic queens.

Tests for distribution normality showed all colony worker weights to fall under normal distributions (p>.01 for all tests). Standard deviations of worker weights from the mean were similar (PLEO 2 = 0.46 mg, PLEO 1 = 0.42 mg, HAPLO = 0.44 mg). Mean weight for PLEO 2, PLEO 1 and HAPLO workers was 2.27 mg, 2.32 mg, and 2.62 mg respectively. Independent sample t-tests showed that means were not significantly different between PLEO 1 and 2 workers, but were significantly different between PLEO 2 and HAPLO workers (p=0.001), and PLEO 1 and HAPLO workers (p=0.003). Queen weights were similar in all colony types: PLEO 2: 11.72 mg and 12.64 mg (average = 12.18), PLEO 1: 12.22 mg, HAPLO: 12.48 mg.

Temperature tolerance

The mean running speeds (in cm/s) of workers that completed 7 or more trials were used to construct histograms for temperature tolerance analysis (PLEO 2 n=20, PLEO 1 n=15, HAPLO n=15) (Fig. 4).

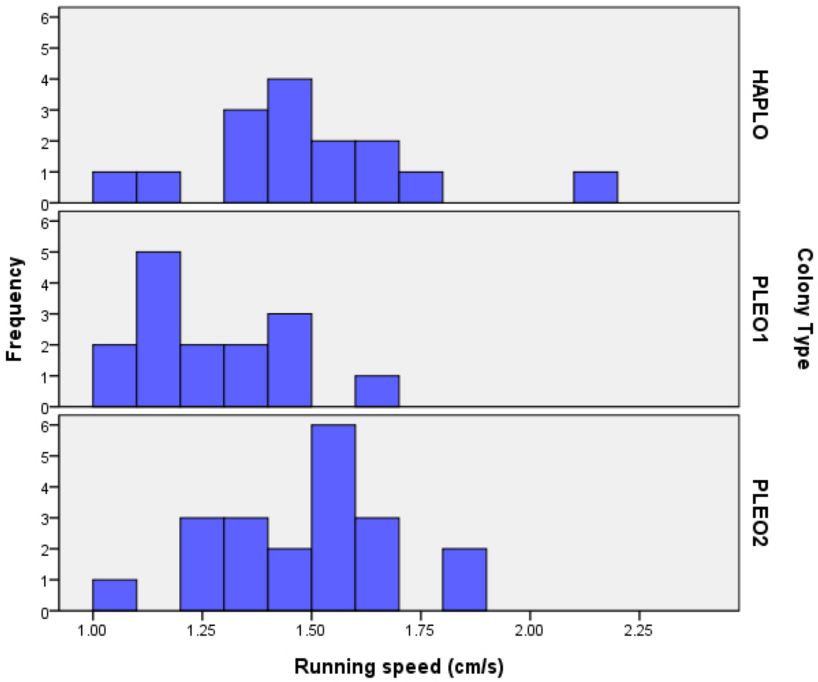

Figure 4: Histograms of running speed distribution in three colony types. Colonies of *P. californicus* were tested for running speed in cm/s at 40°C. Running speed distribution looks roughly normal for all colony types. Mean running speeds are similar for PLEO 2 and HAPLO workers and slower in PLEO 1 workers.

Tests for distribution normality showed all running speeds of workers to fall under normal distributions ($p > 0.1$ for all tests). Standard deviations of worker running speeds from the mean were similar across colony types (PLEO 2 =0.217 cm/s, PLEO 1 = 0.170 cm/s, HAPLO = 0.260 cm/s). Mean running speed for PLEO 2, PLEO 1 and HAPLO workers was 1.47cm/s, 1.25cm/s, and 1.40cm/s, respectively. Independent sample t-tests showed that means were not significantly different between PLEO 2 and HAPLO workers, but were significantly different between PLEO 2 and PLEO 1 workers ($p=0.003$), and HAPLO and PLEO 1 workers ($p=0.006$).

