SIMULTANEOUS SELECTION FOR REDUCED VARROA LEVELS, HYGIENIC BEHAVIOR, BROOD VIABILITY, BROOD PRODUCTION, HONEY PRODUCTION AND GENTLENESS IN EUROPEAN HONEY BEE (*APIS MELLIFERA* L.) COLONIES USING CONVENTIONAL QUEEN PROPAGATION AND MATING METHODS

by

NABOR H. MENDIZABAL

(Under the Direction of Keith S. Delaplane)

ABSTRACT

At the UGA honey bee laboratory fifty European honey bee colonies were established to develop a selection program for six traits at the same time during 2003-2004. The selected traits were low Varroa mite levels, hygienic behavior, brood viability, brood production, honey production and gentleness. All traits were measured and their values transformed into *z*-scores in order to normalize units. Using a selection index, positive trait values were added and negative ones subtracted to select the five queens whose daughters were reared to create the next generation in 2004. The population's mean responses for each trait were compared for both generations (2003 and 2004). 2004 population means were significantly higher than 2003 means for honey production, brood production and hygienic behavior. This constitutes preliminary evidence that a multiple trait selection program could work using a selection index based on *z*-scores.

INDEX WORDS: honey bee breeding, multiple trait selection, honey, brood, Varroa mites hygienic behavior, gentleness

SIMULTANEOUS SELECTION FOR REDUCED VARROA LEVELS, HYGIENIC BEHAVIOR, BROOD VIABILITY, BROOD PRODUCTION, HONEY PRODUCTION AND GENTLENESS IN EUROPEAN HONEY BEE (*APIS MELLIFERA* L.) COLONIES USING CONVENTIONAL QUEEN PROPAGATION AND MATING METHODS

by

NABOR H. MENDIZABAL

ING. AGR. Universidad Mayor de San Simon, Bolivia, 2002

A thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2004

SIMULTANEOUS SELECTION FOR REDUCED VARROA LEVELS, HYGIENIC BEHAVIOR, BROOD VIABILITY, BROOD PRODUCTION, HONEY PRODUCTION AND GENTLENESS IN EUROPEAN HONEY BEE (*APIS MELLIFERA* L.) COLONIES USING CONVENTIONAL QUEEN PROPAGATION AND MATING METHODS

by

NABOR H. MENDIZABAL

Major Professor: Keith S. Delaplane

Committee: Joseph McHugh
Nancy Hinkle

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2004

DEDICATION

I would like to dedicate this work to my family, Angelica Chavez, Mario, Abad and Huascar Mendizabal and to Jennifer Lewis for their constant support and encouragement.

ACKNOWLEDGEMENTS

This work would not be possible without the support of the Georgia Beekeepers Association and Dr. Keith Delaplane. I would like to also thank my committee members Dr. Joseph McHugh and Dr. Nancy Hinkle for their insightful suggestions and Jennifer Berry, Selim Dedej, Herbert Yeomans, Carl Hall and Jamie Ellis for all their help.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

CHAPTER 1

LITERATURE REVIEW

Within a span of less than 10 years the U.S. beekeeping industry transitioned from one that was chemical-averse to one that is chemical-dependent. This dramatic reversal was driven largely by the introduction and spread of Varroa mites (*Varroa destructor* Anderson and Trueman 2000, formerly *V. jacobsoni*). These blood-feeding parasitic mites made landfall in North America in the 1980s and 90s and spread to all regions where honey bee hives are kept. Colonies left untreated, with rare exception, die. Synthetic acaricides – chiefly Apistan™ (fluvalinate) and CheckMite™ (coumaphos) – have been developed and provide a good degree of control (Delaplane 1997), but chemical resistance in mites is a growing problem (Elzen et al., 1998, 1999; Milani 1999; Mathieu and Faucon 2000) and there are risks inherent to using acaricides when the host itself is an arthropod. Although alternative controls exist, the adoption of these technologies by beekeepers has remained modest. After four decades of continuous use of the antibiotic Terramycin™ (oxytetracycline) to control American foulbrood disease (AFB), antibiotic resistance in the causative bacterium *Paenibacillus larvae* was confirmed for the first time in this country in 1998 (Miyagi et al. 2000). The upshot is that the American beekeeping industry – responsible annually for $2.2 million in honey production (NASS 2002) and over $14 billion in crop pollination (Morse and Calderone 2000) – is for all practical purposes chemical-dependent.

Varroa and AFB are widely regarded as the two most serious bee disorders in the world. Varroa has been called “the most significant event affecting 20th century apiculture” (Sanford

2001), and for decades it was AFB that virtually defined the mandate of state bee inspection services (Morse 1997). The problem is all the more acute in the southern region where, with its warm climate and long brood-rearing season, both disorders occur at levels equal to or higher than anywhere else in the U.S. Thus, between chemical-resistant mites and chemical-resistant bacteria the argument has never been stronger for American beekeepers to adopt IPM with breeding as a strong component.

