ABSTRACT

WITHROW, JAMES MARTIN. Sharing the Throne: Establishment of Multiqueen Honey Bee (*Apis mellifera*) Colonies. (Under the direction of Dr. R. Brian Langerhans and Dr. Fred Gould).

Western honey bee (*Apis mellifera*) colonies are typically monogynous, with a single reproductive queen whose health and productivity is crucial for colony success. Since at least the 1890's beekeepers have sought to increase colony efficiency and productivity by experimenting with methods of managing colonies with multiple queens at a time. While these efforts have demonstrated success the challenges of managing typical multiqueen systems, with queens physically separated in the colonies, have limited this to being a niche practice among beekeepers. Queen aggression towards rivals, as evidenced in the well-studied duels in which newly emerged virgin honey bee queens fight to the death to monopolize reproduction in the colony, has generally been accepted to preclude the possibility of establishing truly polygynous colonies, with queens living together in a shared brood space. However, multiple honey bee queens are known to coexist naturally in colonies, at least temporarily, during apparent supersedure events and recent literature suggests that queens may lose aggression with age or may be prevented from fighting through mandible ablation.

Here we have conducted a new investigation of honey bee queen aggression, finding that aggression is not circumvented through mandible ablation (queens with ablated mandibles are as likely to fight as queens with intact mandibles) but that aggression does vary with age—pervasive in virgin and newly mated queens but absent in old queens and declining rapidly in young laying queens, with only half of queen pairs engaging in fatal duels by 9 weeks old. By using non-aggressive mature queens and non-aggressive newly emerged workers we have successfully established polygynous colonies. These colonies exhibit multiple queens living and successfully reproducing together, as demonstrated by genotyping analysis of workers produced

in these colonies. This method serves as a proof of concept for the largely overlooked polygynous approach to multiqueen beekeeping, which has simpler management requirements than the traditional double-hive techniques as well as offering a potential new approach to manage queen failure, one of the primary challenges in the apiculture, by having multiple queens present to mitigate the effects of a failing queen.

Additionally, we have conducted a study to assess the potential for beekeepers to adopt this method and inform future research developing the polygynous method. Through a survey of hobbyist and professional beekeepers we have found significant enthusiasm among beekeepers for trying new management techniques, including multiqueen beekeeping, that is consistent with the collaborative nature of beekeeping communities which serve as places to share information and techniques to address the wide array of challenges in beekeeping. In particular, we have identified a profile of large hobbyist beekeepers who would be excellent candidates to directly include in the research process as they are experienced beekeepers with both large operations and innovative dispositions amenable to participating as research partners for multiqueen or other types of honey bee research projects.

Sharing the Throne: Establishment of Multiqueen

Honey Bee (*Apis mellifera*) Colonies

by

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DEDICATION

For the victims and survivors of graduate programs.

BIOGRAPHY

James Withrow's previous studies include a Bachelor of Arts in Music and a Master of Science in Entomology. In his time at NC State James was an avid participant in the university community through student governance and advocacy. He was honored to be a member of the inaugural cohort of North Carolina STEM Policy Fellows, working in the North Carolina Department of Commerce Office of Science, Technology & Innovation and is currently a member of the Foreign Service as an Agricultural Attaché in the United States Department of Agriculture. He remains a philosopher at heart.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Review of multiqueen beekeeping history

Multiqueen systems of beekeeping

The historical record of human management of western honey bees (*Apis mellifera*) extends back well over 4000 years to the Old Kingdom of Ancient Egypt (Crane 1999), and the archaeological record of honey hunting and use of honey bee products is prehistoric (Dams and Dams 1977, d'Errico et al. 2012) with the earliest evidence for widespread use of *A. mellifera* dating back nearly 9000 years (Roffet-Salque et al. 2015). Today honey bees are essential to contemporary agriculture systems as the primary managed pollinator (Klein et al. 2007, National Research Council 2007, Breeze et al. 2011) as well as a model organism for the study of social behavior (von Frisch 1967, Lindauer 1971, Oldroyed et al. 1994, Seeley 1995, Robinson et al. 1997), with complex eusocial organization and behaviors that have drawn the attention of scholars since Aristotle (Aristotle et al. 1862) and Darwin (1859).

Although honey bees are typically monogynous, with colonies containing a single reproductive queen who greatly outlives workers (~1-3 years average for queens vs. ~6 weeks for workers) and is capable of laying over a thousand eggs per day (Winston 1987), beekeepers have experimented with methods for operating multiqueen colonies of honey bees for over a century to create larger, more productive, colonies. The earliest records of multiqueen beekeeping date back to the 1890's, when English beekeeper George Wells described his two-queen system (Cowan and Carr 1893, Wells 1894). The Wells Method establishes a double colony by using a hive design with two side-by-side brood chambers underneath shared supers for storing honey (**Figure i.1**). Each brood area is separated from the rest of the hive by a queen excluder, with openings that allow workers to pass freely but are too narrow for queens, allowing for each queen to be maintained separately while the overall colony operates with the full strength of two individual colonies. By keeping bees in this way Wells established larger colonies with much greater productivity, reporting honey yield from his double colonies nearly four times that produced from regular single colonies (158 lbs. vs 48 lbs.; Wells 1894).

Over the years since Wells' discovery many beekeepers have followed in his footsteps, creating and refining numerous hive designs and methods for managing two-queen colonies. Some, like Wells, placing queens into separate brood nests horizontally (**Figure i.2A**; Ferris 1906, Gouget 1953, Nabors 2016, Hesbach 2016), while others have separated queens with only a vertical queen excluder in a common brood area (**Figure i.2B**; Sladen 1920), or have vertically separated queens and brood areas within the stack of hive boxes (**Figure i.2C**; Medicus 1910, Ferrar 1946, Dunham 1951, Holzberlein 1953). Across all the variations in hive design and management practices, the near-universal achievement reported by proponents of multiqueen beekeeping has been substantial increases in colony productivity.

In the mid-20th century, USDA researcher Dr. Clayton Ferrar quantified the relationship between colony size and productivity, revealing that (regardless of the number of queens) per-capita colony productivity increases as the population increases[1], as larger colonies are able to more efficiently leverage their workforce to exploit the best available food resources (Ferrar 1937). Using multiple queens allows colonies to grow faster early in the season and achieve

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[1] Ferrar determined that "bees in colonies with 30,000, 45,000, and 60,000 bees produced at a rate of 1.36, 1.48, and 1.54 times as much honey, respectively, as colonies with 15,000 bees."

larger overall size, resulting in greater production and efficiency, per bee, than single queen colonies, as well documented by Ferrar and others in the mid-20th Century (Ferrar 1953, Dunham 1953, Miller 1953, Holzberlein 1953, Ferrar 1958).

This increased productivity comes at a cost, however, with multiqueen setups calling for specialized equipment and more demanding management regimens that few beekeepers are well-equipped to utilize (Wade 2021). As a result, two-queen systems have remained an alluring but, ultimately, fringe practice in beekeeping.

Polygynous colonies

An alternative way to leverage the productivity of multiple queens without the cumbersome management of doubled-hive systems is to establish colonies with multiple queens in the shared hive structure. Doing this, however, requires contending with the biological tendency of honey bees to have single-queen colonies. As eusocial animals honey bees arose from a state of ancestral monogamy (Hughes et al. 2008, Boomsma 2009), with the high relatedness of full-sibling colonies headed by a singly-mated foundress enabling the evolution of caste-segregation into nonreproductive workers and reproductive queens. Honey bees have not retained this ancestral monogamy, though. Since evolving through the “monogamy window” they, like many other social species, have abandoned single-mating, resulting in colonies with much higher levels of beneficial genetic diversity (Oldroyd & Fewell 2007, Seeley & Tarpy 2007, Mattila & Seeley 2011, Mattila et al. 2012, Delaplane et al. 2015). While some eusocial species have evolved to have multiqueen colonies, many more, including honey bees, have instead evolved polyandry, with queens mating with multiple males before heading single-queen colonies (Schmid-Hempel & Crozier 1999). In fact, honey bee queens are considered “hyperpolyandrous”, typically mating with more than 10 (and possibly more than 50) drones (Palmer and Oldroyd 2000, Tarpy et al 2015, Withrow and Tarpy 2016).

While the evolutionary history of honey bees to have single, highly promiscuous, queens does not suggest that they would be amenable to being managed in a state of polygyny there are some notable naturally occurring exceptions to the one-queen rule. In fact, it is not unusual for beekeepers to notice two queens together in a colony for a period of time, apparently the result of a supersedure process of a new daughter queen replacing a failing older queen (Doolittle 1889, Brichter 1921, Butler 1957, Neumann et al. 2001). During the supersedure process the nurse bees will raise a small number of new queens with the old queen still present (a different process, emergency queen rearing, takes place when there is no queen in the colony). One of these new queens, likely the first to emerge, will eliminate her rivals and then go on to mate and begin laying in the colony, often while the old queen remains in place. This naturally occurring period of polygyny may last for weeks or even months but is ultimately temporary, and although the circumstances under which the old queen eventually disappears are unknown, this process requires that old queens tolerate the production and emergence of new queens and also that they are not themselves targeted by the aggression by which virgin queens eliminate their rivals.

Another naturally occurring example of polygyny is related to the Cape Honey bee subspecies (*Apis mellifera capensis*), which is best known for engaging in thelytokous parthenogenesis (Onions 1912). Not only are Cape Honey bee workers capable of developing into pseudoqueens and laying diploid eggs, but they are able to successfully mimic a suite of pheromones enabling them to parasitize the nests of the co-occuring (and non-parthenogenic) *Apis mellifera scutellata* (Martin et al. 2002a, Martin et al. 2002b, Wossler 2002, Neumann & Hepburn 2002). In parasitized colonies, a group of *A. mellifera capensis* pseudoqueens will coexist, often alongside the original *scutellata* queen, until the colony collapses and newly produced thelytokous psuedoqueens disperse to find other colonies. While neither of these cases represent a permanent and stable state of polygyny in honey bees they do suggest that, in principle, such a colony structure could be established and maintained by beekeepers.

The earliest documented experiments in establishing polygynous colonies took place in parallel in the United States and in Ireland in the early 20th century. In a series of letters to the journal *Gleanings in Bee Culture*, E. W. Alexander described his method for introducing up to 14 queens in a colony and achieving high productivity with these colonies (Alexander 1907a, Alexander 1907b, Alexander 1907c). Alexander's discovery attracted significant attention from the beekeeping community and other beekeepers also found success following his method (Hall 1907, The National Bee Keeper's Convention Harrisburg 1907, Wright 1907, Davenes 1907, Sherrod 1907, Bussy 1908, Hand 1908, Joice 1911). Also, in response to Alexander's published letters, Irish beekeeper Cruadh described his own method for establishing polygynous colonies, which was subsequently adapted (sometimes with the use of queen excluders[2]) and used in Scotland to optimize production of late-season heather honey (Cruadh 1907, Ellis 1908a, Ellis 1908b, Medicus 1908, Medicus 1910).

Despite the promising experimentation by Alexander, Cruadh, and others in establishing polygynous colonies, multiqueen beekeeping in the 20th century was primarily focused on the

[2] Medicus in particular drew an important distinction between doubled hives, with separate entrances but shared super space, and single-entrance multiqueen colonies, while not regarding whether queens were together or separated by queen excluders as similarly significant.

development of double colony systems in the legacy of George Wells (Wade 2021). In recent years, however, researchers have once again turned attention to the establishment and successful overwintering of polygynous colonies (Zheng et al. 2009). This renewed interest in polygyny suggests a new potential for multiqueen beekeeping with colonies that, once established, could be managed similarly to typical single-queen colonies. If such colonies can be reliably established with an accessible method, then this technique would offer beekeepers an alternative and possibly more widely applicable means to access the benefits of multiqueen beekeeping, including use as an alternative approach to managing queen loss, one of the major drivers of colony and productivity loss is beekeeping (vanEngelsdorp et al. 2010, Neumann and Carreck 2010, Seitz et al. 2016). The following chapters present research examining queen aggression in honey bees, developing a prototype method for establishing polygynous colonies, and assessing the potential for such a method to be successfully adopted by beekeepers.

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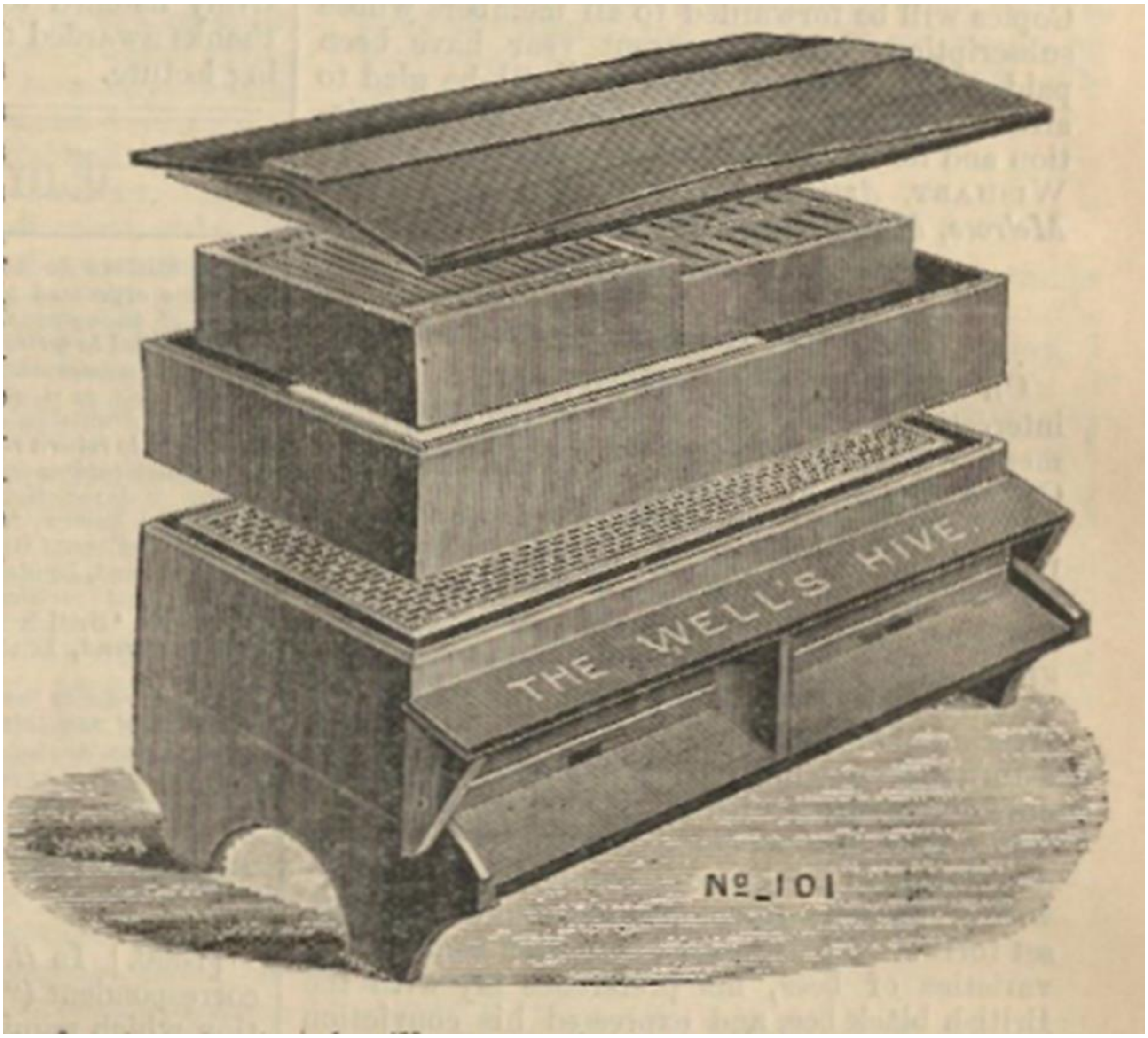

Figure i.1. Illustration of the Wells Hive design

Depicted in the in the March 16, 1893 issue of the British Bee Journal, Beekeepers' Record and Adviser. This "doubled" hive design uses much larger boxes than a conventional Langstroth hives.

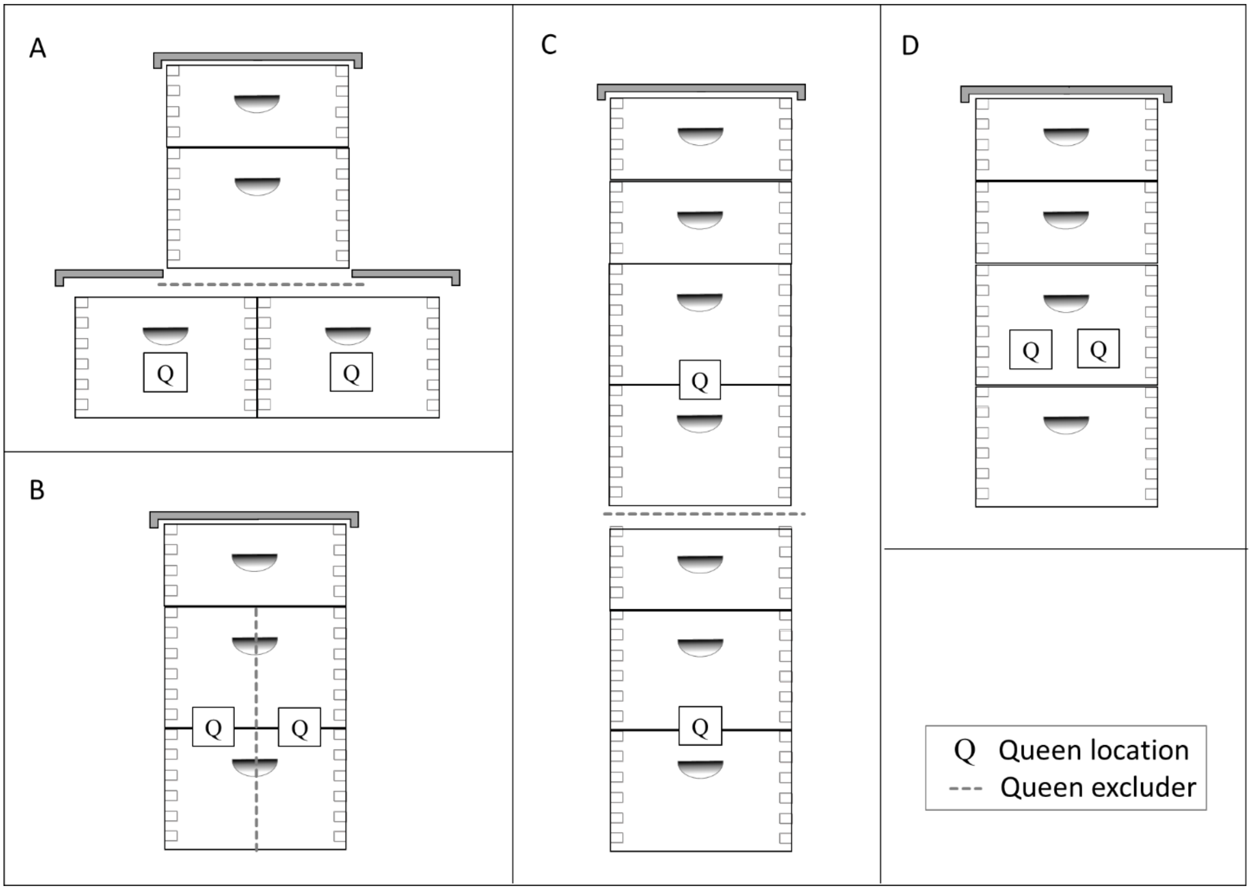

Figure i.2. Multiqueen hive configurations

CHAPTER 1.