Aggression tests

The trimmed mean (5%) aggression scores (see Methods Table 1. for aggression score explanation) were used for analysis, as fight scores often had a few questionable data points. Workers that completed 6 or more trials, or had an $SD < 1$ for 5 trials were used to construct histograms for aggression analysis (PLEO 2 $n=34$, PLEO 1 $n=18$, HAPLO $n=18$) (Fig. 5).

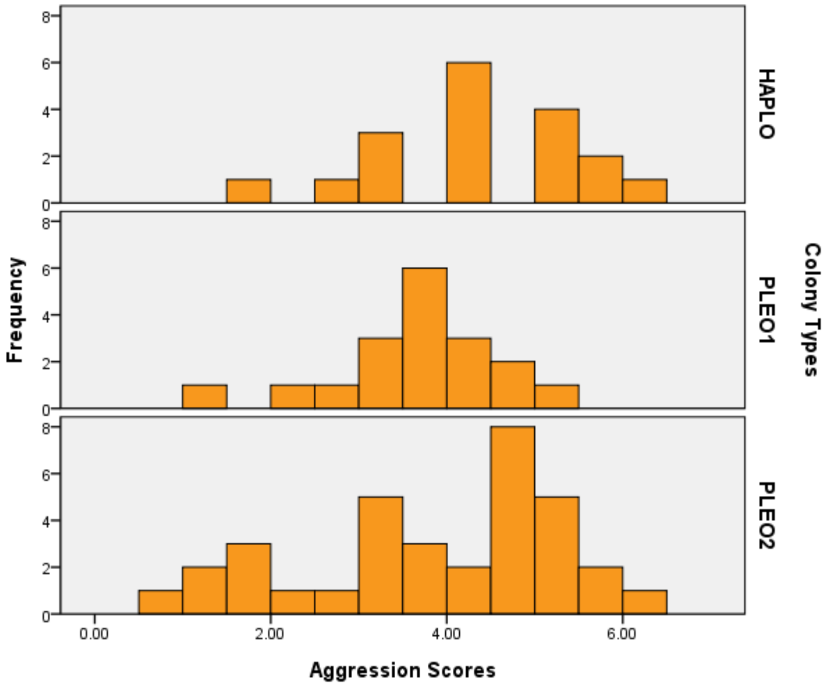

Figure 5: Histograms of trimmed mean (5%) aggression score distribution in three colony types. Colonies of *P. californicus* were tested for aggression displayed against intruding alien ants. Aggression score distribution is normal for all colony types if using stringent p values of 0.01 for significance. Mean aggression scores are similar for all colony types also if using stringent p values of 0.01 for significance.

Tests for distribution normality showed all worker aggression scores to fall under normal distributions when using the more stringent p value of 0.01. Although, PLEO 2 distribution had a p value < 0.05 (p=0.035). Standard deviations from the mean of trimmed mean aggression scores were similar across colony types (PLEO 2 =1.45, PLEO 1 = 0.93, HAPLO = 1.13). Mean aggression scores for PLEO 2, PLEO 1 and HAPLO workers were 3.8, 3.6, and 4.4, respectively. Independent sample t-tests showed that worker aggression score means were not significantly different between any colony type when using the more stringent p value of 0.01, though the difference between PLEO 1 and HAPLO had a significance value < 0.05 (p=0.041).

Comparisons between trials

Tests for Pearson's correlation showed no significant correlations between any variables.

DISCUSSION

Due to small sample sizes and an n = 1 for colony type, this study was mostly only useful in evaluating experimental technique. Significant differences in means were discussed as possible indicators of trends. Of course, no conclusion on population trends or effects of polygyny can be reached.