The Varroa and AFB crises have generated a prodigious output of IPM-based information and technologies by bee researchers in North America and abroad. Some of the most exciting progress has been the recognition of heritable Varroa- and AFB-resistance in honey bees. Various candidate traits have been implicated. So-called hygienic bees are capable of detecting abnormal brood cells – whether Varroa-parasitized or AFB-diseased – and removing the affected larva or pupa (Spivak 1996). Field trials have demonstrated that hygienic colonies have significantly fewer mites for up to one year without treatment (Spivak and Reuter 2001) and no AFB symptoms (Spivak and Reuter 1998). Bees selected for suppressed mite reproduction (SMR) confer a measure of infertility to the mites that parasitize them, through a mechanism that is not yet clearly understood and continues being studied (Harbo and Hoopingarner 1997; Harbo and Harris 1999b). The taxonomic work of Anderson and Trueman (2000) reveals that of the 20 known haplotypes of *Varroa* spp., only the Korea and Japan/Thailand haplotypes of *V. destructor* can successfully reproduce in the brood cells of the western honey bee *Apis mellifera*. In other words, SMR or mite resistance is a general, not exceptional, phenomenon in the *Varroa – A. mellifera* relationship and may prove fundamental to the pursuit of a truly mite-resistant honey bee. Other promising Varroa-resistance factors are shortened capped brood interval (Büchler and Drescher 1990) as well as hygienic behavior.

Selection Must be Reliable and Include Economic Traits

Delaplane (2003) has demonstrated a negative correlation between colony mite levels and the expression of hygienic behavior (Fig. 1). This confirms the efficacy of hygienic behavior, although average colony mite levels were independent of the selection status of queens ($F \geq 0.02$; df =1,74; $P \geq 0.5$). In other words, expression of hygienic behavior was independent of whether the queens were selected (and marketed) as "hygienic". Heritability for hygienic behavior is high ($h^2 = 0.65$ [Harbo and Harris 1999a]), so these kinds of breeding failures implicate problems with selection practices, mating control, or premature release of stocks in which expression is not yet measurably improved.

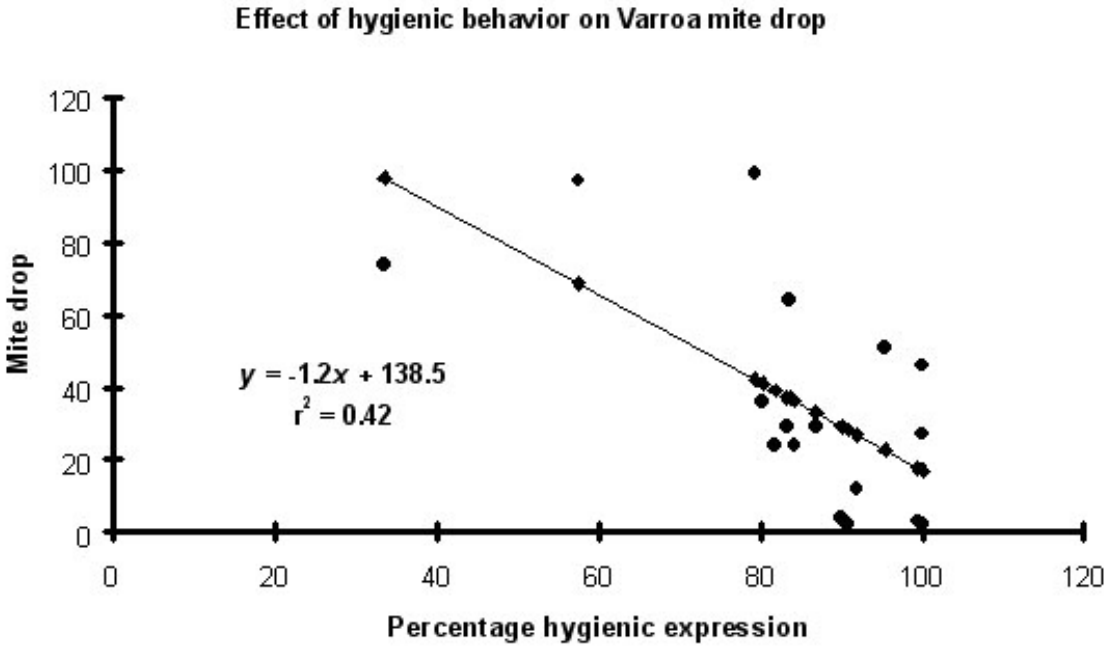

Figure 1: Effect of hygienic behavior on *Varroa* mite drop (Delaplane 2003)

Economic traits

While researchers have not been complacent about the importance of economic traits. The typical pattern has been that selection for mite resistance proceeds to a point that a stock shows resistance, and only then is it subjected to comparative economic trials with non-selected stocks. This was the case with resistant bee stock imported from Yugoslavia (Rinderer et al. 1993), from Russia (Rinderer et al. 2001) and a stock selected for hygienic behavior (Spivak and Reuter 1998). This study proposes selecting for economic characters synchronously with resistant ones. Such an approach has been done in Germany (Büchler 1997), but there are no published projects of this kind in the U.S. The efficacy of such a multiple-objective breeding program has yet to be demonstrated.