Reproductive conflict in honey bees (*Apis mellifera*): Queens lose aggression with age

ABSTRACT

Although eusocial animals are known for high levels of cooperation there remain instances of conflict within eusocial colonies. One such instance is the reproductive conflict among virgin honey bee queens, which engage in winner-take-all duels to monopolize reproduction in their colonies. The aggression of honey bee queens towards rivals is apparently not absolute, however, as beekeepers report instances of multiple queens coexisting in established colonies, typically attributed to supersedure events where an old queen is replaced by a daughter queen. Although poorly understood, these instances of polygyny have contributed to longstanding beekeeper interest in establishing multiqueen colonies, and both queen age and mandible ablation have been identified as determinative factors in queen aggression that may be utilized in multiqueen beekeeping. We have carried this work further, finding that aggression in honey bee queens varies according to age. While aggression is pervasive in virgin and newly mated queens we find that it rapidly declines (with only half of queens pairs fatally dueling by 9 weeks post-emergence) in young laying queens and is absent in old laying queens. This is possibly a consequence of colony-level selection for supersedure of failing queens by replacement daughters. Additionally, we found no effect of mandible ablation on aggressive behavior in queens despite a decrease in fighting success in mated, but not virgin, queens with ablated mandibles.

INTRODUCTION

Eusocial animals are defined by an extraordinary degree of cooperation among nestmates, to the point that the majority of individuals are workers, forgoing their own reproductive potential to instead rear the offspring of the queen(s) (Wheeler 1911, Wilson 1971). While characterized by cooperation, eusociality still retains opportunities for conflict within colonies—i.e., when the selective interests of some individuals are in opposition to those of their nestmates or the overall colony (Frank 1995, Ratnieks et al. 2006). These opposing interests are evident during reproduction, especially in the haplodiploid eusocial Hymenoptera, and include: conflict between queens and workers over the sex ratio of reproductive offspring and the production of males (Trivers and Hare 1976, Ratnieks 1988, Heinz 2004, Olejarz et al. 2017); conflict among workers to produce males and select larvae for rearing as replacement queens (Ratnieks 1988, Tilley and Oldroyd 1997, Monin and Ratnieks 2001, Tarpy et al. 2016); competition among totipotent individuals over the opportunity to become reproductive (Bourke and Ratnieks 1999, van der Westhuizen et al. 2013); and competition among queens to control reproduction, as when, in some species, multiple queens fight to the death until only one remains (Huber 1821, Darwin 1859, Gilley 2001, Schneider et al. 2001, Gilley and Tarpy 2005, Long et al. 2017).

Western honey bees (*Apis mellifera*) are a model system in the study of reproductive conflict within eusocial colonies, including direct queen-queen conflict. In the colony-level reproductive process of swarming the existing queen departs with much of the worker population to found a new colony, leaving the remaining colony with a well-provisioned nest and a cohort of developing new queens to replace her. Following emergence, these virgin queens compete for the ability to monopolize reproduction in deadly, winner-take-all, duels (Huber 1821, Gilley 2001, Tarpy et al. 2004). During these fights, newly emerged virgin queens engage in intense bouts of grappling and, sometimes, spraying of a fragrant ejecta from their hindguts (Post et al. 1987, Bernasconi et al. 2000, Tarpy and Fletcher 2003), until one successfully stings and eliminates its rival. The victorious queen that survives these duels then proceeds onto one or more mating flights, storing sperm for the remainder of her life, and then begins producing eggs as the sole reproductive female in the colony.

While the aggression of honey bee queens towards rivals is well known through study of virgin queen duels, and honey bee colonies are nearly always monogynous, queen-queen aggression is not absolute. Queens may live for several years, possibly departing with a future swarm to found a new colony, but when they grow old and begin to fail the colony will attempt to supersede them with a new virgin queen (Butler 1957, Winston 1987). While the full processes of supersedure remain poorly understood, it often results in a period of temporary polygyny, with mother and daughter queens apparently peacefully coexisting until the old queen disappears. There are also anecdotal reports that queen aggression is lost following mating. For example, Alexander (1907) wrote that "there is not much you can do with virgin queens until they are fertilized and commence laying; then their desire to sting other queens is all gone. I have often kept two or three laying queens under a common drinking-glass on the work-bench for a number of days without their trying to sting each other." These instances of queens tolerating the presence of other queens stand in stark contrast to the aggressive behavior exhibited during virgin queen duels, raising the question of how these different patterns of queen-queen interactions are maintained.

Understanding queen aggression towards rivals is especially relevant in the context of multiqueen management systems. Beekeepers have long been interested multiqueen management systems as a means to create larger, more productive, colonies and provide insurance against queen failure (Wells 1894, Farrar 1937, Farrar 1953, Holzberlein 1953, Farrar 1958, Miller 1953, Moeller and Harp 1965, Walton 1974, Moeller 1976, Zheng et al. 2009, Nabors 2016, Hesbach 2016). While some approaches involve establishing colonies in hives designed with shared food stores but separate broodnests to keep the queens separated, these systems are more challenging to manage than hives with a single brood nest. In contrast, single-nest systems are more difficult to setup as they require queens to peacefully cohabitate but may thereafter be managed similarly to typical single-queen hives. The feasibility of single-nest polygynous beekeeping would be substantially increased if beekeepers had a reliable source of non-aggressive queens to use in establishing colonies. Identifying a consistent relationship between queen age and aggression towards rivals would provide a potential answer to this problem, though the age at which any change in aggression takes place would be important as there would be little value in establishing colonies with queens that are nearing the end of their productivity. Another potential answer lies in a recent suggestion that queens may be prevented from fighting through ablation of their mandibles (Zheng et al. 2009; **Figure 1**), decreasing their ability to grasp each other to land a fatal sting and also cause them to "self-assess" and decline to fight as they are unlikely to win (Dietemann et al. 2008).

To investigate the viability of producing queens suitable for polygynous beekeeping we have conducted a series of trials to investigate the effects of age, mating status, and mandible ablation on aggressive behavior and fighting success in honey bee queens across a series of ages and mating statuses.

METHODS

Experiment 1

We conducted this experiment to determine whether queen-queen aggression varies according to mandible ablation status, mating status, or age by conducting fighting trials of queen pairs across six treatment groups: virgin, virgin ablated, young, young ablated, old, and old ablated.

Queen sources

To produce virgin queens, we grafted larvae according to standard queen rearing procedures (Laidlaw and Eckert 1962). Briefly, we grafted newly hatched (1-day-old) worker larvae into queen cups that we then placed into queenless cell-builder colonies to rear as queens. Once the resulting queen cells were capped for pupation, we transferred them to an incubator set at 34.5 °C to complete development. We allowed the virgin queens to emerge from their cells in the incubator and then placed the queens into individual petri dishes and supplied them with a ~50% sucrose solution diet.

For mated queens, we grafted larvae into cell-builder colonies and transferred capped queens cells to an incubator as with the virgin queens, but, shortly before emergence we placed each cell into an individual mating nucleus hive (containing three total frames of bees with brood, pollen, and honey) and allowed them to emerge and mate naturally. After the queens had mated and begun laying (approximately 2-3 weeks post-emergence), we collected these queens and returned them to the incubator in individual petri dishes supplied with a sucrose solution, as with the virgin queens.

For old queens, we acquired >1 year old queens that were being culled from a commercial operation as a part of systematic annual requeening. We banked these queens according to standard procedure in strong (visually assessed to be healthy, with a large population of worker bees and brood) queenless colonies (Harp 1967, Wyborn et al. 1993, Gençer 2003) then transferred them to petri dishes in the incubator supplied with sucrose solution, as with the virgin and young queens.

For mandible ablation in all three groups (virgin, young, and old), we removed approximately one half of each mandible using microscissors (Dietemann et al. 2008, Zheng et al. 2009; **Figure 1.1 B and C**), then returned them to their petri dishes for at least 24 hours to ensure that we had not caused additional injury.

Queen duels

For queen duels to test aggressive behavior, we used 20 pairs of queens from each of the six groups (virgin, virgin ablated, young, young ablated, old, and old ablated). We first uniquely paint-marked and weighed each queen to the nearest 0.1 mg, then introduced them in pairs into petri dishes and supplied them with a sucrose solution. We observed the queen duels for 5 minutes, then returned the petri dishes to the incubator and checked them every 5 minutes for the first half hour, then every 15 minutes until the second hour, then every half hour until the fourth hour, then hourly until at least the sixth hour. At each time point, we recorded whether queens were fighting and if any queen appeared to be injured, “fatally” injured (lying in a “death pose” with legs curled and unable to move around the petri dish), or dead. Upon observing a fatally injured or dead queen, we removed both queens from observations, froze them in marked

Epindorf tubes, and later measured the head and thorax widths of each to, along with queen weight, control for queen size in outcome of duels.

Experiment 2

We conducted this experiment to determine whether mandible ablation on has an impact on fighting success in dueling pairs of virgin and young mated honey bee queens. We produced queens as in Experiment 1 by grafting larvae into cell-builder colonies and then collecting capped queen cells to either emerge and maintain virgin queens in an incubator or transfer cells to emerge and naturally mate in mating nucleus colonies for young queens. We utilized a total of 22 pairs of virgin and 14 pairs of young mated queens setup to duel in petri dishes as in Experiment 1, except that each pair consisted of one queen with intact mandibles and one queen with ablated mandibles.

Experiment 3

We conducted this experiment to measure the loss of aggression in young queens over time by conducting queen duels at age cohorts of 3, 5, 7, 8, 9, 10, and 12 weeks post-emergence. We produced all queens as in Experiments 1 and 2 by grafting larvae into cell-builder colonies and then transferring cells to emerge and naturally mate in mating nucleus colonies. Once the queens had completed their mating flights and begun laying eggs, we transferred them into individual queen cages and banked them as in Experiment 1 until each cohort reached the designated age for trials. To conduct the trials, we collected and weighed each queen to the nearest 0.1 mg, then introduced them in age-matched pairs into petri dishes supplied with a sucrose solution to duel as in Experiments 1 and 2. We observed the queen duels for 5 minutes, then returned the petri dishes to the incubator and checked them every 5 minutes for the first half hour, then every 15 minutes until the fourth hour. At each time point, we recorded whether queens were fighting and if any queen appeared to be injured, “fatally” injured (lying in a “death pose” with legs curled and unable to move around the petri dish), or dead. Upon observing a fatally injured or dead queen, we removed both queens from observations and froze them at -80 °C in marked Eppendorf tubes. We deemed any queens that did not fight or ceased any initial aggressive behavior, remaining alive together until the end of the observation period, as “not-fighting” and froze them at -80 °C in marked Eppendorf tubes. We used 74 pairs of queens total, with the number of pairs in each cohort ranging from 4-15 due to unequal losses across cohorts in grafting, mating, and banking (**Table 1.1**, **Figure 1.4**). For analysis of age and fighting behavior we conducted a Pearson’s chi-square test and post-hoc pairwise chi-square tests with Bonferroni corrections, and for analysis of queen weight and fighting behavior (**Figure 1.5**) we conducted a logistic regression using R Studio (Version 1.2.1335).

RESULTS

Experiment 1

Among the six treatment groups of queen pairs (virgin ablated, virgin unablated, young ablated, young unablated, old ablated, and old unablated) we found only queen age to be a significant factor in cohabitation time ($F_{5,114}$=24.90, $p<0.0001$), with pairs of old queens surviving together (usually for days) longer than either virgin or young mated queens (**Figure 1.2**). While virgin and young queens generally concluded their duels with the fatal injury or death of a queen within an hour of being placed together, we rarely observed aggressive interactions between old queens and never observed fatal stinging in either of the old queen groups. Additionally, we found no significant differences in the cohabitation times of virgin and young queens and no significant differences in the cohabitation times between ablated and unablated queens in any age category. This suggests that these queens were not modifying their behavior in response to having their mandibles ablated.

Experiment 2

In our examination of the impact of mandible ablation on fighting success we found no effect in virgin queens, with half the duels won by ablated and half won by unablated queens. Ablation did have a significant affect in young queens, however, where unablated queens won the majority of duels (χ^2 =4.071, $p<0.05$; **Figure 1.3**). Notably, five pairs of young mated queens did not engage in fatal dueling, with queens ceasing any observed fighting and surviving together for more than 24 hours.

Experiment 3

Queen aggression as measured by the percentage of queen pairs that engaged in fatal duels ranged from 18.18%-81.82% significantly decreasing as queen age increased, with a significant difference between age cohorts (χ^2 = 24.8, df = 6, $p <0.001$), specifically between the oldest age cohort and the youngest two cohorts (pairwise χ^2 tests with Bonferroni correction, p = 0.0352 for 3 week-old queens versus 12 week-old queens and p = 0.0130 for 5 week-old queens versus 12 week-old queens, all other comparisons were non-significant), **Table 1.1**, **Figure 1.4**). A total of 44 queen pairs engaged in fatal duels, with an average fight time of 40.23 min (SD ± 38.35 min). A total of 30 queen pairs survived the entire observation period together (with both queens considered to be “not-fighting”) and either exhibited no aggressive behavior at all or quickly ceased any signs of aggression without fatal injury. Additionally, we found no effect of queen size on whether queens won or lost fights (logistic regression $p = 0.603$) but did find that larger queens were more likely to fight (logistic regression, $p = 0.003$, **Figure 1.5**)

DISCUSSION

While the winner-take-all duels of virgin honey bee queens have been a topic of scientific inquiry since Darwin (1859) hardly any notice has been given to the fact that this aggression towards rivals is not absolute, despite widespread anecdotal reports of beekeepers finding temporary instances of multiple queens present together in a shared hive. Though limited, the previous literature has implicated queen age and decreased fighting condition as a result of experimental mandible ablation as likely drivers for lost aggression (Dietemann et al. 2008, Zheng et al. 2009). In testing these variables through the present experiments, we have found queen age and not mandible ablation to be the primary driver lost queen-queen aggression in honey bees, though we do find some effects of mandible ablation on the fighting ability of ablated queens.

Although it may be unsurprising that mandible ablation impedes the fighting ability of young queens (presumably by making it more difficult for them to grasp and successfully sting their rivals), it is unclear why this effect would not also be present in virgin queens. Under natural conditions, these queen duels occur between virgin queens during swarming or, more rarely, during supersedure events. Mating triggers substantial physiological changes in queens as they transition into egg production, thus it may be that fighting ability is impacted even before there is an observable decline in aggression and mandible ablation may exacerbate this change. The five pairs of unablated vs. ablated queens that (apparently) stopped fighting without fatal stings also points to a mating- or age-related change exhibited in this group, though we are unable to make any inferences because of the lack of differences in aggression or fighting times among the matched pairs of virgin and young queens; pairs of young ablated queens were neither less aggressive nor slower to finish their fights than the unablated young queens or either group of virgin queens. Additionally, while larger size has been found to provide an advantage in queen duels (Tarpy and Mayer 2009), we did not find this to be the case here (logistic regression $p = 0.603$), though we did observe that larger queens were more likely to engage in duels (logistic regression, $p = 0.003$, **Figure 1.5**).

One potentially confounding variable in Experiment 1 is that the "old" queens came from a different source than the virgin and young queens, as they were queens from a commercial operation rather than locally produced. While sourcing in this manner was primarily a matter of necessity as it was not feasible to generate a sufficient number of "old" queens locally, doing so also served as a proof of concept for establishing multiqueen colonies to test whether "cull" queens from commercial colonies might be a viable source of the necessary surplus queens. While the expected failure rate of these >1 year old queens is great enough to justify systematic requeening in a commercial setting where there is a relatively low risk tolerance for the lost productivity and potential colony loss caused by unexpected queen failure, the majority of such queens are healthy, with significant remaining productive life. Though from a different source, these queens were of equivalent Italian commercial stock to our existing population and unlikely to exhibit any behavioral differences due to their origin. While such effects cannot be fully ruled out for Experiment 1, the consistency of results between Experiments 1 and 3 provide strong evidence that it is indeed age and not any other confounding variables driving the loss of aggression in older queens. As a whole, this study indicates that not only is age the primary factor in honey bee queen aggression but that, as seen in Experiment 3, aggression declines rapidly, from universal aggression in newly emerged virgin queens to only half of queens pairs fatally dueling by 9 weeks and ~18% fighting at 12 weeks post-emergence. This significant decline in aggression is in accordance with observations that six month old queens can successfully cohabitate in polygynous colonies (Zheng et al. 2009).

It is important to note that these experiments observe aggressive behavior at the level of queen pairs and not individual queens. While any queen who successfully kills her rival may clearly be regarded as "aggressive" we are not able to infer the status of losing queens. Therefore, the results of Experiment 3 are best understood as the rate at which queen pairs contain no "aggressive" queens across the series of ages. Aggression measured at the level of individual queens rather than queen pairs would likely reveal an even more rapid decline by accounting for the aggression status of "losing" queens. While aspects of queen aggression may be measured at the individual level (Farkhary et al. 2017), whether or not aggressiveness reaches the threshold of fatal dueling is necessarily a group phenomenon. As such, it is likely determined by the interaction of the individuals at the group level as well as by their individual thresholds of aggression, with particular behaviors serving to escalate or, potentially, deescalate the overall interaction (Clark and Fewell 2014), which may explain why some intermediate-aged queens sometimes exhibit aggression upon initial contact but relatively quickly abandon fighting and begin cohabitating (apparently) peacefully. Due to these group-level interactions it may be that to fully understand aggressive behavior in individual honey bee queens will require trials with three or more queens to observe how aggressiveness at the group level scales according to queen number.

The overall observation that honey bee queens lose aggression after reaching maturity makes sense in the context of colony survival, as established queens are unlikely to encounter another rival unless she is failing and the colony is attempting to replace her with a new (daughter) queen. In such instances, aggression from the old queen could result in failed supersedure, jeopardizing the likelihood of colony survival. While we have not tested aggression between old and young (virgin or mated) queens, it seems possible that young queens might not perceive old queens as rivals or exhibit aggression towards them either, as old and new queens are observed to cohabitate temporarily during the supersedure process and the actual mechanism by which old queens die during supersedure is unknown. This study has focused on queen aggression in terms of an age threshold above which queens may safely be introduced together for use in establishing multiqueen colonies and not on aggression between queens of different life stages, though future research along these lines might provide additional insight into the actual process of queen supersedure.

This experiment informs not only our knowledge of honey bee queen behavior but also insight into the application of establishing polygynous honey bee colonies. The potential utility of multiple honey bee queens coexisting together in the same colony is bolstered by a rapid loss of aggression, indicating that queens may be successfully introduced together while still relatively young, allowing for a longer productive time together. This is especially helpful given that polygynous colonies add an additional level of interaction in that the workers must accept multiple queens in addition to the queens tolerating each other. The process by which workers collectively decide to accept or reject queens remains largely unknown but using higher quality queens (of which age is a factor, as reproductive quality declines with age) seems likely to increase their acceptability to workers, in addition to increasing the potential lifespan of the queens if they are accepted (Butler 1957, Rangel et al. 2016, Tarpy et al. 2020).

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Table 1.1 Contingency table of age and fight outcomes

Age affects the outcome of the fight ($\chi^2 = 24.8$, df = 6, $p < 0.001$), this is driven mainly by significant differences between the oldest queens and the two youngest groups of queens (pairwise χ^2 tests with Bonferroni correction, p = 0.0352 for 3 week-old queens versus 12 week-old queens and p = 0.0130 for 5 week-old queens versus 12 week-old queens, all other comparisons were non-significant).