Mass

Results showed that the pleometrotic queens produced significantly smaller workers than the haplometrotic queens. Trends of queens in polygynous colonies producing smaller workers than their monogynous counterparts is well documented (Goodisman & Ross, 1996; Schwander et al., 2005 and refs therein) and often queen number is negatively correlated with worker size. That is why it is surprising that PLEO 1 workers were nearly the same average size as the PLEO 2 workers. Reasons given for the correlation between worker size and queen number mention additive effects of queen pheromone, and a decrease in available food due to additional brood and queens – neither of which can be evoked to explain the low numbers in the single queen PLEO 1 colony.

The most obvious explanation for the low mean worker weight in the PLEO 1 colony is that it was a feature of this particular colony and not indicative of a trend in the actual population. Also, perhaps the matrilineal genes contained a defect which prevented the workers from obtaining adequate nutrition. If weight is a heritable trait and is predicted by queen size, then the mean worker masses correlate perfectly with the average of queen masses in the colony (Average queen masses for PLEO 2, PLEO1, and HAPLO are 12.18 mg, 12.22 mg, and 12.48 mg respectively; the average worker masses are 2.27 mg, 2.32 mg, 2.62 mg respectively).

Otherwise, small worker size may be an adaptation to the environment within their habitat – the area where the pleometrotic population is located is at a slightly higher elevation, and exhibits more precipitation and biomass than the surrounding habitats.

Or, perhaps workers in colonies where polygyny is obligatory (as suggested for this species: Bespalova, 2011) are naturally selected to be a little bit smaller. There are currently few hypothetical reasons why this would be the case, since a diversity of sizes seems to be more beneficial

(Gravish et al., 2011). However, the extent of queen-worker size dimorphism (how much bigger is the queen compared to the workers) sometimes is suggestive of the reproductive ability of the workers (workers that are much smaller than the queen are less likely to be able to lay eggs), and some suggest that selecting against worker reproduction leads to better cooperation among individuals and less energy expended through worker policing (Fjerdingstad & Crozier, 2006).

Also, it is likely that if both queens are producing workers for the PLEO 2 colony - and it is feasible that this is the case as a high colony growth rate was observed for this colony - the first brood produced by queens is usually extremely small (they are even termed “minima” because they are so different in size from later brood). It is likely that this colony had a higher proportion of minums as compared to other colonies, pulling the average down.

No conclusion for whether weight is heritable can be reached, but the interesting correlation between average worker weight and average queen weight per colony is worth looking into.

Temperature

Running speed at a particular temperature is a measure of degree of behavioral response to a set stimulus. Because there was a normal distribution of weights for all colonies, I would say that running speed in response to temperature change makes for a reliable measure of a behavior that can be distinguished between colony types (that is, if the distribution was random, it would be a poor measurement to consider because it would mean that running speed was random). However, whether running speed is a heritable trait or not is still subject to speculation. PLEO 1 workers did exhibit a significantly slower mean running speed than either other colony type. If you consider PLEO 1 workers to be representative of a single matriline, you could say that the matriline had a different mean running speed compared to the HAPLO matriline. However, if running speed was heritable you could also expect the running speed of the PLEO 2 workers to show a wide, or bimodal distribution, as compared to the PLEO 1 workers (unless the two matrilines had incredibly similar mean running speeds). The standard deviation is a little bit higher in the PLEO 2 workers compared to the PLEO 1 workers (0.217 cm/s compared to 0.17 cm/s), but interestingly the

HAPLO workers showed the widest deviation (0.26 cm/s). This higher diversity in running speeds could be explained by a higher genetic diversity within the HAPLO colony, because the number of males each queen mated with prior to collection is unknown. However, the HAPLO queen would have to have effectively mated with seven males, while the PLEO 2 queens had only effectively mated with two males, in order for the HAPLO workers to exceed the PLEO 2 workers in genetic diversity (because each queen adds two sets of chromosomes while each male adds only one). This is a fairly big difference, but such a scenario is not improbable.