		Outcome			**Totals**
		Win	**Lose**	**Draw**	
Age (weeks)	**3**	13	11	6	30
	5	8	8	2	18
	7	10	10	6	26
	8	7	7	10	24
	9	4	4	12	20
	10	1	1	6	8
	12	2	2	18	22

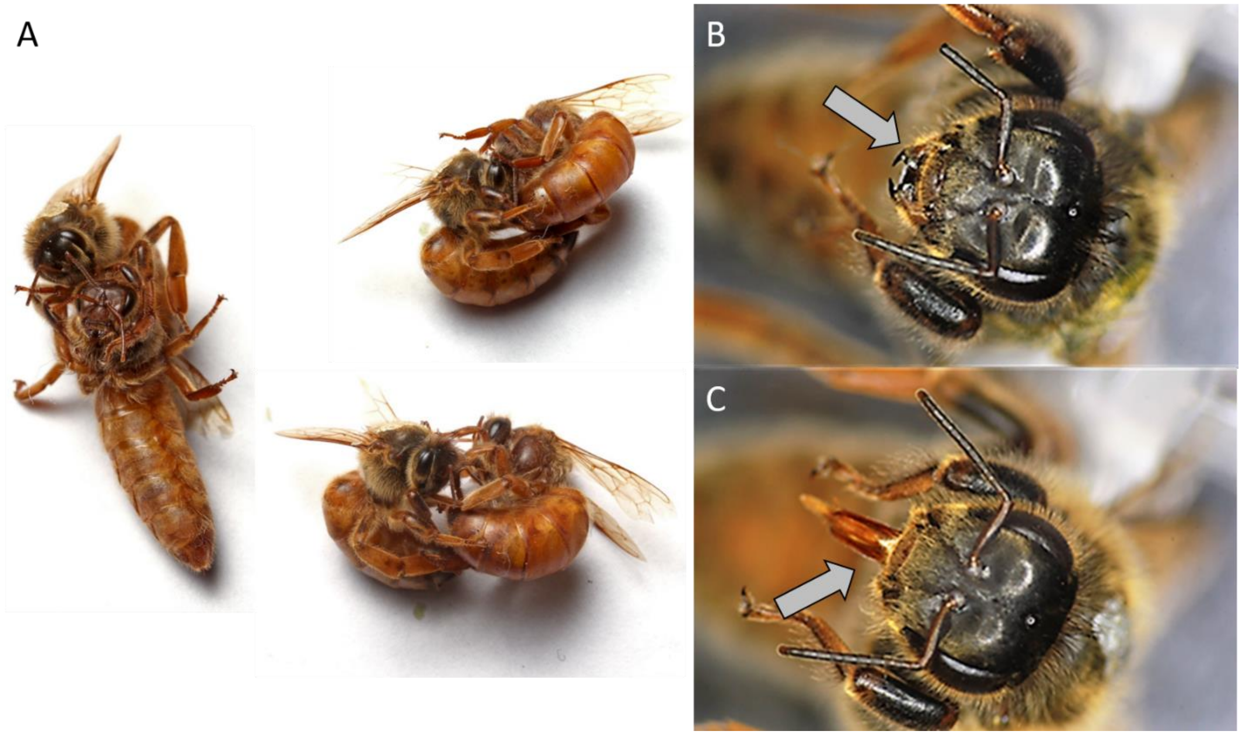

Figure 1.1 A: Dueling honey bee queens grasp each other with their legs and mandibles while attempting to sting each other. B: Queen with intact mandibles. C: Queen with mandibles ablated using microscissors.

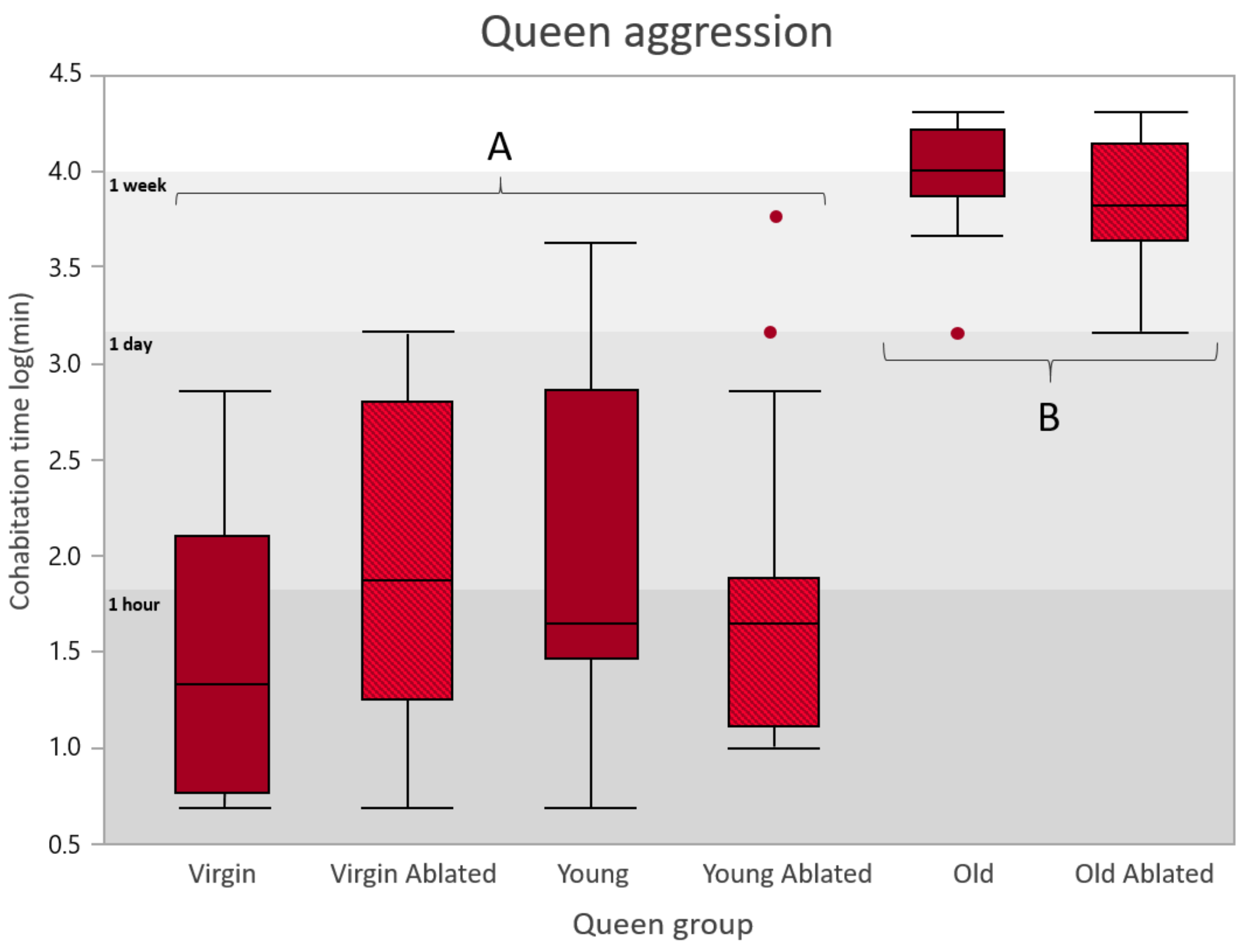

Figure 1.2. Aggressive behavior as measured by cohabitation time in queen duels across six groups based on age—virgin, young (newly mated), and old (>1 year)—and mandible ablation (unablated and ablated). Length of queen cohabitation is shown in log(minutes), with hour, day, and week threshold indicated. Old queens (both unablated and ablated groups; "A") survived significantly longer together than all groups of virgin and young queens ("B"; ($F_{(5,114)}$=24.90, $p<0.0001$). There was no significant difference between survival time of unablated and ablated queens in any age group.

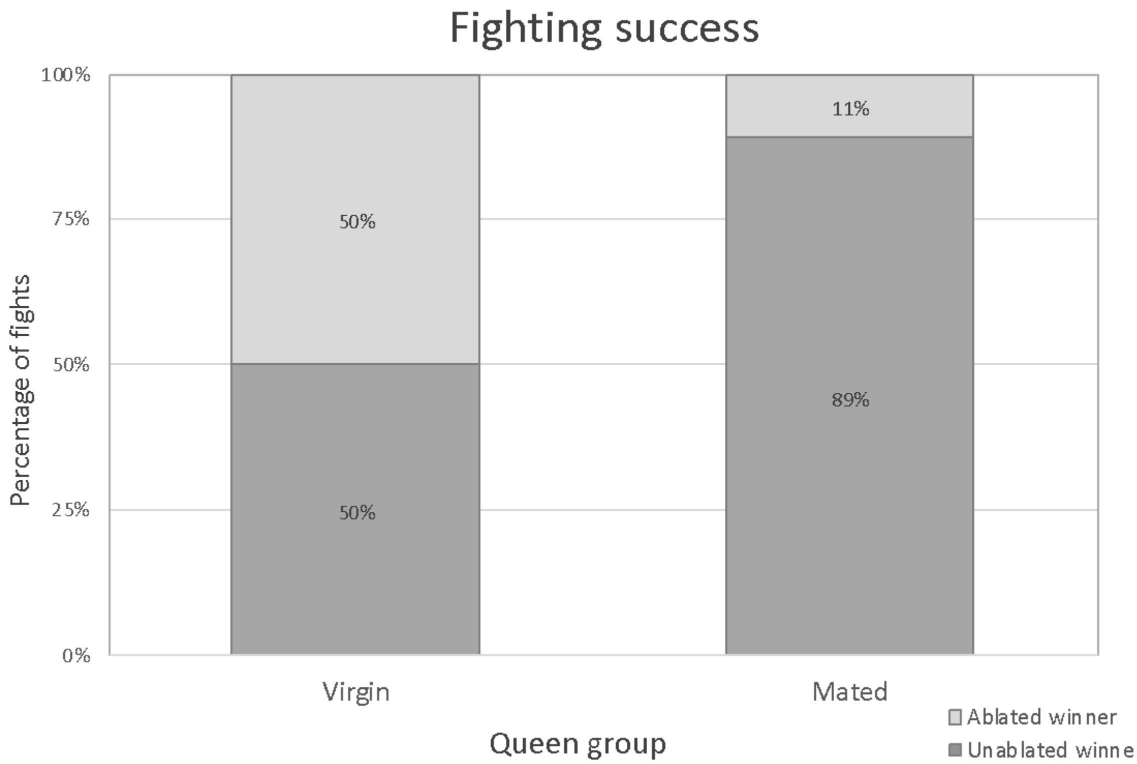

Figure 1.3. Effect of mandible ablation on fighting success in queen duels between queens with unablated mandibles and ablated mandibles in two age groups (virgin and young mated). Virgin queens had equal numbers of unablated and ablated winners, significantly different from duels of young queens, of which 89% were won by the unablated queen ($\chi^2 = 4.071$, $p < 0.05$).

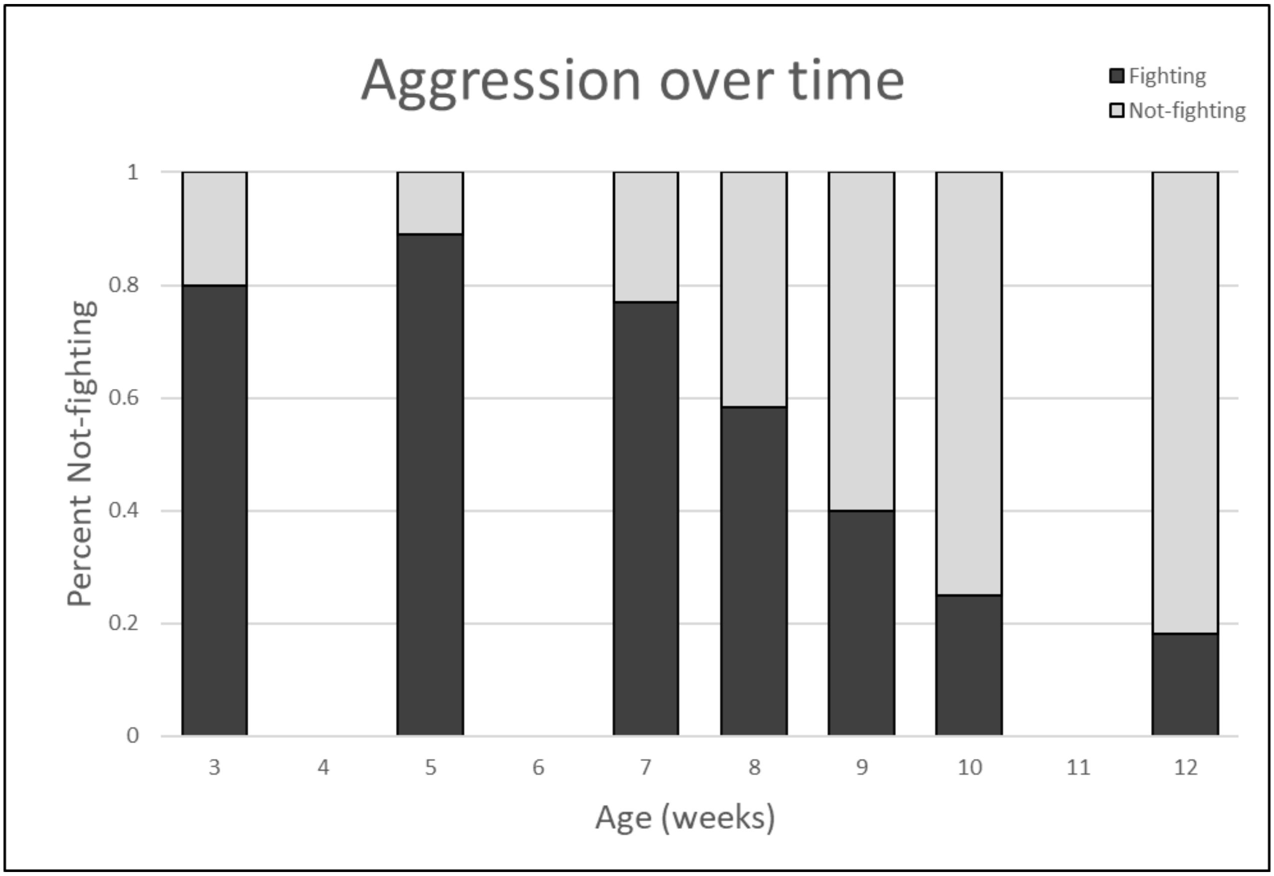

Figure 1.4. Percentage of queen pairs engaging in fatal duels (dark bars) or not (light bars) over time. Age affects the outcome of the fight ($\chi^2 = 24.8$, df = 6, $p < 0.001$), this is driven mainly by significant differences between the oldest queens and the two youngest groups of queens (pairwise χ^2 tests with Bonferroni correction, p = 0.0352 for 3 week-old queens versus 12 week-old queens and p = 0.0130 for 5 week-old queens, all other comparisons were non-significant).

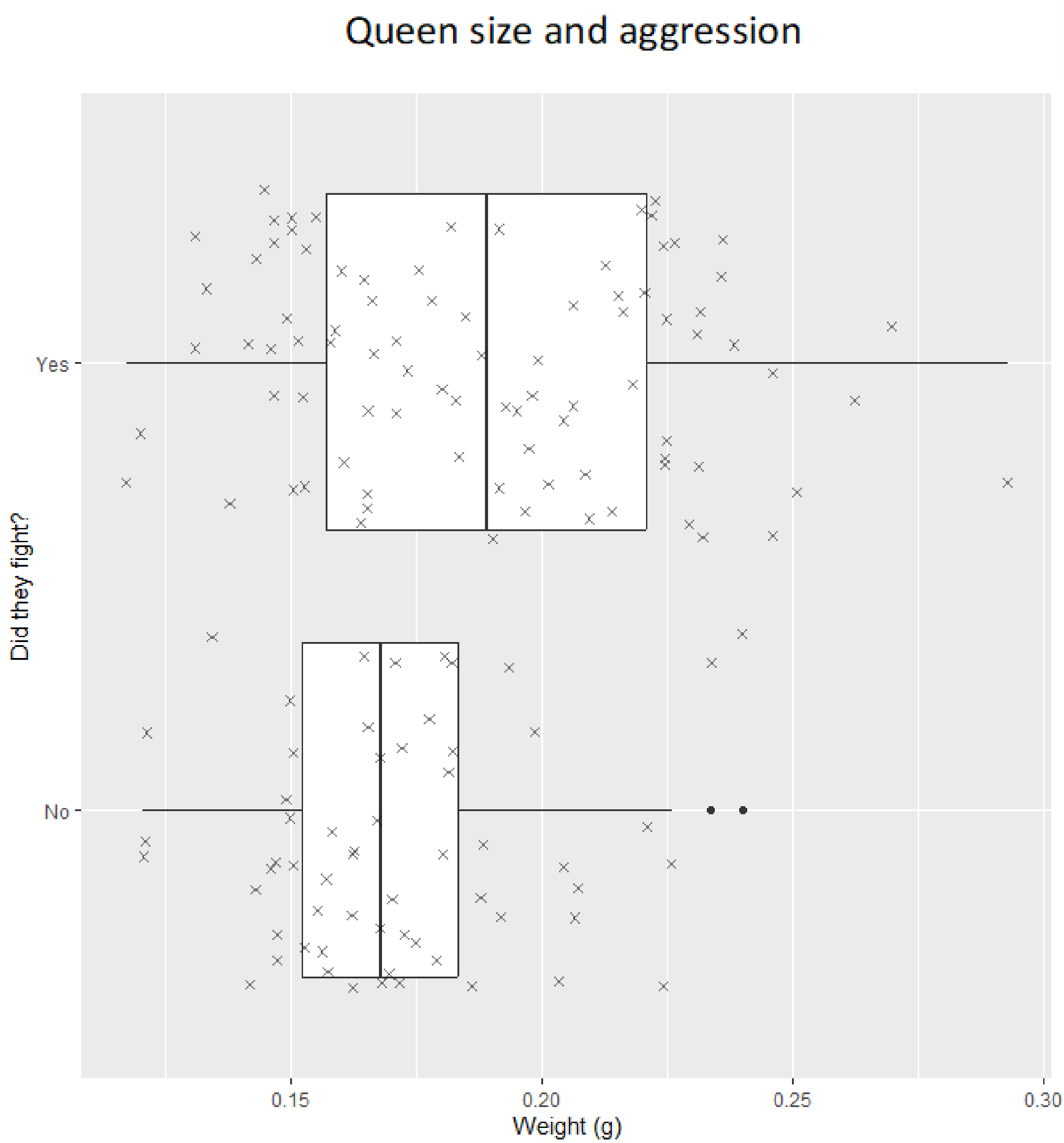

Figure 1.5. Larger queens are more likely to engage in fights (logistic regression, p = 0.003).

CHAPTER 2.

Sharing the throne:

Establishment of multiqueen honey bee (*Apis mellifera*) colonies

ABSTRACT

Western honey bee (*Apis mellifera*) colonies are typically monogynous, with a single reproductive queen whose health and productivity is crucial for colony success. Beginning with George Wells in the late 19th century, beekeepers have sought to increase colony efficiency and productivity by experimenting with methods of managing colonies with multiple (usually two) queens at a time. Although these efforts have demonstrated success in principle, they have failed to find widespread use due to prohibitive management requirements and remain a niche practice with limited applicability. By utilizing new information about honeybee queen aggression towards rivals we have developed a new approach to multiqueen beekeeping. Using non-aggressive mature (>1 year old) queens and newly emerged workers we have established polygynous colonies, with multiple queens freely living together in a shared broodnest and conducted microsatellite analysis of the resulting brood demonstrating true polygynous reproduction. By establishing colonies in which multiple queens share the same brood nest this method offers the potential for more widespread usage as the management of these colonies is more similar to that of traditional single queen colonies. The development of polygynous colonies also presents the potential for a new approach to managing queen failure, one of the primary challenges in the apiculture industry, by having multiple queens present to mitigate the effects of a failing queen.

INTRODUCTION

The Western honey bee (*Apis mellifera*) is both a model organism for the study of social behavior and evolution as well as an essential component of commercial agriculture as the primary species managed for pollination services (Klein et al. 2007, National Research Council 2007, Breeze et al. 2011). In recent years, the apiculture industry has been strained by prohibitive rates of annual colony losses (Neumann and Carreck 2010; Potts et al. 2010, vanEngelsdorp and Meixner 2010, vanEngelsdorp et al. 2010, vanEngelsdorp et al. 2012, Seitz et al. 2016), raising concerns for the future of commercial agriculture generally (Alzen and Hardin 2009, Calderone 2012). One major approach for addressing problems in honey bee health and survival is through improving queen quality, as queens are the sole reproductive in each colony and queen and colony success are inextricably intertwined (Winston 1987). Although high rates of queen failure are implicated as a major contributor to colony losses (Pettis et al. 1991, vanEngelsdorp et al. 2013, Sandrock et al. 2014) the causes of queen mortality remain unclear (Delaney et al. 2011, Tarpy et al. 2011, Traynor et al. 2016).

Honey bee research and queen breeding programs focused on improving genetic traits have produced lines with improved disease and parasite resistance, hygienic behavior, reduced defensiveness, and increased honey production (Harbo and Harris 1999, Rinderer et al. 2001, Spivak and Reuter 2001a, Spigak and Reuter 2001b, Büchler et al. 2010, Rinderer et al. 2010, O'Shea-Wheller et al. 2022). Researchers have also focused on environmental factors affecting queen fecundity, including environmental exposure to pesticides and chemical treatments directly applied to honey bee colonies to combat the parasitic *Varroa destructor* mite (Haarman et al. 2002, Rangel and Tarpy 2015). Despite progress in these areas, queen loss remains a persistent challenge for beekeepers, with over 1 million replacement queens purchased annually in the US and many operations engaging in annual routine queen replacement even though queens are capable of remaining healthy and productive for several years (Winston 1987). Due to the persistence of these problems, we have investigated the potential for managing honey bee colonies with multiple queens as an alternative approach to mitigating queen problems.

Among social insects, polygyny effectively decreases the importance of individual queens. With multiple queens sharing the reproductive burden there is less pressure on individual queen fecundity as well as reduced impact on the colony when a queen dies. Although honey bees are not typically polygynous, beekeepers have long been interested in creating multiqueen management systems as a means to leverage the increased productivity of larger colonies (Farrar 1937, Farrar 1953, Holzberlein 1953, Farrar 1958, Miller 1953, Moeller and Harp 1965, Peer 1969, Walton 1974, Moeller 1976, Zheng et al. 2009, Nabors 2016, Hesbach 2016). While these various approaches to multiqueen beekeeping have had limited success they provide a foundation for new research.

The primary challenges to establishing multiqueen colonies are queen aggression—individual queens must tolerate the presence of rivals—and worker aggression—workers must accept and attend to multiple queens in the colony. As discussed in the previous chapter, honey bee queens are known for engaging in deadly duels to monopolize colony reproduction but, in vitro at least, this aggression dissipates soon after emergence, suggesting the potential for establishing polygyny among mature queens. Worker rejection of queens is comparatively poorly understood. When queens are placed in individual protective cages workers will attend to them indefinitely and in this way many queens at a time can be “banked” in colonies until they are needed elsewhere. However, workers are also known to directly attack and “ball” queens (**Figure 2.1**), especially newly introduced foreign queens (though Spoja [1953] reported success with multiqueen acceptance in colonies with workers of all age groups). This collective behavior to reject queens appears to be moderated by older workers in the colony (Robinson 1984), a fact which may be exploited to establish multiqueen colonies (Zheng et al. 2009).

We have conducted this study to establish a method of creating multiqueen honey bee colonies by using mature queens and young workers to avoid the problems of queen-queen and worker-queen aggression.

METHODS

Queen source

To avoid queen-queen aggression we used >1 year old cull queens of Italian stock that were being systematically replaced in a commercial beekeeping operation. Upon receipt we banked these queens until use (Harp 1967, Wyborn et al. 1993, Gençer 2003). We conducted trials to verify that these queens were not aggressive towards each other by placing groups of them together in petri dishes as in the previous two chapters. Before placing them in colonies we weighed each queen and paint marked it for individual identification.

Colony establishment

To set up polygynous colonies we first tried a young worker method, adding queens to colonies consisting of frames of open larvae and nurse bees set up similar to Zheng et al. (2009), by collecting frames of open brood from established colonies and shaking them to remove older workers while younger workers remain clinging to the frame. We then added 10 caged queens for 24 hours before releasing them into the colonies.

To further reduce worker aggression, we next used colonies with only callow (newly emerged) workers by collecting frames with emerging worker brood, brushing all bees off of them, and placing them in an incubator set at 34.5 °C for 24 hours. We then made up six colonies by combining three frames of emerging brood with newly emerged workers on them with a frame of honey to sustain them until they could begin foraging in five frame nucleus hives. We then added 10 queens each and kept each colony in the incubator with the entrance closed for an additional 24 hours to stay warm while more workers emerged. We then moved them to them to an apiary site and opened the entrances to allow the workers to begin foraging as well as providing each colony with a ~50% sugar solution to ensure that they had an adequate food supply and enable colony growth.

Brood analysis

To test for true polygyny in these colonies, with multiple queens reproducing as well as cohabitating, we collected samples of emerging brood to genotype. We sampled 192 workers from each colony by removing one metathoracic leg and cutting it into several pieces using microscissors, then performed DNA extractions in 0.2 ml strip tubes, using 150 µl 5% Chelex® 100 (BioRad) in dH_2O, plus 5 µl 10mg/ml Proteinase K. We ran reactions in a thermocycler with the following program: 60 min at 55 °C, 15 min at 99 °C, 1 min at 37 °C, 15 min at 99 °C, then held at 4 °C.

For genotyping, we conducted multiplex PCRs to amplify 7 microsatellite markers (A24, A76, A88, A113, Ap43, Ap81, and B124; Estoup et al. 1993, Delaney et al. 2011, Evans et al. 2013) using 1 µl of extracted DNA template, 5 µl multiplex PCR Mastermix (Kapa), 0.67 µl combined fluorescent-tagged primers, and 3.33 µl lab grade H_2O, for a total reaction volume of

10 μl. We conducted the reactions with the following program: 3 min at 95 °C, followed by 48 cycles of 15 s at 95 °C, 30 s at 57 °C, 45 s at 72 °C, with an elongation step of 5 min at 72 °C and held at 4 °C. For fragment analysis we combined 1 μl of PCR products (diluted 10:1 with lab grade H_2O) with a 9 μl solution of Gene Scan Liz 500 sizing standard (Thermo Fisher) in Hi-Di Formamide (50 μl Liz 500 per 900 μl Formamide), denatured for 5 min at 95 °C, and had the samples sequenced on a 3730 DNA Analyzer (Applied Biosystems) by the NCSU Genomic Sciences Laboratory (Raleigh, NC).

To estimate the number of matrilines present we scored the microsatellite markers of each sample using GeneMapper® v4 software (Applied Biosystems) and conducted genotype analysis of the scored microsatellite loci using Colony 12, a program for conducting pedigree analysis that designed to estimate relatedness (parent/offspring as well as full and half-siblingship) from population samples of unknown relatedness and is designed to accommodate variables such as haplodiploidy and polygamy in one or both parents (Wang and Santur 2009, Jones and Wang 2010). First, we ran Colony analysis on offspring samples only with an unspecified number of matrilines and patrilines within each matriline. Next, we collected actual queen markers from the established colonies by non-destructively sampling queen wingtips for DNA and amplifying the markers according to the same PCR protocol. We then ran Colony analysis a second time, including these known queen samples to refine the estimate of polygynous reproduction. Due to variability in DNA extraction and PCR amplification, the usable data for each colony consisted of 156-187 worker samples and 4-6 microsatellite markers per colony (Appendix A).

RESULTS

Colony establishment

We found low success with the young worker method, observing signs of worker-queen aggression upon queen release and finding that most queens were eliminated (0-4 queens remaining per colony) within one week of colony establishment. The newly emerged worker method was more successful, with each colony having 7-9 queens after one week. One colony failed entirely before any new workers emerged and was not included in the remaining analysis but the remaining five colonies remained polygynous, with queens coexisting and observed laying eggs in a common broodnest (**Figure 2.2**) until they were combined with stronger colonies at the end of the season, approximately 3 months after establishment, in accordance with standard beekeeping practice.

Brood analysis

The first round of Colony 12 genotype analysis of the five multiqueen colonies using only worker samples produced estimates of 8-14 queens per colony, with estimated mating numbers for each queen ranging from 1-7 with a mean value of 3.3 (**Figure 2.3**). The second round of analysis incorporating queen samples produced lower estimated queen numbers in all but one case, ranging from 7-14 queens per colony, with higher estimated mating numbers, ranging from 1-14 mates per queen with a mean value of 4.6 (**Figure 2.4**). As each colony was established using 10 queens, it is clear that even the second round analysis is not an entirely accurate estimate of the number of matrilines per colony.

DISCUSSION

The results of this study provide the clearest documentation to date of polygyny in honey bees and a method for establishing polygynous colonies. The success of this method of establishing multiqueen colonies using queens with intact mandibles provides further, *in vivo*, support for our previous conclusion that mandible ablation is not a determinative factor in queen-queen aggression, which is driven instead by queen age (Chapter 1). The use of >1 year old queens in this study served primarily to avoid any potential queen-queen aggression as a variable, but also to demonstrate a potential application for polygynous beekeeping by utilizing queens which would otherwise be mass-culled. If future development reveals the establishment of polygynous colonies to be practical, then the large-scale beekeepers who engage in systematic requeening would have the option to use these non-aggressive queens to establish polygynous colonies rather than writing them off as a loss. Based on the queen aggression data from Chapter 1 we do expect that it will be possible to establish polygynous colonies using much younger queens, allowing for broader applications of this technique, but the practical limits of queen age and aggression for polygyny will need to be established in future study, along with assessment of the growth rate and productivity of polygynous colonies established in this manner.

Similarly, the use of only callow workers effectively eliminates the potential for workers to reject queens during the initial colony establishment as these workers are guaranteed to be too young to exhibit aggression and, in fact, not sufficiently hardened to be capable of stinging. This presents a significant tradeoff, however, as callow bees are both challenging to obtain (requiring an incubator), and the lack of older bees significantly delays the growth of colony productivity as all of the workers in the colony must age into tasks such as foraging. Because of this, further investigation into the role of worker age in worker rejection of queens is a high priority for further development of this method. The ability to use even moderately older workers that could more easily be collected, such as described by Spoja (1953) and Zheng et al. (2009), would substantially increase the practicality of this method. Given the reported success of these previous researchers, such an improvement in this method seems likely to be achievable.

The observation of egg laying by multiple queens along with the results of the brood analysis provides strong evidence that queens were reproducing and not merely cohabitating in these colonies. While the pedigree analysis from Colony 12 provides support for the presence of polygynous reproduction it is clearly not an exact estimation of the relationships within the polygynous colonies as, in some cases, it calculated more matrilines than the possible number of queens present. Even in the second round, where the inclusion of queen markers reduced the estimated number of matrilines within the population of each colony, the calculation appears to still be overestimating the number of queens and underestimating the number of drones each queen mated with. misattributing paternal genetic diversity as increased maternal genetic diversity. Honey bee queens are “hyperpolandrous”, typically mating with more than 10 and possibly more than 50 drones each (Palmer and Oldroyd 2000, Tarpy et al 2015, Withrow and Tarpy 2016). This means that each polygynous colony contains the offspring of not only multiple queens but also of the dozens, or potentially even hundreds, of drones who mated with those queens. While the Colony program is designed to accommodate data from polygamous species, it appears to be misattributing the extremely high paternal genetic diversity as excess maternal genetic diversity. Higher samples sizes would likely improve the calculation and provide a more accurate pedigree analysis, as appears to be the case with patriline analysis in single-queen colonies (Withrow and Tarpy 2016), however, these flawed estimates still provide evidence of polygynous, rather than monogynous, reproduction in these colonies.

Achieving this state of stable polygyny with multiple queens living and producing offspring in a shared brood nest requires circumventing several natural processes by which honey bees eliminate queens, thereby returning to a state of monogyny. Maintaining cohabitation of multiple queens requires that both workers and other queens tolerate the presence of additional queens without exhibiting direct (lethal) aggression towards them. However, true functioning polygyny, with multiple queens reproducing together in a shared brood nest, requires more. This level of polygyny requires that queens are not subject to indirect (non-lethal) aggression or interference that would prevent them from reproducing. It is conceivable that the presence of rival queens might pheromonally disrupt a queen's normal reproductive behaviors, as the presence of queen pheromone plays an essential role in colony functioning, including suppressing reproductive development in workers (Butler and Fairey 1963, Velthuis 1970, Hoover et al. 2003). While the data here indicate that the presence of multiple queens are not preventing each other from producing eggs in this manner, further analysis of the pheromonal impact of multiple queens on each other and on colony functioning are warranted. Similarly, while it appears here that workers are not neglecting queens or selectively cannibalizing the eggs of some queens (worker policing of eggs laid by fellow workers is a component of the evolution of eusociality in honey bees; Ratnicks 1988, Ratnicks and Reeve 1992, Olroyd and Osborne 2006) the factors driving worker preferences for and rejection of queens even in single-queen colonies remain largely unknown (Butler 1957, Rangel et al. 2016, Tarpy et al. 2020). Whatever pheromonal and behavioral impacts that the presence of multiple queens may have it seems clear that they are not a barrier to the practical functioning of these colonies.

Multiqueen beekeeping has remained a tantalizing prospect since Wells (1894) first wrote on the subject. Yet, despite the widely attested benefits to productivity, the management constraints have been a barrier to significant adoption. Polygynous multiqueen colonies, in the legacy of Alexander (1907), present an alternative approach that, once established, are easier to manage and provide a built-in safeguard against queen failure. Despite these advantages, polygynous colonies have received comparatively little experimentation by beekeepers and very little formal research. These findings suggests that, contrary to popular belief, the chief barrier to polygyny in honey bees may be worker, and not queen, aggression. Under normal (monogynous) circumstances, a failing queen is an existential threat to colony survival and, further, queen replacement through supersedure is one of the relatively few options that honey bees can take in response to adverse circumstances. Because of this, the collective decision to reject a queen is multifaceted and driven by multiple colony-level variables. While it is possible to preclude worker-queen aggression by restricting the colony population to only young workers as investigated here, a reliable method based on overall colony condition and provisions would be a significant breakthrough in making polygynous colonies practical (Alexander 1907, Spoja 1953, Butz and Dietz 1994, Brother Adam 2018). Unraveling the mystery of queen rejection would not only address the problem of unwanted supersedure but also greatly reduce the barrier to establishing multiqueen colonies, making this technique more applicable beekeepers.

The prototype method developed in this study serves as a proof of concept for polygynous beekeeping, which offers a means to achieve the benefits of multiqueen beekeeping without the management challenges of the typical doubled-hive approaches. As previously discussed, future development of this method should assess using younger queens and older workers to find the actual age boundaries for establishing polygynous colonies, which will be an important factor in determining the practicality of polygynous beekeeping. Additional topics for future research include assessment of the life-cycle and longevity of polygynous colonies, including overwintering success and examination of whether queen lifespan or failure rates are impacted by living in polygynous colonies. Ultimately, while this experiment suggests that polygynous beekeeping may provide a more accessible approach to multiqueen beekeeping, the primary value of this research paradigm and any further development of this method depends on there being a reasonable chance that this technique would actually prove useful to beekeepers. An assessment of beekeeper interest in polygynous beekeeping and the potential barriers to its adoption would provide the clearest insight into how future development of this method might best proceed.

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Figure 2.1. Ball of honey bee workers attacking a rejected queen.

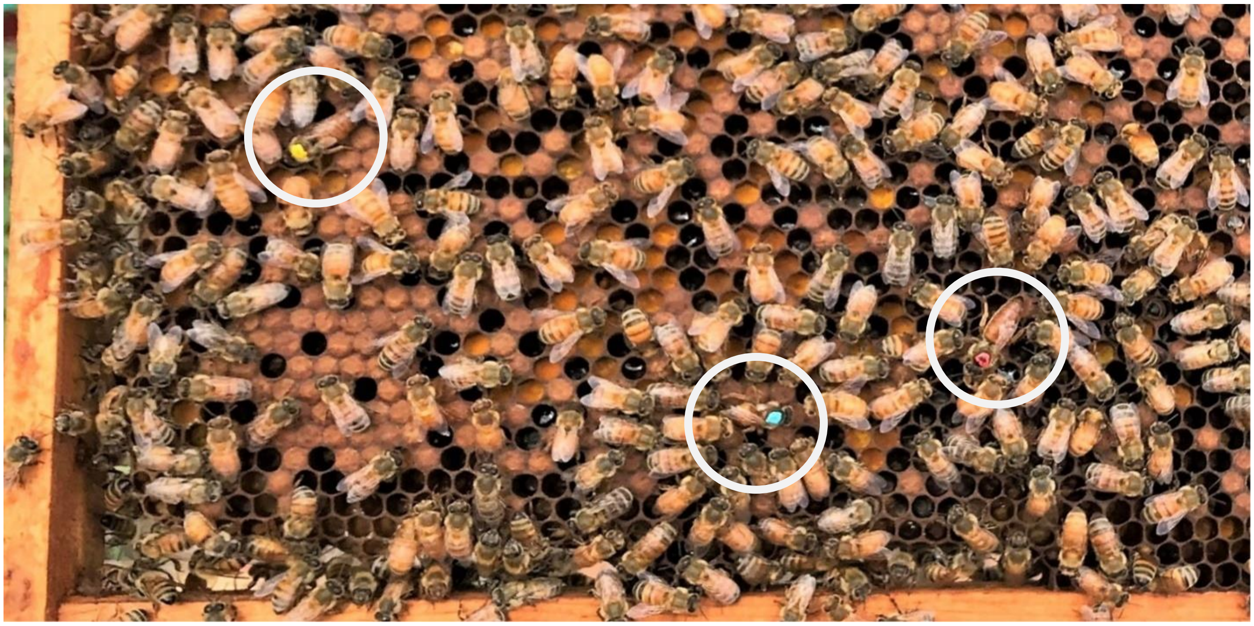

Figure 2.2. Group of honey bee queens in a shared broodnest.

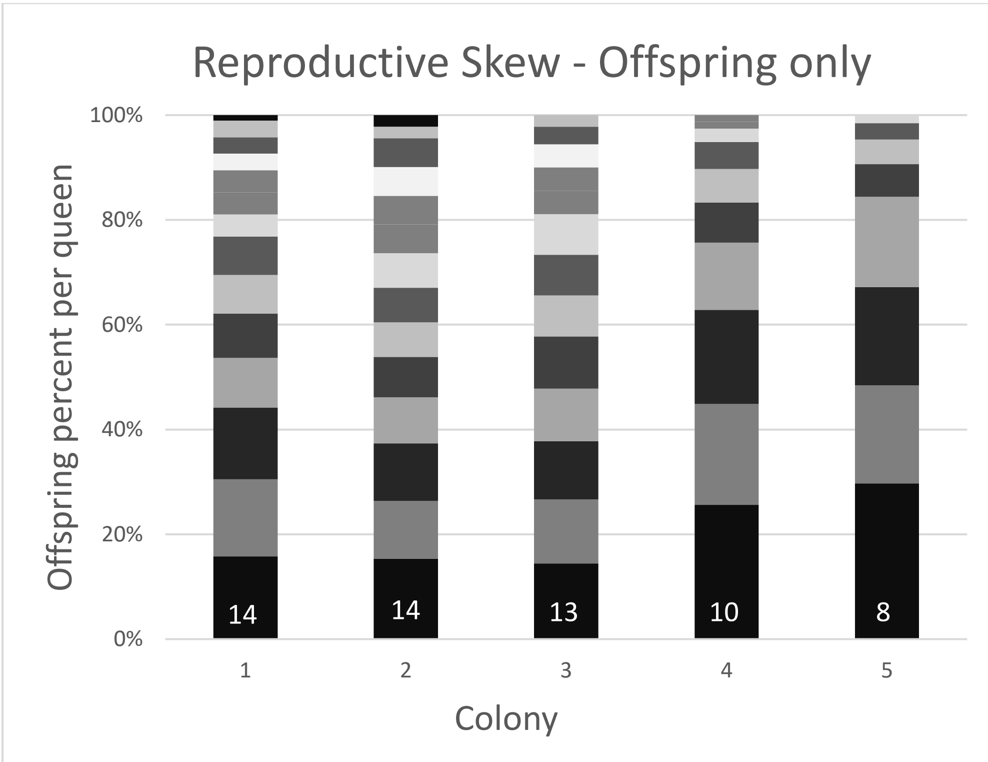

Figure 2.3. Worker offspring grouped into purported matrilines based on microsattelite analysis of offspring DNA. Number of purported matrilines per colony given, grouped from most to least abundant.