Some of the lower running speeds which are contributing to the large SD's may actually represent incorrect measurements of running speed, due to a flaw in the experimental design. The small test tubes used for this experiment proved to be a problem as ants would often not run straight forward but at an angle, scrambling at the sides of the test tube in an attempt to escape. Such runs were not used in my analysis, but finding a point at which they ran in a straight line was tedious and sometimes only two or three milliseconds of a run could be used for analysis. This may have provided reduced running rates for some trials. For future experiments, it would be simpler to use a small petri dish with sides coated in INSECT-a-SLIP, or a similar container allowing for free running space. With more space to run freely and with the tools available in Image J, tests may be more accurate.

This technique could also be used to check for different running start thresholds. After ants acclimated to a tube, they were observed sitting, immobile. When the slide warmer was turned on, they would start running and run faster as the temperature increased. The point at which they went from resting to running, and perhaps even the acceleration in running speed over time, could be measured and provide interesting values for comparison. Even a study of responses to temperature - where running start temperature and acceleration rates were compared to look at whether acceleration was correlated with start threshold - would be informative. Since I started taping at a fairly high temperature, and didn't wait for ants to acclimate before heating began, I couldn't analyze start temperatures for this experiment. It would also be interesting, for future experiments, to look at responses to gradual cooling

Aggression

If less stringent p values were considered, the weakly non-normal distribution of the PLEO 2 aggression scores compared to the normal scores seen in PLEO 1 and HAPLO workers, as well as the almost bimodal distribution seen in the histogram of PLEO 2 worker scores could be taken as a suggestion that aggression was heritable and could vary across matrilines. Aggression is heritable in bees (Page et al., 1995), and pleometrosis is generally considered to arise from a mutation that affects queen aggression, so it would not be surprising if aggression was heritable in ants. Furthermore, the weakly significant difference (again, if less stringent p values are considered) of the mean aggression scores between the PLEO 1 and HAPLO workers could be an indication of heritability, and of differences in response between matrilines. Again, aggression response here was a measure of behavioral response to a set stimulus, not the measure of the threshold level for the response. An accurate measure of threshold would be the amount of stimulus it takes to get a worker to respond aggressively. The time it takes for a *Pogonomyrmex californicus* worker to notice an enemy ant could be an indicator of stimulus, but there are also many conflicting variables in such a scenario – the distance between worker and enemy could be such a factor, as well as strength of enemy smell. Furthermore, when I attempted to quantify the amount of time it took a worker to respond, I found that the tendency of *Monomorium minimum* to hide or crouch low to the ground when placed in the aggression testing arena reduced the accuracy of the test. A better way to look at response thresholds would be something like the technique established by Guerrieri & d'Ettorre (2008). This study found that aggressive response could be measured by applying the scent of an enemy to the tip of the antenna, and measuring how wide the mandibles opened. Graduated concentrations of enemy smell could be used to check for the onset of an aggressive response.

One important thing the running speed test and the aggression test shows is that the dishwashing metaphor fails to mention that there may be a gradated response to a stimulus. That is, ants could notice a pile of “dishes” but only wash two or three of them unless the stimulus increases. How much the gradations differ between individuals, and whether gradation at all changes the way labor is divided as proposed by the threshold hypothesis is yet unclear. Tests such as the ones outlined here may help elucidate the relationship.

Another thing that is important to note is that aggressive response seems not only to vary in degree of aggression, but in what aggressive behavior is elicited. If you look at the aggression assay I created for this test (Methods and Materials, Table 1) you can see that between “ignore” and “fight” was not only a gradation but a multitude of varieties of aggressive behaviors. In fact, it was difficult to decide how some things should be grouped; behavior where an individual bent her gaster while baring her jaws was different from one where an individual made several short charges at an enemy – but how does one accurately quantify which is more or less aggressive? It would be interesting to look at descriptions of all the different kinds of behavioral responses seen in the individual populations, then see whether different populations exhibit different behaviors, and whether more diverse populations exhibit a larger variety of behaviors. A finding such as that could give support for my second hypothesis.