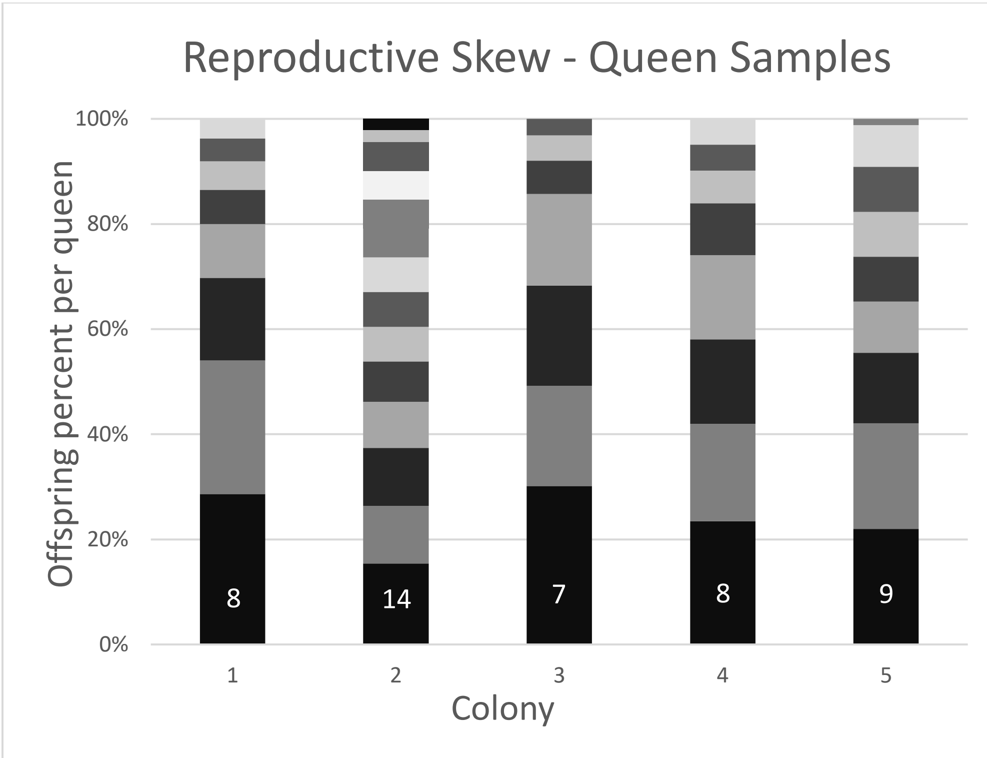

Figure 2.4. Worker offspring grouped into purported matrilines based on microsattelite analysis of offspring DNA and queen DNA extracted from queen wings. Number of purported matrilines per colony given.

CHAPTER 3.

Adoption potential of multiqueen beekeeping

ABSTRACT

The success of an applied research paradigm requires not only that the results of the project provide a solution or improvement to the focal problem, but also that such a solution is reasonably implementable by the stakeholders. This study explores technology adoption among beekeepers, using a novel management technique of polygynous multiqueen beekeeping as a case study to both inform future research projects about the potential viability and barriers to adoption of this technology and to provide broad information on the patterns and approaches of beekeepers to the adoption of new technology for the use of the key stakeholder groups of researchers, extension agents, and beekeepers themselves. Through analysis of survey data from 94 hobbyist and professional beekeepers this study finds that beekeepers are highly amenable to the adoption of new methods and technologies, being organized in communities (local and online) dedicated to sharing information and techniques to address the myriad of management challenges in beekeeping. Participants exhibited overall enthusiasm for the idea of multiqueen beekeeping, including responding especially strongly to the novel framing of using polygynous colonies as a potential means of mitigating queen problems, while providing useful insight into the barriers to adoption that should be addressed by any future research developing this method. In particular, this study identifies a profile of large (managing 50+ hives) hobbyist beekeepers who would be excellent candidates to directly include in the research process as they are experienced beekeepers with both large operations and innovative dispositions amenable to participating as research partners.

INTRODUCTION

This study examines technology adoption behavior among beekeepers as well as the adoption potential of a new management technique for beekeeping: polygynous mutliqueen honey bee colonies. Beekeepers face a range of challenges that can be addressed through the development and adoption of new management practices. One of these major problems is queen failure, when queens exhibit a variety of problems ranging from reduced reproductive capacity to death. This study is focused on beekeeper interest in a new approach to multiqueen beekeeping (the establishment of honey bee colonies with multiple reproductive queens instead of just one) as an example of technology adoption among beekeepers.

To understand the significance of this new technology we need to consider the context of beekeeping. Beekeeping is an important industry at both large and small scales. Migratory beekeeping is essential for commercial food production, with large-scale operations transporting thousands of hives around the country each year to provide pollination services, but beekeepers with smaller sideline and hobby operations are widespread in urban, suburban, and rural environments (vanEngelsdorp and Meixner 2010, Seitz et al. 2016). While many management practices and concerns vary widely across the different types of beekeeping, some problems, including queen failure, are common for all types of beekeepers (vanEngelsdorp and Meixner 2010, Neumann and Carreck 2010, vanEngelsdorp et al. 2012, Seitz et al. 2016).

As the only reproductive female in a honey bee colony, a healthy and productive queen is essential to the success of the colony (Winston 1987). While honey bee queen research and breeding programs often focus on producing high quality queens, an alternative means to alleviating the concern of queen problems is to establish colonies with multiple queens, where the success of each individual queen is no longer critically important to the colony. While multiqueen beekeeping is uncommon, beekeepers have experimented with multiple systems for doing this, typically with the goal of creating larger and more productive colonies (Wells 1894, Farrar 1937, Farrar 1953, Holzberlein 1953, Miller 1953, Farrar 1958, Moeller and Harp 1965, Peer 1969, Walton 1974, Moeller 1976, Zheng et al. 2009, Nabors 2016, Hesbach 2016. In particular, this study is focused on a new prototype method in which colonies are established with multiple queens living freely together in the same brood nest. Current research on this method demonstrates the possibility of establishing these "polygynous" multiqueen colonies but many questions remain about the feasibility of this method, including whether there are beekeepers who would be sufficiently interested in and able to utilize this method.

Through a survey of professional and hobbyist beekeepers, this study evaluates receptiveness and potential challenges to the implementation of multiqueen beekeeping among different types of beekeepers to evaluate the potential impact of this technique as well as to identify a target population of beekeepers for whom further development of this beekeeping method would be most applicable. This study also identifies significant concerns in beekeeping and patterns of technology interest and adoption that are informative for beekeepers, honey bee extension, and honey bee researchers. In particular, my research aims to lay a groundwork for future research developing multiqueen beekeeping in partnership with the beekeepers who are most likely to benefit from the implementation of this technique.

METHODS

To assess beekeeper interest in this novel method of multiqueen beekeeping I created a survey, beginning with a one-page informational flyer [See Appendix A]. The survey was conducted via Qualtrics with all responses anonymous. The survey asked participants about the size and type (hobbyist or professional) of their operations, their familiarity with multiqueen beekeeping and whether they had ever attempted to establish multiqueen colonies. In addition, Likert-type scale questions probed participants about their perceptions of queen problems in beekeeping and the efficacy of current honey bee research, their general disposition towards adopting new technology, and their interest in the method proposed in the one-page flyer. Finally, free response questions to solicit additional information on four topics: (1) Sources of information used by beekeepers, (2) Problems in beekeeping, (3) Questions about the method described at the beginning of the survey, and (4) Challenges to adoption of this method [see Appendix B for survey instrument].

For this study I targeted beekeepers in North Carolina and California. North Carolina was selected because of its proximity and large number of independent beekeepers (with 82 county and regional constituent chapters of the North Carolina State Beekeepers Association) and because the long-standing history of apiculture extension at NC State would provide a basis for trust and likely increase the response rate. California was selected to increase variation of the sample, as the largest beekeeping state (USDA-NASS Honey Bee Colonies Report, 2021) with a large commercial beekeeping presence.

To recruit participants, I identified local beekeeping clubs listed as affiliated chapters of State level beekeepers associations as well as web searches for county-level clubs. I contacted clubs through their websites and Facebook pages with a message requesting that they distribute the information and survey to their members [See Appendix C]. To solicit additional professional beekeepers I also contacted individual beekeepers publicly listed as members of the California Bee Breeders Association. Responses indicating that the person did not agree to participate (2 responses) and those with less than 44% completion (14 responses) were removed from the dataset prior to analysis. All 14 responses removed for incompletion were abandoned prior to answering questions about the size and type of operations, making it impossible to determine any characteristics of this group.

I maintained the data in an Excel spreadsheet. To analyze participant responses, I examined closed-ended questions to identify patterns of respondent types, particularly relating to those most and least likely to try new beekeeping methods. For open-ended questions I coded the data according to relevant response clusters. In keeping with our primary and secondary objectives to identify barriers to adoption of multiqueen beekeeping and inform future research priorities based on beekeeper needs I developed code groups aligned with the four free-response questions: (1) Sources of information used by beekeepers, (2) Problems in beekeeping (3) Questions about the method described at the beginning of the survey, and (4) Challenges to adoption of this method. In the first round of coding, I adopted a provisional code set based on anticipated response types drawn from the researcher's experience discussing research with beekeepers in extension settings (Saldaña 2016, p. 168), modified and supplemented by in vivo coding of emergent features (Saldaña 2016, p. 105). Validity was enhanced by regular peer debriefing. After an initial pass with a list of 5-9 codes per topic I applied pattern coding in a second pass, reorganizing our codes into 4-5 clusters per topic (See Appendix D for the final list of codes and definitions). Finally, I combined the two analytic strategies to identify the concerns most relevant to the high and low-adopting populations to better understand their respective needs and develop recommendations.

RESULTS

I present results in three main sections: (1) general results, (2) attitudes toward innovation, and (3) analysis of most and least-likely adopters. The general results begin with an overview of the participants, including assessment based on profession (professional or hobbyist beekeepers) and size of operation. This section also includes evaluation of the sources of information beekeepers rely on and the major problems they face in beekeeping. The attitudes towards innovation section covers the participants' attitudes towards innovation generally as well as their response towards multiqueen beekeeping, including evaluation of participants questions about the method presented and challenges to adoption of this method. The analysis of most and least-likely adopters presents patterns of innovation tendency among participants and analysis of the high-adopting ("Experimental") beekeepers in comparison to the low-adopting ("Skeptical") beekeepers.

General results

A total of 112 participants initiated the survey. Of these 2 selected that they did not wish to participate and an additional 14 were removed from analysis due to having completed less than 44% of the survey. The responses of the remaining 94 participants, including 8 incomplete responses, constitute the data for this analysis. While this study did not evaluate the representativeness of the participant population, the survey collected demographic information on occupation (professional or hobbyist beekeepers) and size of operation as well as participant age and gender (**Table 3.1**). These demographics are fairly diverse overall, however, the relatively small number of professional and large beekepers, as well as lack of information on experience level (such as length of time as a beekeeper), and inability to assess regional patterns (e.g. North Carolina beekeepers vs California beekeepers) due to IRB restrictions on collecting participant information are limitations to this study.

Because of the real-world management and priority differences between professional and hobbyist beekeepers as well as large and small operations, I examined these groups for differences in their survey responses. Size of beekeeping operation is closely related to occupation, with all 8 professional beekeepers reporting that they have large operations (at least 20 hives) whereas the majority of hobbyists managed small operations. Only 8 hobbyist beekeepers reported managing at least 20 hives and, of the hobbyist beekeepers managing smaller operations, half reported managing very small operations of 1-5 hives. In general, professional and hobbyist beekeepers were very similar in their responses to this survey, not exhibiting clear distinctions related to adoption behavior, perception of queen problems in beekeeping, opinion of research priorities, and coded responses to the free-response questions. Similarly, since most of the large beekeepers in the study also reported being professional beekeepers, I did not find participants to be distinct in these general comparisons based on size of operation, despite the largest beekeepers managing at least 10x as many hives as the smallest beekeepers. However, professional beekeepers did indicate greater familiarity with the idea of managing multiple queens in a single brood area; all of them indicated that they had at least heard of this practice. By contrast, a majority of hobbyist beekeepers indicated that they had never heard of this. Professional beekeepers were also more polarized than hobbyists in their interest in multiqueen beekeeping, with half indicating interest and the other half disinterest while most hobbyists indicated interest.

The first two free response questions were written and coded to examine participant's opinions on (1) Sources of information used by beekeepers and (2) The most significant problems in beekeeping. **Tables 3.2 and 3.3** provide the code lists and number of responses for each of these questions. The key results are as follows:

Sources of information used by beekeepers

When asked about sources of information they rely on, participants gave more responses than for any of the other free response questions. A majority cited local clubs and mentors and many also referenced industry sources (such as trade journals and information from regional and national conferences), internet and popular figures in beekeeping, and research and extension sources. e.g.:

> *"Our [County Name] Beekeepers Club and members. I am awaiting my first copy of American Bee Journal*."
>
> "*Other beekeepers I respect, Randy Oliver [well known beekeeper with a large online presence], ABJ [American Bee Journal]*"
>
> "*Bee clubs and youtube*."
>
> "*Bee club members, reading, my own intuition*"

It is clear that beekeepers are aware of and utilize a wide range of resources to inform their beekeeping practices but that they rely most heavily on the advice and experience of people they know. While this survey does not examine how beekeepers synthesize or prioritize information from various sources, these results suggest that partnering with local beekeepers to try a new method or technology would be the most effective way to facilitate successful implementation of that technology.

Most significant problems in beekeeping

The most common management problem cited by participants was disease, including disease vectoring parasitic *Varroa destructor* mites. Participants also made significant references to queen problems, swarm management, and environmental concerns such as weather and resource availability. e.g.:

> *"Varroa and the frequency of treatments needed to keep the mites below threshold."*
>
> "*Early queen failure is always an issue. Good nutrition during the honey dearth is also one of my biggest problems. Many hives do poorly on a sugar syrup diet and current supplements don't seem to help much*."
>
> "*Not being able to predict or prevent swarming*."

These are typical of problems reported by beekeepers, and responses citing swarming and basic management concerns are especially typical of newer beekeepers.

Attitudes toward innovation

As willingness to try technology and management techniques is a central part of this study, the survey asked participants multiple types of questions about their approach to trying to techniques. Using Rogers (2003) typology of adopters to categorize the participants approximately 5% of participants are classified as Innovators, approximately 40% each as Early Adopters and Early Majority, approximately 10% as Late Majority, and approximately 1% as Laggards.

Overall responses regarding participants' tendencies to try new techniques indicate a consistent pattern of technology adoption behavior among participants. Throughout the survey, participants who self-identified as more innovative indicated greater interest in trying new beekeeping methods generally, greater interest in the experimental method of multiqueen beekeeping presented in the survey and were more likely to indicate actual experience attempting to establish multiqueen colonies in the past.

While this survey presents an overview of a novel technique for multiqueen beekeeping this is a practice that already exists, albeit uncommonly, in multiple forms. Many participants were familiar with the concept of multiqueen beekeeping, and a minority reported having attempted it themselves. While these beekeepers with firsthand multiqueen experience indicated much greater familiarity with both vertical and horizontal systems than other beekeepers, majorities of both groups reported never having heard of polygynous systems before.

Despite this unfamiliarity, a majority of participants in all categories of innovativeness except the slowest adopters indicated interest in multiqueen management to both address queen problems and to increase colony productivity. However, for queen problems, more innovative participants indicated greater interest in multiqueen management than less innovative participants while, for increased productivity, interest was more stable across innovativeness. Though not explicitly linked, many participants identified queen failure as a significant challenge in beekeeping, including in free responses, as seen in **Table 3.3**, e.g.:

"*Losing queens and varroa mites*"

"*Frequent supercedure. Badly mated queens.*"

In contrast, another significant management challenge, losing bees to swarming, would potentially be exacerbated by having faster growing and/or larger colonies, e.g.:

"*Swarming, failure to thrive of post-swarm colonies*.",

"*Managing spring buildup. The balance between swarming and being strong when honey flow starts is challenging*.", and

"*I have lost several queens this year and have had a couple of swarms. I would like to hear more about multiqueen but have the following 2 concerns: are multi queen colonies more likely to swarm, I run single deep brood nests, how much more time and effort does it take to establish a multiqueen colony*?"

While participant interest in multiqueen beekeeping was fairly high, this method is not well suited to all types of beekeeping operations, as is further explored in the next set of open-ended questions.

The second pair of open-ended questions targets participants' attitudes towards the experimental method of multiqueen beekeeping presented in the survey and were written and coded to evaluate: (3) Questions about the experimental method of multiqueen beekeeping, and (4) Challenges to adoption of this method. **Tables 3.4 and 3.5** provide the code lists and number of responses for each of these questions. The key results are as follows:

Questions about the multiqueen beekeeping method described at the beginning of the survey

Most of the questions asked about the new method described in the survey related to basic understanding of how to set up the colonies, e.g.:

"*What special equipment is needed (incubator)"*

"How much new equipment is necessary to have multiple queens?"

"With multiple queens I assume the brood configuration has to be enlarged? More queens, more brood. More bees, more frequent harvesting?"

Others, by contrast, also asking about longer-term management (including swarming), wanting to see efficacy data, and questioning whether this method would be applicable for them. e.g.:

> *"How would you do it on a smaller scale? The study talks about 10 queens. Can you do it w/ just 2 or 3? How do you handle a new season, with swarming, etc?"*

> *"Unsure it's impact on honey crop, our major source of revenue. I wouldn't monkey with multiqueen techniques until I knew its impact on honey crop."*

> *"I am intrigued but have been moving in the direction of keeping smaller colonies (even at the expense of honey production) and breaking the brood cycle frequently to help with varroa management. I worry that having two older queens would increase the likelihood of swarming."*

While this question was designed to get at the longer-term management questions beekeepers have that would determine whether a technique like this would be appropriate for them, the more basic answers are unsurprising given that the one-page informational flyer provided a general overview of the method and not specific instructions.

Challenges to adoption of this method

For challenges to adopting this method, the most commonly cited concerns were about managing multiqueen colonies and other significant responses related to queens (especially to obtaining the queens to setup these colonies), and needs for bees and equipment to use this technique. e.g.:

> *"The biggest challenge for me would be to have colonies ran with different management styles. Keeping employees on the same page with multiple systems in play can be confusing."*

> *"The lack of incubator and getting the timing on everything right."*

> *"Sourcing multiple Queens old or new at one time."*

I expected equipment and access to appropriate queens to be significant factors in determining the types of operations for which this method would be suitable but had not thought about the challenge of having to manage different colonies in different ways. While this is not an insurmountable challenge given that some operations already manage different colony types (mating nucs, queen banks, etc.) it is an added burden that would have to be justified by the success of the method.

Interestingly, while a few beekeepers raised concern about using an “unnatural” method, e.g.:

“*It seems so unnatural!*”

others referenced anecdotal accounts of naturally occurring multiqueen colonies, e.g.:

“Is there evidence that this occurs in "the wild"? (I think there is...)”

But, overall, this was not a major concern raised by participants.

Most and least likely adopters

To understand the characteristics of beekeepers who would be most and least likely to try this method of multiqueen beekeeping I identified two subgroups based on answers to the questions “Based on the information presented I would be interested in trying to manage multiqueen colonies”. Fourteen beekeepers answered “Strongly Agree”, twelve answered “Disagree” and four answered “Strongly Disagree” (with the remainder answering “Agree” or “Neutral”). I considered the fourteen strongly agreeing beekeepers as an “Experimental” group most likely to test this method and the sixteen disagreeing beekeepers as a “Skeptical” group who would be least likely to try this method.

On the whole the Experimental group tended to have more clustered responses and the Skeptical group to be more heterogeneous. The overall profiles of these groups were similar across most responses in the survey, with a few observable differences. Experimental beekeepers had almost unanimous interest in the potential for multiqueen colonies to address queen failure and to promote colony growth and productivity, as well as belief that this method could be useful to beekeepers like them, while Skeptical beekeepers were more divided and tending toward disagreement on all of these questions. Similarly, the Experimental beekeepers were all neutral to agreeing that queen failure is a major problem in honey bee management, while Skeptical beekeepers were more divided, with about a third disagreeing. Experimental beekeepers all identified as being generally interested in trying new management techniques and were more likely to identify as early adopters, with about a third identifying as preferring to wait until they have seen others try new techniques first. Skeptical beekeepers were again more heterogeneous and more likely to identify as slower adopters.

In free response questions both groups had a similar range of management complaints, but Experimental beekeepers were more likely to mention queen problems, for example:

> "*Early queen failure is always an issue. Good nutrition during the honey dearth is also one of my biggest problems. Many hives do poorly on a sugar syrup diet and current supplements don't seem to help much*"

Experimental beekeepers were also more likely to make a connection between *Varroa* complaints and queen complaints, such as:

> "Varroa *and hive beetle are my most common problems. I have lost hives at the end of summer and early fall, which I attribute to Varroa, it could be queen failure. I tend to requeen in the spring, with queens raised in my hives and locally matted. Dr Tarpy* [NC State honey bee Researcher and Extension Apiculturist] *mentioned a study a few years old, where purchased queens had a higher rate of failure. It is also my experience.*"

A few Skeptical beekeepers also mentioned issues that imply or outright state a non-conventional management style. For example:

> "*The problems I face with my colonies are different than the standard problems. I practice treatment free beekeeping. That means I use no chemicals in the hive other than a smoker and some oil to protect against ants. I do not treat colonies for pests such as varroa or small hive beetles. I also do not house more than a few hives in an area to avoid overstepping the carrying capacity. My largest apiary only houses 6 colonies (yet I manage 150 around town). I have a few that have only been opened a couple times a year. My average colony loss last year was less than the national average. I do have colonies that abscond, struggle with mites, small hive beetles, and robbing. However, rather than treating the colonies I let nature play out. Some colonies are able to bounce*

back, others may die out. If they die out, I repopulate the hive with a swarm from a stronger colony."

For sources of Information, Experimental beekeepers were more likely than Skeptical beekeepers to reference Research and Extension sources, while Skeptical beekeepers rely more on local clubs and mentors than Experimental beekeepers.

Both groups had similar types of questions about the method of polygynous beekeeping, but, when asked about challenges Experimental beekeepers were more likely than Skeptical beekeepers to reference material concerns (access to appropriate queens and equipment) whereas Skeptical beekeepers were more likely than Experimental beekeepers to reference management concerns. Skeptical beekeepers were also stated more concerns about polygynous beekeeping being unnatural.

Interest in trying polygynous multiqueen beekeeping is not the only factor that determines whether a beekeeper would be a good candidate to try this method. The equipment and management requirements of this method are not suitable for all operations. Based on a combination of operation type and general innovativeness, the best candidates for future research and adoption of polygynous multiqueen beekeeping are large hobbyist beekeepers. While only a small group (only 5 of these participants completed the entire survey) in this study, the large hobbyist beekeepers are highly cohesive across their answers, being highly innovative, concerned with queen problems, and interested in this method. All of them are familiar with the idea of multiqueen beekeeping generally and more than half of them have attempted a form of it themselves, further demonstrating their interest in experimentation and to the potential use of multiqueen beekeeping. They tended to write lengthy and thoughtful questions about the method and only one of them wrote anything for challenges to adopting the method. They represent a clear profile of beekeepers who would be ideal candidates to implement and further develop this method.

DISCUSSION

The discussion will follow the order of: (1) characteristics of beekeepers, (2) factors promoting innovation in beekeeping, and (3) analysis of most likely adopters of polygynous multiqueen beekeeping. The characteristics of beekeepers discussion will discuss the significance of different types of operations and experience levels on innovativeness. The factors promoting innovation section will discuss reasons why beekeepers are innovative. The discussion of most likely adopters will consider interest in this technology and how future work could proceed to increase the likelihood of successful adoption of the method. The final section will summarize the primary conclusions of this study for the audiences of: researchers, extension, and beekeepers.

Characteristics of beekeepers

There is great diversity in beekeeping communities, ranging from small backyard operations of a few hives to large commercial operations with hundreds or thousands of hives, beekeeping in urban, suburban, and rural settings, operations focused on honey production, migratory operations that truck bees across the country for pollination services, bee breeding operations, brand new beekeepers, and operations that have been handed down through generations. The results in this study reflect much of this diversity, with participants indicating through their responses that they include beekeepers with just a few hives all the way up to those managing hundreds of hives, beekeepers in suburban and rural settings, beekeepers with diverse management approaches ("natural" and conventional), and wide-ranging experience from beginners to seasoned professionals. Despite these differences among beekeepers, participants in this study have many commonalities across demographics, in particular revealing high interest in innovation as a unifying characteristic of beekeepers. The majority of participants were also interested in multiqueen beekeeping as presented to them, though not always for the same reasons as others.

In relation to multiqueen beekeeping techniques, professional beekeepers exhibited both greater familiarity with the idea and methods and lower interest in trying these methods than hobbyists. While this may suggest an inverse relationship, with learning more leading to lower interest, this may be best understood as reflecting core differences between professional and hobbyist beekeepers. Experimenting is inherently a lower-stakes proposition for hobbyist beekeepers than professional ones, who have a greater need for efficiency and predictability in their operations. Almost by definition, professional beekeepers are more experienced than hobbyists in general (and thus more likely to be familiar with a practice like multiqueen beekeeping) and productivity-focused multiqueen beekeeping is traditionally more relevant to commercial operations as well. All together it makes sense for hobbyists to be driven more by curiosity and interest and professionals to be driven more by an assessment of how well this technology would fit in their operation. For example, one commercial beekeeper wrote:

> "*In terms of multi-queen beekeeping, I'd be interested in two specific aspects as a commercial beekeeper:*

1. Running two queens freely. We have all heard the stories about 1000+ hive operations run with 2 queens free in the 80s, but it seems like either their techniques were lost or kept secret. Or it just fell out of favor. But I could see that being a giant asset for honey production and nuc production.

2. Dual brood chambers (separate) with common supers for wintering small colonies like nucs."

Although this survey does not show general differences between professional and hobbyist beekeepers in innovativeness and technology adoption tendencies, more specific questions about this particular technology reveal the differences, at least for this particular practice.

Experience plays a huge role in beekeeping and how greater experience informs interest in innovation is an important question. Do beekeepers tend to become less interested in trying new techniques as they become more confident in their existing management practices or do they tend to retain a high interest in adopting new practices? While the survey did not collect participants experience level directly, it is possible to make some inferences about experience from the information they did provide.

In particular, some beekeeping problems are closely related to knowledge and experience. Commonly cited issues with management and swarming are directly related to or even exacerbated by management decisions, as exemplified in the beekeeping adage that "the biggest killer of honey bees is bad beekeepers". For example, swarming is the natural biological process of colony-level reproduction (with the old queen and many of the workers departing to establish a new colony, leaving the remainder of the colony to replace her with a new queen) that can be relatively easily managed by monitoring for signs of swarming and taking actions like adding extra space to the hive for the colony to grow and splitting colonies to effectively create a controlled swarm without losing the population of bees to a natural swarm. These types of problems predominantly impact newer beekeepers and beekeepers with less active management styles. Because of that, management and swarming concerns serve as a partial proxy for experience level among the participants of this study. These potentially newer beekeepers do show greater reliance on clubs and mentors and more challenges to adopting polygynous multiqueen beekeeping, presenting a consistent profile. However, similar patterns of innovativeness, interest in multiqueen beekeeping, and questions about the method among these and other beekeepers suggests that these groups are broadly similar in their interest in technology adoption.

While the data in this study is consistent with an expected pattern of more experienced beekeepers having fewer problems and relying less heavily on external management advice there is not evidence that beekeepers lose interest in innovation as they gain experience. Fully answering this question is outside the scope of this study but the existence of serious management problems in beekeeping with inadequate solutions, such as *Varroa* mites and queen failure, provides a reason for even experienced successful beekeepers to remain interested in finding new management solutions for these problems. Regardless of their proportion among beekeepers overall, however, the most likely candidates to be early adopters of novel techniques are experienced beekeepers with operations suitable for a technique and who are disposed towards innovativeness

Factors promoting innovation

Participants in this study exhibited broad interest in learning about and potentially trying new management techniques. This high enthusiasm for innovation is a major unifying theme across beekeeper types and operations. Several factors contribute to this high interest in innovation, including a steep learning curve requiring habitual problem solving, the existence of serious management concerns in need of better solutions, and robust communities and networks for learning and sharing techniques.

High interest in developing and learning about new techniques makes sense for a discipline with a steep learning curve where beekeepers must learn and adopt many new (to them) practices in order to succeed. As previously discussed, many of the beekeeping problems referenced by participants, such as swarm control, can be remedied with knowledge and experience. And, while it’s possible for hobbyist beekeepers to accept some degree of lowered productivity in exchange for less-active management, the minimum requirements for keeping bees alive are high. Having even one colony survive a full year is an accomplishment for a new beekeeper. Learning and trying new management techniques isn’t so much a benefit as it is a necessity for beekeepers, contributing to the high degree of innovativeness.

While beekeepers can learn to effectively manage many of their concerns, the most significant problems in beekeeping are more difficult. These problems stem from fundamental biological and environmental causes that are common to all beekeepers and do not have satisfactory management solutions. As seen in the survey results, *Varroa* mites and their associated diseases are the most pervasive complaint as they are a now ubiquitous invasive parasite that causes serious harm, and the management options are difficult and/or expensive and not fully effective. Queen problems (encompassing a variety of different types and causes but all ultimately resulting in colony-jeopardizing queen loss and/or replacement) are also widespread and lack clear management solutions. The costs and frustrations of dealing with these problems provide strong motivation for beekeepers to seek out and adopt new management practices to improve, if not solve, these problems.

Beekeepers rely on many sources of information to help them solve their problems and have extensive social networks. Local beekeeping clubs are ubiquitous and there are regional and national organizations geared towards the different types of beekeepers. In the 21st century this has expanded to include numerous online communities in Facebook groups and other forums. While this study does not probe how information flows among and between the various beekeeping communities it does reflect the broadly interconnected nature of beekeeping and identify some general patterns of technology adoption within beekeeping. In particular, the influence of trusted local peers and mentors stands out. While some beekeepers are naturally innovative and experimental, many more are likely to try something when they are able to learn how it works and see the results (even anecdotally) from someone they know. Successful adoption of a technology requires both innovators willing to try new things and efficient means of sharing information about new techniques that work. Beekeeping communities exhibit both of these features, increasing the likelihood that successful new techniques will be widely adopted. These chances could be further increased by coordinating with innovative and influential early adopters to begin the process of adoption.

Most likely adopters

Participants' general interest in new management practices also extended to specific interest in multiqueen beekeeping. Although these positive attitudes are widespread, it is also clear that there will be barriers to adoption. The clearest barriers are the basic management requirements for equipment (e.g. incubator) and bees (e.g. extra queens) that are fundamental to this method and make it only suitable for certain types of operations. That is why it is particularly important to identify beekeepers who would be good candidates for participating in future research developing this method and early adoption of the method. Identifying these beekeepers began with an examination of the beekeepers most and least interested (Experimental and Skeptical, respectively) in multiqueen beekeeping.

The Experimental group of participants most interested in trying polygynous multiqueen beekeeping are much more cohesive than the Skeptical group. While they come from across the different types and sizes of operations, Experimental beekeepers tend to be innovative and concerned with queen problems and, therefore, interested in an unconventional practice designed to mitigate the effects of queen loss. Experimental beekeepers' greater familiarity with multiqueen beekeeping practices than other beekeepers probably also contributes to their openness to this specific method and provides actual evidence that beekeeper interest in this method would grow if it were to be more widely known.

In contrast, Skeptical participants don't share as much of an overarching profile but do have some different response patterns than general or Experimental participants. As a group they have lower interest in trying new management techniques generally, are less concerned with queen problems, and have lower interest in potential benefits of multiqueen beekeeping. But these differences are the result of Skeptical beekeepers being more divided in their answers than other beekeepers. They are all disinterested in trying polygynous multiqueen beekeeping but likely for a variety of different reasons. Some of them are generally disinterested in trying new techniques while others may see it as not a good fit for them even if it is for some beekeepers and a few appear to see it as an unnatural practice and dislike the idea for that reason. Based on free response questions, though, management concerns about this method are a significant barrier for Skeptical beekeepers, which makes sense and accurately reflects the reality that this is a method that would not be suitable for every operation.

It is notable that the Experimental participants stand out for being less reliant on local clubs and other beekeepers as sources of information and instead are more attuned to research and industry sources. Clubs and mentors were cited more than any other code in the entire study, with 70% of participants (and 75% of Skeptical participants) making that kind of reference in the free response question, but only cited by ~40% of Experimental beekeepers. It is unclear whether Experimental beekeepers are more independent from other local beekeepers or if they are still involved but more in mentors than mentees. Regardless, though, they are interested in multiqueen beekeeping and in research and extension generally so would make good candidates for researchers to partner with.

The Experimental beekeeper group is defined by their interest in trying polygynous multiqueen beekeeping but that does not mean that they are all well equipped to use this method in practice. Ideal candidates to beta test and become potential early adopters of this method are naturally innovative and interested in the specific technique but they also need to have experience and an operation that is large enough to have the kind of materials and equipment needed. The large hobbyist beekeepers match this profile the best. As large beekeepers they have experience, resources, and significant personal investment (managing 50+ colonies is a substantial undertaking) to successfully carry out a project like this while also having fewer operational constraints than professional beekeepers. The characteristics of these beekeepers make them stand out as the ideal candidates to partner with for future research on multiqueen beekeeping and, likely, other projects as well. While the survey does not provide data for identifying these beekeepers large hobbyist beekeepers could be found through clubs and extension work, as they appear active in both, and these beekeepers are also likely to be the type of beekeepers who are involved in larger scale state and regional beekeeping associations that cater to hobbyist beekeepers.

Conclusions

This study finds beekeepers to be generally interested in learning about and potentially adopting new technology and management techniques and affirms that beekeeping is a practice with many interconnected communities based around sharing information and mentoring which is favorable to the spread of successful techniques. Participants also expressed significant interest in the idea of polygynous multiqueen management even though this is an early-stage method with important questions remaining to be answered. This study confirms that there is interest and a potential user base for this method while providing guidance for future research developing it. Specific conclusions are presented for the following audiences: researchers, extension agents, and beekeepers.

Researchers

While muiltiqueen beekeeping has traditionally been viewed as a means to increased colony productivity, beekeepers responded especially strongly to the novel framing of polygynous colonies as a potential means of mitigating queen problems. Because baseline interest is relatively high the major challenges to the implementation of this method are the information and resources needed to establish colonies and more information about medium to long-term management and efficacy, especially related to overwintering, swarming, and disease management. While some challenges to this method are inherent and make it appropriate for limited operation types, there is a promising opportunity to conduct future research developing this method in partnership with beekeepers who would be interested in the method and well suited for early adoption, namely the beekeepers operating large hobbyist operations.

Extension

The major beekeeping concerns shared by beekeepers in this study are managing *Varroa* mites and disease, swarming, queen problems, and environment and resource concerns. While beekeepers have well developed networks to share information and learn from each other there are still clear needs for research-based training and advice. Extension is especially important for the difficult management concerns like managing *Varroa* and diseases, with complicated treatment options and regulatory concerns. Extension provides a link between research and practice and can help beekeepers determine whether a new practice, like multiqueen beekeeping, would be suitable for them to try and could be helpful in connecting potential collaborations between researchers and beekeepers.

Beekeepers

The sheer number of participants citing local clubs and mentors as a primary source for information speaks to the importance of local beekeeping communities and the influence of experienced local beekeepers. There are many beekeepers with concerns that can be addressed through learning established management techniques, and they are overwhelmingly looking to other local beekeepers. This suggests that the formal and informal mentoring programs, bee schools, etc. offered by local clubs are working to meet these needs. But there are also more difficult management concerns, like *Varroa*, with limited and sometimes hazardous treatment options. In a community like beekeeping, with high innovativeness and interest in trying new management techniques across the board, this means that experienced and influential local leaders and mentors have an added responsibility of helping to protect the safety of beekeepers and their bees by advocating for the use of proper extension and regulatory guidelines. It is these community leaders who have the greatest potential to shape and improve beekeeping practices and to coordinate with extension services to promote innovation and responsibility in beekeeping.

Altogether, this study finds beekeepers to be highly innovative and engaged in communities focused on sharing information and problem solving to promote success in beekeeping, in keeping with the variety and severity of beekeeping problems. Overall, this is an environment that is highly conducive to the adoption of new technologies that meet the needs of beekeepers. This general enthusiasm for new management solutions also extends to specific interest in polygynous multiqueen beekeeping. While there are many outstanding questions about this method that make it difficult to determine whether it would be successful, this study provides clear direction for future research developing the method.

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Table 3.1. Summary of participant demographics

Occupation	**Number**
Professional	8
Hobbyist	86
Size of Operation	
1-5	39
5-20	39
20-50	5
More than 50	11
Age	
Under 40	16
40-60	34
Over 60	36
Gender	
Female	24
Male	53
Wrote something else or left blank	17

Table 3.2. Summary of response codes for sources of information used by beekeepers

Code	Definition	Response Number
Clubs and Mentors	Local beekeepers known personally	67
Industry Sources	Beekeeping-specific presentations, conferences, and publications at a regional or national level	42
Internet and Popular Figures	Websites or other information from widely known figures in beekeeping, online sources unless further specified as another code	42
Research and Extension	Presentations and publications from academic and extension sources, books unless further specified as another category	34
Other	Other or unspecified	6

Table 3.3 Summary of response codes for significant problems in beekeeping

Code	**Definition**	**Response Number**
Mites and diseases	*Varroa* mites and diseases generally, including treatment and treatment cost	48
Management and Swarming	Swarming and general management	25
Queen Problems	Queen problems including failure, supersedure, longevity, cost of queens	23
Environment and Resources	Weather, nectar flow, resource availability, feeding	18
Other	Other or unspecified	14

Table 3.4. Summary of response codes for questions about the method of multiqueen beekeeping

Code	**Definition**	**Response Number**
Basic Understanding	Questions about setup and bee biology	40
Efficacy	Questions about how well the method works, how long it lasts, data	10
Management	Questions about ongoing management after establishment, including swarm management	18
Modifications	Ideas for modifications or additional uses	3
Applicability	Suggestion that this method would not work well for a particular beekeeper, regardless of how it could work for others	8
Other	Other or unspecified	16

Table 3.5. Summary of response codes for challenges to adoption of this method

Code	Definition	Response Number
Queens	Difficulty acquiring suitable queens or other queen-related concerns	19
Bees and Equipment	Concerns about worker bees, equipment, and setup	19
Management	Concerns about management after setup, including time investment	33
Unnatural	Unnatural system of beekeeping	4
Risk Aversion	Concern about failure, losing time and money, general desire to see others do this first	7
Other	Other or unspecified	22

APPENDICES

Appendix A

Genotype Data

Colony 1 summary

A113		A24		A88		Ap43		Ap81		B124	
Allele	Count	Allele	Count	Allele	Count	Allele	Count	Allele	Count	Allele	Count
214	65	100	39	142	31	145	164	135	175	212	145
202	25	92	79	140	111	137	36	127	77	216	37
220	127	102	107	148	159	143	26			220	37
216	4	104	21	146	14	135	8			214	25
222	18	106	59			139	5			224	17
226	10	108	1			181	9			218	20
228	1					149	30			222	4
224	4					179	1			232	8
										226	8
										228	1
										234	1