In future tests, it might be more informative if aggressive responses toward an actual enemy of *P. californicus* were measured, such as against the ant *Messor pergandi* (Johnson, 2004). Though it would probably be wise to use a tethering technique like the one proposed by Fénéron (1996) in order to incapacitate the enemy and prevent aggression towards *P. californicus*. Fatal injuries reduce the number of trials that can be completed, which reduces n values very quickly. *M. minimum* was a fortunate choice for an enemy, in that it typically tried to avoid *P. californicus*, though it surprisingly was able to do quite a bit of harm when it was aggressive.

Comparisons across tasks

Though it may just be a random occurrence, it is interesting that PLEO 1 workers varied at least with weak significance from HAPLO workers in both mean running speeds and fight scores, while PLEO 2 workers never varied in mean from the HAPLO workers. If this was observed across larger colony sample sizes, it could be indicative of selection for specialization in the matrilines of the pleometrotic population. I would expect HAPLO workers of the same matriline to be more average in their response, because I would expect genetic lines to be more generalized when they must survive on their own, since in a solitary line, the ability to deal with all environmental situations should be selected for. Similarly, grouped pleometrotic queens may have matrilines that specialize in a particular task, but together they should produce a more generalized colony (remember the bee study: Page & Robinson, 1995).

Additional tests

I attempted a digging test, where I knocked individuals to the bottom of a one cm diameter test tube, buried them under two cm of finely sifted dirt, and then timed how long it took them to dig themselves out. After five trials, I noticed results were highly variable, and also that they may conflate behavior and size (perhaps smaller individuals were lifted as sand was poured on top of them). I found another digging test that may lead to better measurements. *Pogonomyrmex occidentalis*, a close relative of *P. californicus*, will dig to rescue trapped compatriots (Spangler, 1968). In this test, a nestmate is pinned down with mesh and then covered with one or two millimeters of sand. The trapped ant will send out a distress signal, and the behavior of another ant towards the trapped ant could be measured, such as the length of time until she comes to the rescue and the length of time it takes to dig and reach the trapped individual.

Food preference by different matrilines could be tested with the protocol presented by Dussutour & Simpson (2009), who have a recipe to make foods of variable ratios of protein to sugar. Individuals could be tested on preference for protein or sugar, and for foraging rate. Potentially, methods could be worked out which measure how large a pile of a certain food has to be before an individual will recruit others to help her carry it.

Finally, the behaviors of individuals outside of the colony environment should be compared to their behaviors within the colony. Tests of the response of an entire colony to an enemy invader could be compared with tests of an individual's aggressive behavior. Tests of how individuals with faster running speeds at high temperatures function within colonies which are made to forage at different temperature could also be looked into. Measures of the preference of a particular nutrient type by an individual could be compared to foraging specialization by that individual within her colony. Digging responses and the efficiency of the digging response in an individual could be compared to the likelihood of that individual digging for a lost worker when the entire colony is presented a chance to act on the stimulus.

CONCLUSION

Although traditional hypotheses consider high genetic relatedness to be necessary for social cohesion in the Hymenoptera, and the addition of unrelated individuals to be the cause of social tension, empirical evidence shows that this may be too hasty a conclusion. High genetic relatedness may attest for behaviors seen in lower-level social Hymenoptera, but recent empirical evidence combined with new trends in thought is showing evermore the ways in which diversity could be the explanation for the efficiency and resiliency exhibited by the most derived of the Hymenopterans.

Response thresholds may provide an excellent explanation for how genetic diversity creates a division of labor which is more efficient and better maintains homeostasis. However, threshold levels aren't the only attribute that can be regulated by genes. There may be other benefits to genetic diversity that have as of yet been given little attention.

I have proposed two hypotheses for how genetic diversity can benefit a colony. Firstly, differences in a variety of abilities, not just thresholds, could account for the increase in productivity seen across the board in polyandrous and polygynous species. Secondly, cooperation can compensate for weaknesses in genetic lines and lead to a lessening of a genetic bottleneck, causing a rapid increase in diversity and preservation of unique abilities. This can, in turn, generate more ingenious workforces.

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