Colony 1 genotypes

1			100	106	142	148	145		135		212	
2			92	106	140	148	145		127	135	212	220
3	214	220	100	102	140	148	145	149	135		212	222
4	214	222			140	148	145		127		212	220
5	202	214	92	100	148		137	145	135		216	
6	214	220	100		142	148	145		135		212	
7	214	220	102		140	148	145		135		212	216
8	202	214	92	102	148		137	145	135		216	
9	220	222	102	106	142	148	137	145	127	135	212	214
10	214	220			140	148	137	149	127	135	220	224
11	220	226	92	106	140		145	149	135		212	220
12	214	220	92	102	140	148	143	145	135			
13	202	214	92	102	140	148	145		127	135	212	
14					148		137	145	135		216	
15			92	106	140		145		127	135	212	220
16	220	222	102	106	140	146	145	181	135		212	216
17	214	220	92	106	140	148	145		127	135	212	220
18	214				140	148	137	149	127	135	216	224
19	220		100	102	140	148	143	145	127	135	212	
20	214	220			148		143	145	135		214	216
21	202	220	100	102	148		143	145	135		212	214
22	220	226	102		140	148	137	145	127	135	212	214

23	214	220	100	102	146	148			127	135	212	216
24	220		100	102	142	148	145		135		212	
25	202	220	92	102	140	148	145		127	135	216	232
26	220		102	106	142	148	145		127	135	212	218
27	214	220			140	148	143	149	127	135	212	
28	202	220	92	102	140	148	145	149			212	224
29	214	220	100	102	142	148	145		135		212	
30	214	220	102	106	140	148	137	145	135		212	220
31	220	222	92	102	140		145		127		220	
32			100	106	140	148	145		127	135	216	218
33	214	220	92	102	148		145		135		212	216
34					140	142	145		135		212	
35	220		100	102	146	148			127	135	212	
36	214	220	92	102	148		145		135		212	216
37			100	106	142	148	143	145	135		212	
38					140	148	145	149	135		212	
39	220	226	102		142	148	137	145	135		212	214
40			104	106	148		145	149	127	135	212	232
41	214	220			140	146	145	181	135		212	226
42	220	222	102	106	142	148	137	145			212	214
43			92	104	140	148	145		135		212	224
44	220		92	102	148		137		135		212	
45			92	102	140	148	145	149	135		212	
46			104	106	148		145		135		212	
47	220				140		145		135		212	220
48			92	104	140	148	135	137	135		212	220
49	220	226	102		142	148	137	145	127	135	212	214
50	202	220	104		140	148	145		135		212	
51	214	222	102	106	140	146	145	181	135		216	
52			104	106	148		137	145	135		212	
53			92	104	146	148	139	145	135		212	214
54	220	226	102		140	142	143	145	135		212	228
55	220		102	106	142	148	145		127	135	212	218
56	220	222	92	102	140		145		135		212	220
57	214	220			142	148	145		127	135	212	
58			104	106	148		137	145	135		216	
59	214	226	92	106	140		145		127	135	212	220
60	214	220	100	102	148		143	145	135		214	216
61	220		100	102	140	148	181		127	135	212	216
62	214	220	92	102	140	148	145		127	135	212	232
63	220		104	106	140	148	145	149	135		224	226
64	220		92	106	146	148	139	145	135		214	218
65					142	148	145		127	135	212	

66			92	106	148		137	149	127	135	212	
67	216	220	92	102	140	148	145		135		212	224
68	214	220	104	106	140	148	145		135		212	
69			104	106	140		145		135		212	
70	214	220	92	102	148		137	149	135		218	
71	220		102	106	140	148	143	149	127	135	212	224
72	214	220	92	102	140	148	139	145	135		212	216
73	214	222	92	106	140	148	145		127		212	220
74	214	222	102	106	140		145	181	135		216	
75	220		92	102	148		135	145	135		212	220
76	220	222	102	106	142	148	137	145	127		212	214
77	220		92	102	142	148	145		127		212	
78	202	220	92	102	140	148	145		127	135	212	232
79	202	220	100		140	148	145		127	135	216	218
80	220	228	92	102	148		145		135		212	
81					140	148	145		127	135	212	
82	214	220	102		140	148	145		127	135	212	218
83	202	220	92	100	140	148	143	145	135		216	234
84	216	220	104		140	148	145	149			212	
85			92	106	148		145		127	135	212	220
86	202	220	100		148		143	145	135		212	214
87					148		143	145	135		214	216
88	214		92		148		145	149	135		212	
89	220	222	102	106	142	148	137	145	127	135	212	214
90			104	106	140	148	145		127	135	212	218
91			102	106	140	148	145		127	135	212	232
92	214	220	104	106	140	148	145		135		212	
93			104	106	140		145		135		220	224
94			104	106	146	148	145	181	127	135	212	
95	216	220	104		142	148	143	145	127	135	212	
96	220		92	100	140	148	143	145	127	135	212	
97	214	216	92		140	148	145		127	135	212	224
98	202	220	100	102	148		143	145	135			
99			92	106	140		145	149	135		220	224
100	214		102		148		145		135		214	216
101	220	224	92	102	140		145		135		212	220
102	220		92	102	148		137		135		212	218
103	220	226	102		140	142	143	145	127	135	212	220
104	214	220	100	102	142	148	145		127	135	212	
105	214	220	92	106	140	148	145		127		212	220
106	220		92	102	140	148	137		135		212	
107	214	224	92		140		145		127	135	220	224
108	220		102		140	148	137	145	135		220	224

109	220		92	102	148		137	145	135		212	218
110	214	220	92	100	140	148	143	145	127	135	212	224
111	220	224	92	102	140		145		135		212	
112	220	226	102	106	140	142	143	145	127	135	212	220
113	214	220	92	102	140	148	145	149	127	135	218	232
114	214	220	100	102	140	148	145	145	127	135	212	
115	214		102	106	146	148	145	181	127	135	212	216
116	202	220	92	106	140	148	135	149	127	135	212	220
117			92	104	140	148	145		127	135	212	220
118	202	220	100		148		145		135		212	216
119	214	220	92	106	148		145		135		212	216
120	214	220	100	102	142	148	145	149	135		212	222
121	202	220	100		148		143	145	135		214	216
122			92	104	140		137	145	135		212	220
123	220		92	102	148		137		135		212	218
124	214	220	92	102	148		137	145	135		212	216
125	214	220	102	106	140	148	137	145	135		212	220
126	214	220	102	106	140	148	145		135		212	226
127	214		102		140	148	145	179	127	135	212	
128	220		92	102	140	148	137		135		212	
129	220		92	102	140	148	145		127	135	212	218
130	214	220	92	102	140	148	137	149	127	135	220	224
131	202	220	92	100	148		143	145	135		212	214
132			104	106	142	148	145		135		212	226
133	214	220	100		140	148	145		127	135	212	218
134	220		92	102	140	148	135	145	135		212	220
135	220	226	102		142	148	137	145	135		212	214
136	214	220	92	102	148		145		135		212	
137	220		102	106	142	148	143	145	135		212	
138	220	222	102	106	140	148	145		127	135	220	
139			106		148		145		127	135	212	218
140			92	106	140		135	149	127	135	212	
141	220		92	102	146	148	139	145	135		212	214
142	220		102	106	142	148	145		135		212	218
143	202	220	92		140	148	135	149	127	135	220	224
144			102	106	148		143	145	135		212	214
145	214		102	106	140	148	137	149	127	135	212	216
146			102		140	148	145				212	216
147	214	220	92	100	140	148	143	149	127	135	212	224
148	214	220	102		146	148	137	145	135		212	216
149	214		102	106	140	148	145		127	135	212	
150	220	222	92	102	140	148	145		135		212	226
151	202	220	92	100	140	148	143	145	135			

152	214	220	100		140	148	145		135		212	
153	220	222	92	102	140	148	145		127	135	212	220
154	220		92	102	140	148	145		127	135		
155	214		100	102	148		145		135		212	214
156	202	220	102		140	148	145		127	135	216	218
157	214	220	100	102	140	148	145	149	127	135	212	222
158	220		92	102	140	148	137	145	135		212	
159					140		145		135		212	
160	220		92	102	142	148	145		127		212	220
161	214	220			140	148	145		127	135	212	
162	220		100	102	140	148	145		135		212	
163	220	222	92	102	140	148	145		135		212	226
164	214	220	92	100	140	148	145		127	135	216	232
165	220	226	102	108	140	148	137	145	127	135	212	214
166	214	224	92	102	140		145	149	135		212	220
167	214	220	102		140	148	145	149	135		212	
168	220		102	106	142	148	145		135		212	218
169	220		92	102	140	146	137	139	135		214	218
170	202	220	100		148		143	145	127	135	212	214
171	220	222	92	102	140	148	145		135		212	226
172	220		102	106	140	148	145		127		212	
173	214	220	100	102	140	148	145	149	135		212	222
174	202	220	92		140	148	135	149	127	135	212	220
175	220	222	102		140	146	145	181	135		212	216
176			92	106	140		145		135		220	224
177	202	214	100		148		145		135			
178	220	222	92	102	142	148	145		135		212	226
179	214	220	102		148		145		135		212	
180	214		92	102	148		145	149	135		212	
181			104	106	140	146	145	181	135		216	
182			102	106	142	148	145		135		212	218
183			102	106	140	148	145		127	135	212	220
184	202	220	102	106	140	148	145		135		212	216
185	202	220	92	100	140	148	145		127	135	216	232
186	202	220	100		148		143	145	135		214	216
187	220		92	102	148		135	137	135			
Q1	214	220	102	106	140	146	145	145	127	135		
Q2	220	220	102	106	140	148	145	145	127	135		
Q3	214	220	102	106	140	148	145	145	127	135		
Q4	220	220	102	106	148	148	145	145	127	135		

Colony 2 Summary

A113		A24		A88		Ap43		Ap81		B124	
Allele	Count	Allele	Count	Allele	Count	Allele	Count	Allele	Count	Allele	Count
208	8	102	130	140	77	145	177	127	102	214	56
214	82	92	69	148	157	143	48	135	234	212	185
220	147	100	54	146	94	137	10			216	21
202	24	106	32	136	2	149	77			220	15
218	6	108	15	150	4	135	1			226	18
212	5	104	2			169	8			232	9
222	8					165	9			228	1
228	2					175	2			230	5
216	2									218	8
										222	2

Colony 2 genotypes

1	208	220	102	102	140	148	145	149	127	127	214	226
2	214	220	102	102	148	148	145	145	127	135	212	232
3	214	220	102	102	146	148	145	149	135	135	214	226
4	220	220	102	102	146	148	145	169	127	135	212	214
5	214	220	92	100	148	148	145	149	135	135		
6	220	220	100	100	146	148	145	149	135	135	212	228
7	220	220	100	100	140	148	145	145	135	135	212	212
8	202	220			148	148			127	135	214	214
9			102	102	140	148	145	145	127	135	214	230
10			92	100	148	148	145	145	127	135	214	218
11			102	102	148	148	143	145	127	135	212	212
12			102	106	140	148	143	145	135	135	214	218
13	220	222	102	102	146	148	145	145	127	135	212	212
14	202	220	92	102	146	148	145	145	127	135	212	212
15			100	100	140	150	137	143	127	135	212	220
16	202	202	102	102	140	148	145	145	135	135	212	216
17					146	148	145	149	135	135	214	230
18			102	108	146	148	143	145	135	135	214	220
19	202	202	102	102	148	148	145	145	135	135	214	216
20	220	220	102	102	146	146	143	169	127	135	212	214
21	220	220	92	102	148	148	145	145	127	127	212	214
22			92	100	140	146	143	145	135	135	212	214
23			92	100	140	146	145	149	135	135	212	226
24			100	104	140	150	143	145	127	135	212	220
25	202	220	102	102	140	148	145	145	135	135	214	216

26	220	220	102	102	146	148	143	145	127	127	212	214
27	202	220	100	100	140	148	145	145	127	135		
28	220	220	102	102	140	148	145	149	127	127	212	226
29	214	220	92	92	140	146	149	149	135	135	212	226
30			102	102	146	148	145	145	127	135	212	212
31			92	102	146	148	137	145	135	135	212	226
32			102	102	148	148	145	145	127	135	212	212
33	202	214	92	100	146	148	143	145	135	135	214	216
34	214	220			146	148	145	149	135	135	212	212
35	214	214	100	106	146	148	145	149	135	135	214	220
36	220	220	102	102	148	148	143	145	127	135	212	218
37	214	220	102	102	140	140	145	145	127	135	212	230
38			100	104	140	140	145	149	135	135	212	216
39	220	220	92	102	140	146	145	145	127	135	212	212
40	202	220	92	102	140	148	145	145	127	135	214	230
41	214	220	92	102	140	148	145	145	127	135	212	216
42	214	214	100	100	146	148	143	165	135	135	212	214
43	214	220	92	102	140	146	143	145	135	135	212	214
44	220	220	102	102	140	148	145	149	127	135	212	226
45			92	102	140	148	145	145	127	135	212	212
46			92	102	148	148	145	145	127	135	212	212
47			102	102	146	148	149	149	127	135	212	214
48	220	220	92	102	148	148	145	149	127	127		
49	202	220	92	102	146	148	143	145	127	135	212	212
50	214	220			148	148	143	149	135	135	212	214
51	214	220	102	102	140	148	145	149	127	127	212	226
52	214	222	92	100	140	148	137	145	135	135	212	226
53	220	220	92	102	140	146	143	145	127	135	212	212
54			92	102	146	148	145	149	135	135	212	216
55	220	220	100	102	148	148	145	149	127	135	212	212
56	220	220	92	102	146	148	143	169	127	135	212	214
57	220	220	102	102	146	146	149	149	135	135	212	232
58	214	222	102	108	140	148	143	145	127	135	212	212
59	220	220	92	100	146	148	145	149	135	135	212	226
60	220	220			140	148	145	145	127	135	212	214
61			102	102	146	146	143	149	127	135	212	212
62	208	220	92	102	146	148	145	149	127	135	212	232
63			102	102	140	148	145	149	127	135	212	226
64	220	220	102	106	146	146	145	169	127	135	212	214
65	214	214	92	100	140	146	145	145	135	135	212	216
66	220	220	92	102	140	146	143	145	127	135	212	212
67	214	220	102	102	146	148	145	145	135	135	212	232
68	214	220	92	102	146	148	145	145	127	135	212	216

69	214	220	102	106	146	148	145	149	127	135	212	214
70	208	220	100	106	140	148	145	149	135	135	212	216
71	214	220	100	108	146	148	149	165	127	135	212	214
72	214	220			140	140	149	149	127	135	216	226
73	214	220	92	106	140	148	149	149	135	135	212	212
74	220	220	102	106	146	146	143	169	127	135	212	214
75	214	220	100	106	146	148	143	165	135	135	212	214
76	214	220			140	148	137	149	127	127	212	226
77	220	222	102	106	146	148	145	145	127	135	212	212
78	214	214	92	108	140	148	145	149	127	135	212	216
79	202	220	100	102	140	148	145	145	127	135	212	220
80	208	214	92	108	148	148	143	145	127	135	212	212
81			100	106	146	148	143	145	135	135	212	226
82	208	220			146	148	145	145	127	135	212	212
83					148	148	145	145	127	127		
84	202	214	92	102	148	148	145	145	135	135	214	220
85			100	108	146	148			135	135	212	214
86	220	220	92	106	140	146	145	149	135	135	212	214
87			92	108	146	148	143	149	135	135	214	220
88	214	220			146	148	149	149	135	135	212	212
89	214	220	102	108	146	146	143	169	127	135	212	214
90	208	220	102	106	148	148	145	145	127	127	212	214
91			100	102	140	140	149	165	135	135	212	212
92	214	220	102	106	140	140	143	145	127	135	212	212
93	220	220	100	102	146	148	149	149	135	135	212	214
94	214	220	102	106	146	148	149	165	135	135	212	212
95	220	220	92	106	146	148	149	149	127	135	212	212
96	208	220	92	102	146	148	145	145	135	135		
97	220	220	102	106	146	148	143	145	127	135	212	214
98	208	220	92	102	148	148	145	145	127	135	212	214
99	220	220	92	108	140	148	149	165	135	135	216	232
100	220	222	102	108	146	148	145	145	127	135	212	212
101	220	220	102	106	146	146	145	149	135	135	212	232
102	220	222	102	102	146	148	143	145	127	135	212	212
103	214	220	92	102	140	148	143	145	135	135	212	218
104	214	220	92	102	148	148	145	145	135	135	214	220
105	214	214	100	106	146	148	149	165	135	135	212	214
106	220	220	92	102	140	148	143	145	127	135	212	212
107	202	214	92	102	146	148	149	149	135	135	212	220
108	220	228	100	102	140	150	137	143	127	135	212	220
109	218	218	100	102	146	148	145	149	127	135	212	212
110	212	218	92	100	146	148	149	149	127	135	212	212
111	220	220			146	148	145	149	135	135	214	226

112	214	222	102	106	140	140	135	137	135	135	212	212
113	214	220	92	108	140	148	145	149	135	135		
114	220	220	102	108	146	148	143	145	127	135	212	214
115	214	220	102	108	148	148	145	149	127	135	212	214
116	202	214			148	148	143	145	135	135	212	216
117	220	220	102	106			145	169	127	135	212	214
118	220	220	92	106	140	146	145	145	127	135	212	212
119	214	220	92	102	148	148	145	145	127	135	212	212
120	220	220	92	102	146	148	137	149	127	135	212	232
121	202	220			148	148	145	149	135	135	212	214
122	202	214	92	102	140	146	143	145	135	135	212	216
123	214	214			146	148	143	175	135	135	214	222
124	214	214	92	100	148	148	137	145	135	135	212	212
125	202	220	100	106	148	148	143	145	135	135	212	218
126	202	220	92	102	148	148	145	145	127	135	212	212
127	220	220	100	106	146	148	149	149	135	135	212	212
128	202	214	92	100	140	146	145	149	127	135	216	226
129	214	220	92	102	140	146	145	149	127	135	212	220
130	214	220	92	102	140	148	145	149	127	135	212	214
131	220	228			140	150	143	145	127	135	212	220
132	202	220	100	102	140	148	145	145	127	135	212	212
133	214	220	92	100	140	146	145	145	135	135	212	216
134	214	220	100	106	146	148	143	145	135	135	214	220
135	214	220	92	102	148	148	149	149	127	135	214	216
136	214	220	102	106	146	146	145	169	127	135	212	214
137	202	220	92	100	146	148	145	149	135	135		
138			92	102	136	140	143	149	127	135	212	218
139	214	218	100	102	146	148	145	145	135	135	212	212
140	214	220	102	106	148	148	143	145	127	135	212	212
141	214	216	100	102	140	146	143	145	135	135	212	218
142	220	220	100	106	140	146	145	165	135	135	212	212
143	214	220	100	102	140	148	145	145	135	135	212	212
144	214	220	92	100	140	146	145	145	135	135	212	212
145	220	220	102	106	140	148	137	149	127	127	212	226
146	212	212	100	100	146	148	143	165	135	135	212	212
147	214	220	92	106	140	148	145	145	135	135	220	232
148	202	214	102	106	136	148	145	145	127	135	212	214
149	220	220	92	102	140	140	137	145	135	135	212	216
150	220	220	102	102	148	148	143	145	127	135	212	226
151	214	220	92	102	146	148	145	149	127	135	212	214
152	214	220	102	106	146	148	145	149	135	135	212	214
153	214	220	92	102	146	148	143	149	127	135	212	232
154	212	218	102	102	140	140	143	145	127	135	212	230

155	220	220	92	102	140	148	145	145	127	135	212	212
156	214	214	100	106	146	148	143	175	135	135	214	222
157	214	220	92	102	140	148	149	149	135	135	212	216
158	202	214	92	100	140	148	145	145	127	135	212	220
159	214	220	92	100	146	148	145	145	127	135	212	216
160	214	220	102	108	146	148	145	145	127	135	212	212
161	214	220	92	102	146	148	145	149	135	135	212	212
162	212	218	100	102	140	148	145	149	135	135	212	212
163	214	222	92	102	140	148	145	145	127	135	212	212
164	214	220			148	148	145	145	127	135	212	212
165	214	216	100	102	140	146	143	145	135	135	212	218
166	214	214			146	148	143	145	135	135	212	214
167	214	214	102	108	146	148	145	149	135	135		
168	214	220	92	92	140	140	145	149	135	135	212	212
Q1			100	106	148	148	145	149	127	127		
Q2	214	220	100	106	146	146	143	149	127	135		
Q3	220	220			146	148	145	145	127	127		
Q4	214	220	100	106	140	146	145	145	127	135		
Q5	214	220	100	106	140	140	145	145	127	135		
Q6	202	214			140	148	143	145	127	135		
Q7	220	220	100	106	146	148	145	149	127	135		

Colony 3 summary

A24		A88		Ap43		B124	
Allele	Count	Allele	Count	Allele	Count	Allele	Count
88	13	150	3	149	17	212	163
106	176	140	114	145	185	214	72
92	4	148	172	137	15	218	13
90	5	146	15	143	42	220	2
100	13	142	2	135	6	216	10
102	97	134	2	139	2	226	44
104	4	152	2	152	4	228	4
				165	6		
				169	24		
				181	11		

Colony 3 genotypes

1	88	104	150	152	149	152	212	214
2	106	106	140	148	145	145	212	214
3	88	104	140	150	149	152	212	214

4	106	106	148	148	145	145	212	218
5	106	106	140	140	137	145	214	226
6	92	106	140	148	145	149	218	226
7	88	106	148	148	137	145	212	226
8	88	106	140	148	143	145	218	226
9	90	106	140	140	143	145	212	212
10	88	106	140	148	143	143	214	228
11	100	106	148	148	145	145	212	212
12	106	106	140	148	145	145	212	214
13	106	106	140	150	149	152	214	228
14	106	106	148	148	143	145	212	218
15	106	106	140	148	145	165	212	212
16	106	106	148	148	145	145	212	218
17	100	106	140	148	143	145	212	218
18	92	106	140	140	145	169	214	226
19	100	106	140	148	145	145	214	226
20	88	106	146	148	143	145	214	226
21	88	106	140	148	145	145	212	214
22	100	106	146	148	137	143	212	214
23	106	106	140	148	145	169	214	226
24	106	106	140	148	143	145	212	218
25	106	106	148	148	145	181	212	212
26	92	106	148	148	145	145	212	212
27	106	106	140	148	145	181	212	226
28	106	106	148	148	145	145	212	218
29	106	106	140	148	143	145	212	226
30	106	106	148	148	143	145	212	214
31	88	106	140	146	143	145	214	226
32	106	106	148	148	145	149	212	212
33	106	106	148	148	145	181	212	212
34	88	106	140	148	145	169	212	214
35	102	106	140	140	145	169	214	226
36	100	100	140	148	145	169		
37	88	106	140	140	143	145	212	226
38	104	104	140	152	149	152	218	226
39	106	106	140	148	145	145	212	220
40	106	106	140	148	145	181	212	226
41	106	106	148	148	143	145	212	214
42	106	106	148	148	145	181	212	212
43	106	106	148	148	145	181	212	226
44	100	100	140	148	137	143	212	218
45	106	106	146	148	143	145	214	226
46	92	106	140	148	143	145	212	214

47	106	106	140	140	137	145	212	214
48	106	106	148	148	145	149	212	212
49	102	106	140	148	143	143	220	228
50	100	106	140	148	137	145	214	226
51	106	106	140	140	145	169	212	214
52	90	106	140	140	145	169	212	214
53	88	106	142	148	145	181	212	226
54	102	106	140	148	145	169	212	214
55	88	106	140	148	143	145	212	212
56	102	106	140	148	143	145	212	212
57	100	106	142	148	137	145		
58	100	106	140	148	145	149	218	228
59	90	106	140	146	143	145	212	214
60	88	106	140	140	145	169	212	214
61	100	106	148	148	137	145	212	214
62	100	106	140	140	137	145	212	214
63	90	106	148	148	145	149	212	212
64	90	106	146	148	143	145	214	226
65	102	106	148	148	145	149	212	212
66	102	106	140	140	145	169	214	226
67	102	106	140	140	145	169	212	214
68	102	106	148	148	135	145	216	226
69	102	106	148	148	145	149	212	212
70	102	106	148	148	145	145	214	226
71	102	106	148	148	145	145	212	214
72	102	106	140	140	137	145	212	214
73	102	106	140	140	145	169	212	214
74	102	106	148	148	137	145	212	226
75	102	106	148	148	145	181	212	226
76	102	106	140	148	145	145	212	216
77	102	106	140	148	145	165	212	212
78	102	106	140	148	145	145	214	226
79	102	106	140	148	137	145	212	214
80	102	106	140	148	145	169	214	226
81	102	106	148	148	145	145	212	214
82	102	106	140	140	145	169	214	226
83	102	102	140	140	143	145	212	212
84	102	106	148	148	145	149	212	212
85	102	106	148	148	145	145	212	212
86	102	106	140	148	145	145	212	216
87	102	106	140	148	145	169	212	214
88	102	106	140	148	137	145	212	214
89	102	106	148	148	145	149	212	218

90	102	106	140	148	145	165	212	212
91	102	106	140	148	137	145	214	226
92	102	106	148	148	145	145	214	226
93	102	106	148	148	135	145	212	216
94	102	106	148	148	145	145	212	214
95	102	106	140	148	139	145	212	212
96	102	106	148	148	145	145	212	212
97	102	106	140	140	145	169	212	214
98	102	106	148	148	143	145	212	216
99	102	106	146	148	143	145	212	214
100	102	106	140	148	143	145	212	212
101	102	106	148	148	145	145	212	212
102	102	106	134	148	145	145	212	212
103	102	106	148	148	145	181	212	226
104	102	106	140	148	145	169	214	226
105	102	106	140	148	143	145	212	212
106	102	106	140	140	145	169	212	214
107	102	106	140	148	145	181	212	212
108	102	106	140	146	143	145	214	226
109	102	106			143	145	212	214
110	102	106	140	148	143	145	212	212
111	102	106	140	148	145	145	212	212
112	102	106	140	148	135	145	212	216
113	102	106	140	148	145	145	212	212
114	102	106	140	146	143	145	214	226
115	102	106	140	148	139	145	212	212
116	102	106	140	148	145	169	214	226
117	102	106	140	140	145	169	212	214
118	102	106	148	148	145	149	212	218
119	102	106	148	148	145	145	212	214
120	102	106	140	148	145	169	214	226
121	102	106	140	148	145	165	212	212
122	102	106	140	146	143	145	214	226
123	102	106	148	148	145	149	212	212
124	102	106	148	148	145	145	212	212
125	102	106	140	148	143	145	212	226
126	102	106	140	148	145	145	212	216
127	102	106	148	148	145	149	212	212
128	102	106	140	148	145	145	212	214
129	102	106	140	148	145	169	212	214
130	102	106	148	148	145	149	212	212
131	102	106	140	148	145	145	212	212
132	102	106	140	148	145	145	212	214

133	102	106	146	148	143	145	212	214
134	102	106	140	146	143	145	212	214
135	102	106	134	148	145	145	212	212
136	102	106	140	148	135	145	212	216
137	102	106	148	148	145	145	212	214
138	102	106	140	148	143	145	212	212
139	102	106	140	148	145	165	212	212
140	102	106	148	148	135	145	212	216
141	102	106	140	148	145	169	212	214
142	102	106	148	148	135	145	216	226
143	102	106	148	148	137	145	212	214
144	102	106	140	140	143	145	212	226
145	102	106	148	148	145	145	214	226
146	102	106	140	148	145	145	212	212
147	102	106	140	146	143	145	214	226
148	102	106	140	148	145	145	212	212
149	102	106	146	148	143	145	214	226
150	102	106	140	148	145	145	212	212
151	102	106	148	148	145	145	212	214
152	102	106	140	148	145	169	212	214
153	102	106	146	148	143	145	212	214
154	102	106	140	148	145	165	212	212
155	102	106	140	148	143	145	212	226
156	102	106	148	148	145	181	212	212

Colony 4 summary

A113		A24		A88		Ap43		B124	
Allele	Count	Allele	Count	Allele	Count	Allele	Count	Allele	Count
216	12	104	57	148	242	145	177	212	41
208	34	102	49	140	88	135	32	216	33
220	205	106	196	142	14	139	31	218	25
214	45	94	45	150	2	143	12	214	23
206	16	88	3			147	8	220	25
234	14	92	4			137	60	226	29
226	12					165	22		
						149	14		

Colony 4 genotypes

1	216	220	104	106	148	148	145	145	212	226
2	208	220	104	106	140	148	135	147	216	220
3	208	220	102	106	140	140	145	145	212	216

4	208	220	102	106	148	148	139	145	212	216
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25	206	220	104	106	148	148	139	165	218	226
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92	220	220	106	106	148	148	137	145			127	127
93	220	220	104	106	148		137	137			135	135
94	220	220	102	106	148	148	137	165			127	135
95			106	106	140	148	145	145			127	135
96	220	220	94	102	148	148	137	145			127	135
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99	214	220	106	106	148	148	143	145			127	135
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136	220	226	102	106	140	148	135	145			127	135
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167	216		94	106	140	148	135	145			127	127
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176	214	214	106	106	140	148	137	145			127	135
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Q4	220	220	102	106	148	148	137	137			127	135
Q5	216	220	102	106	140	148	145	165			127	135
Q6	206	220	102	106	148	148	145	145			127	135
Q7	206	214	102	106	140	148	135	145			127	127
Q8	216	226	102	106	140	148	145	145			127	135

Colony 5 summary

A113		A88		Ap43		Ap81	
Allele	Count	Allele	Count	Allele	Count	Allele	Count
208	9	148	245	137	101	135	230
214	131	136	47	143	13	127	92
216	30	140	33	135	7	145	6
220	55	138	1	145	187		
218	71	150	2	139	2		
226	4			165	4		
224	5			149	8		
222	5						

Colony 5 genotypes

1			148	148			135	135
2			136	148	137	145	135	135
3			136	148	137	145	127	135
4			148	148	143	145	127	127
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6			136	148	135	145	135	135
7			148	148	145	145	127	135
8			148	148	137	143	127	127
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18	208	220	136	140	145	145	135	135
19	214	218	140	148	145	145	127	135
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56	214	220	148	148	137	145	135	135
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93	216	222	148	148	145	145	127	135
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148	216	218	148	148	143	145	127	135
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161	214	218	148	148	143	145	135	135
162	216	224	148	148	137	145	135	135
163	216	218	140	148	145	145	127	135
164	214	218	148	148	137	137	135	135
Q1	214	218	148	148	137	145	127	135
Q2	214	218	148	148	145	145	127	135
Q3	214	218	136	148	137	145	135	135
Q4	214	218	136	148	145	145	127	135

Appendix B

One-page Informational Flyer

Multiqueen Beekeeping

Queen failure is one of the major causes of colony loss in managed honey bee colonies (*Bee Informed Partnership: beeinformed.org*). Researchers at NC State University have developed a new method for managing colonies with multiple queens living together in the same brood nest to address queen failure and increase colony productivity.

CHALLENGES

There are two main challenges to setting up colonies with multiple queens:

- Queen aggression – New honey bee queens attempt to kill rival queens before they mate and lay eggs
- Worker aggression – workers will often reject (ball and kill) extra queens when more than one are introduced to the colony

SOLUTIONS

By studying honey bee aggression, researchers have found solutions based on the age of the bees:

- Queens lose aggression as they age
- Workers become aggressive as they age

Colonies established with "old" (more than 6 months) queens and newly emerged workers remained intact, with multiple queens laying eggs in the same nest area

METHOD

The multiqueen colonies in this study were established by:

- Placing frames of emerging workers in an incubator for ~24 hours
- Making hives with 3 frames of newly emerged workers and 1 frame of honey
- Adding 10 "old" queens

Genetic analysis of workers from these colonies shows multiqueen reproduction

Appendix C

Survey Instrument

Adoption of multiqueen beekeeping

Start of Block: Survey Consent

Q1 Thank you for participating in this research! I'm going to ask you some questions about yourself, have you read a one-page information sheet on multiqueen honey bee colonies, and answer some questions about beekeeping. I expect that this survey will take about 20 minutes of your time. You are welcome to set your browser to private/incognito mode while taking the survey.

Consent Form

You are being asked to complete a survey for research purposes. The survey is about beekeepers' (hobbyist and professional) interest in multiqueen honey bee colonies. Completing this survey is voluntary and you can stop at any time by closing your web browser.

You must be 18 years of age or older, reside in the United States, and are a beekeeper (professional or hobbyist) to participate in this study.

There are minimal risks associated with your participation in this survey. You will not receive any payment for completing this survey. In order to ensure that your responses remain confidential, please take the survey in a private location with your browser in private/incognito mode and close your browser after you finish the survey.

If you have any questions about the survey itself, how it is implemented, or survey compensation, please contact the student researcher, James Withrow, at jmwithro@ncsu.edu and (828) 447-6427. You can also contact the faculty advisor for the protocol, Dr. Jean Goodwin, at jean_goodwin@ncsu.edu and (919) 515-8423. Please reference study number 21020 when contacting anyone about this project.

If you have questions about your rights as a participant or are concerned with your treatment throughout the research process, please contact the NC State University IRB Director at IRB-Director@ncsu.edu, 919-515-8754, or fill out this confidential form online.

If you consent to complete this survey, please select "Yes, I want to be in this research" below.

○ **Yes, I want to be in this research study.** (1)

○ **No, I do not want to be in this research study.** (2)

Skip To: End of Survey If Thank you for participating in this research! I'm going to ask you some questions about yourself,... = No, I do not want to be in this research study.

End of Block: Survey Consent

Start of Block: One pager

Q8 Here is some information about multiqueen beekeeping. Please review it so that you may answer the survey questions.

[See Appendix A for information sheet]

Q20 ***Additional information on multiqueen beekeeping:***
Bee Culture: The Horizontal Two-Queen System
American Bee Journal: Comparing Two-Queen Colony Management Methods

End of Block: One pager

Start of Block: Adoption questions

Q5 **Are you a professional beekeeper (beekeeping is your primary occupation) or hobbyist beekeeper?**

○ Professional (1)

○ Hobbyist (2)

Q6 **How many hives do you typically manage?**

○ 0 (1)

○ 1-5 (2)

○ 5-20 (3)

○ 20-50 (4)

○ More than 50 (5)

Q13 **For each of the mutliqueen management systems listed below please indicate your familiarity with the system**

	Please select familiarity		
	I have never heard of this (1)	I have heard of this but never seen it in person (2)	I have seen this in person (3)

Hive stacked with separate brood boxes on bottom and top with shared honey supers between (1)	○	○	○
Hive with separate brood boxes next to each other with shared honey supers on top (2)	○	○	○
Hive with multiple queens freely moving in a single brood area (3)	○	○	○

Q9 **Have you ever tried establishing multiqueen colonies?**

○ Yes (1)

○ No (2)

Page Break

Q7 **Please indicate how strongly you agree or disagree with the following statements:**

	Please select response				
	Strongly Disagree (1)	Disagree (2)	Neutral (3)	Agree (4)	Strongly Agree (5)

I am interested in the potential of multiqueen colonies to address the problem of queen failure (1)	○	○	○	○	○
I am interested in the potential of multiqueen colonies to increase colony growth and productivity (2)	○	○	○	○	○
Based on the information presented I think this method could be useful to beekeepers like me (3)	○	○	○	○	○
Based on the information presented I would be interested in trying to manage multiqueen colonies (4)	○	○	○	○	○

Based on the information presented I would be interested in trying multiqueen management but I think this method would not work well in my operation (5)	○	○	○	○	○

Q11 **Please indicate how strongly you agree or disagree with the following statements:**

	Please select response				
	Strongly Disagree (1)	Disagree (2)	Neutral (3)	Agree (4)	Strongly Agree (5)

Queen failure is a major problem in honey bee management (1)	○	○	○	○	○
Queens are failing more frequently than they used to (2)	○	○	○	○	○
Existing research and/or breeding programs are focused on the problems related to queen failure (3)	○	○	○	○	○
Existing research and/or breeding programs are improving queen survival and/or productivity (4)	○	○	○	○	○
More research should focus on addressing queen problems (5)	○	○	○	○	○

In general I am interested in trying new management techniques (6)	○	○	○	○	○
In general I am interested in trying new management techniques when someone I know recommends them (7)	○	○	○	○	○

Q12 **Which of the following best describes how likely you are to adopt new management techniques?**

○ I am usually the very first to try new techniques (1)

○ I tend to be among the earlier people trying new techniques (2)

○ I will try new techniques, but I like to wait until I've seen the early reviews and people I know have tried it first (3)

○ I tend to wait until many people I know have tried out new techniques before trying it myself (4)

○ What I have works just fine; I don't tend to try out new techniques (5)

End of Block: Adoption questions

Start of Block: Open ended questions

Q14 **What are some major problems in honey bee management that you are facing with your colonies?**

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Q15 **What sources do you rely on regularly to get information about honey bee management, if any?**

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Q16 **What questions do you have about multiqueen beekeeping?**

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Q18 **What would be the biggest challenge for you to try establishing multiqueen colonies with the method described in this survey?**

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End of Block: Open ended questions

Start of Block: Demographic questions

Q3 **Final Questions**

Please select your age category:

- ○ Under 40 (1)
- ○ 40 to 60 (2)
- ○ Over 60 (3)

Q4 **Please write in your gender identity:**

____

End of Block: Demographic questions

Appendix D

Recruitment Message

Hello,

My name is James Withrow, and I am a graduate student at NC State University. I am working on a project to improve management and productivity of honey bee colonies. The goal of the project is to learn whether professional and hobbyist beekeepers would be interested in using multiqueen honey bee colonies in their operations and how researchers can best support beekeepers like you. In order to figure this out, I am conducting a research study. I would like your input.

To participate, you must be 18 years old or older, live in the United States, and are a hobbyist or professional beekeeper. If you choose to participate in this research, you will complete an online survey where you will read a brief summary of multiqueen colony management and answer questions about your perceptions of beekeeping.

The survey should take about 15 minutes of your time. Participation is completely voluntary and all of your responses will be kept confidential. To ensure confidentiality, please take the survey in a private, quiet location and, if you would like, you are welcome to set your browser to private/incognito mode.

To participate, please click here to access the survey.

If you have questions about the research, please contact me at jmwithro@ncsu.edu or 828-447-6427, You can also contact the faculty advisor for this protocol, Dr. Jean Goodwin, at jegoodwi@ncsu.edu or 919-515-8423.

Thank you for your time and consideration!

Sincerely,
James Withrow
Graduate Student in Biology
North Carolina State University

Appendix E

Final list of codes and definitions

Sources of information used by beekeepers	
Code	**Definition**
Clubs and Mentors	Local beekeepers known personally
Industry Sources	Beekeeping-specific presentations, conferences, and publications at a regional or national level
Internet and Popular Figures	Websites or other information from widely known figures in beekeeping, online sources unless further specified as another code
Research and Extension	Presentations and publications from academic and extension sources, books unless further specified as another category
Other	Other or unspecified
Significant problems in beekeeping	
Code	**Definition**
Mites and diseases	*Varroa* mites and diseases generally, including treatment and treatment cost
Management and Swarming	Swarming and general management
Queen Problems	Queen problems including failure, supersedure, longevity, cost of queens
Environment and Resources	Weather, nectar flow, resource availability, feeding
Other	Other or unspecified

Questions about the method of multiqueen beekeeping	
Code	**Definition**
Basic Understanding	Questions about setup and bee biology
Efficacy	Questions about how well the method works, how long it lasts, data
Management	Questions about ongoing management after establishment, including swarm management
Modifications	Ideas for modifications or additional uses
Applicability	Suggestion that this method would not work well for a particular beekeeper, regardless of how it could work for others
Other	Other or unspecified
Challenges to adoption	
Code	**Definition**
Queens	Difficulty acquiring suitable queens or other queen-related concerns
Bees and Equipment	Concerns about worker bees, equipment, and setup
Management	Concerns about management after setup, including time investment
Unnatural	Unnatural system of beekeeping
Risk Aversion	Concern about failure, losing time and money, general desire to see others do this first
Other	Other or unspecified