An historical pattern has been the release of selected stock to industry queen producers who then produce open-mated daughters from the selected breeder mothers. The latest example of this is a Cooperative Research and Development Agreement between the USDA ARS and a limited number of industry collaborators. The beekeepers are provided breeder queens from which they produce daughter queens for profit (Rinderer et al. 2000). The implication is that this approach will result in genes for resistance being inserted into stocks that have already been demonstrably productive. Although this is reasonable it is not self-evident and the track record is not reliable. American beekeepers rejected the Yugoslavian stock in the 1990s largely because beekeepers reported high supersedure rates and no improvement in Varroa resistance (personal communication, Joe Graham, editor American Bee Journal). Similarly, problems have appeared with brood production in stocks selected for SMR (Harbo and Harris 2001). Simultaneous selection of resistant and economic traits is a prudent safeguard against these kinds of demoralizing industry rejections. In the past, European stocks of honey bees have been selected

to express resistance to parasites and disease (Spivak 1996, Harbo and Harris 1999b). There is sufficient genetic variability for resistance to diseases and pests to make selective breeding a viable component of commercial honey bee management (Laidlaw and Page 1997).

Resistance Selection Parameters are Difficult

Harbo and Harris (1999b) emphasize that the most efficient way to select for mite resistance is to select for specific behavioral or physiologic traits known to confer resistance. Unfortunately, most of the modes of resistance involve parameters that are difficult for non-specialists to measure. For example, shortened capped brood interval requires precise monitoring of brood cells (Büchler and Drescher 1990). Suppressed mite reproduction, perhaps the most important resistance factor known, is determined by examining 30 cells of bee brood (purple-eyed pupae stage), each of which is invaded by only one foundress mite, and determining the percentage of cells in which the mite failed to reproduce. At this stage of development failure is indicated if (1) the foundress is dead with no offspring, (2) the foundress is alive but has no offspring, or (3) progeny are present but they are too young to reach maturity before the host bee emerges (Harbo and Harris 1999a). These kinds of observations require microscopes and specialized knowledge of arthropod ontogeny.

Szabo (1998) argues that the sum effect of all known resistance factors is reduced colony mite levels – a criterion long determined by the simple use of bottom board sticky sheets. Moreover, in using this criterion he has successfully selected a stock that supports smaller colony mite levels (Szabo and Szabo 2002). Thus, simple colony mite counts with bottom boards – a tool readily available in bee supply catalogs – are sufficiently predictive of resistance.

Selection and Instrumental Insemination

There is no question that instrumental insemination is the only way to absolutely control bee mating, except for those breeders fortunate enough to have access to isolated islands. It is the mating mechanism employed in the most sophisticated and efficient breeding schemes, specifically the closed population model developed by Page and co-workers (Page and Laidlaw 1982, 1985). Moreover in the applied beekeeping literature it is touted as a necessary feature of bee breeding (Taber 1995). Unfortunately the expense and expertise required for this technology are insurmountable for most beekeepers. Moreover, there are queen performance problems associated with the instrumental insemination process; instrumentally inseminated queens frequently exhibit reduced egg production and survivorship (Harbo and Szabo 1984, Harbo 1986a).

CHAPTER 2

INTRODUCTION

The American beekeeping industry is highly chemical-dependent. Applications of the synthetic acaricides fluvalinate (Apistan™) and coumaphos (CheckMite™) are used to control Varroa mites (*Varroa destructor).* The antibiotic oxytetracycline (Terramycin™) is used to control *Paenibacillus larvae*, the bacterium responsible for American foulbrood disease (AFB). Since 1997 chemical resistance in both *V. destructor* and *P. larvae* has been increasing in the United States. The extreme virulence of these disorders threatens the American beekeeping industry, which provides over $14 billion annually in crop pollination services (Morse and Calderone, 2000) and inestimable pollination benefits to natural ecosystems.

Genetic resistance in bees has emerged as a vital component of IPM programs for reduced-chemical beekeeping. Heritable hygienic behavior in bees can result in reduced colony AFB levels (Spivak, 1996). Numerous traits are implicated in genetic resistance to Varroa, including shortened brood capped interval, suppressed mite reproduction, and hygienic behavior (Delaplane, 1997).

Although there is evidence that bee breeding can reduce chemical use in beekeeping, experience has shown that adoption of selected stocks by beekeepers has been poor. There are at least three obstacles that contribute to this problem: (1) Resistance is not always reliably expressed, or has rarely been selected along with other traits of economic importance. (2) Multiple modes of Varroa resistance and consequent multiple selection parameters have created unnecessary confusion among breeders. (3) Instrumental queen insemination, long advocated as

indispensable to a successful selection program, has proven infeasible for the vast majority of U.S. beekeepers and may discourage queen producers from initiating selection programs.

Objective

Specifically, this project appraises the practicality of selecting a bee stock simultaneously for reduced colony Varroa levels, hygienic behavior, brood viability, high brood production, high honey production, and gentleness by using conventional queen propagation and mating methods (maternal selection, drone saturation, and open mating).

Hypothesis

The null hypothesis is that no measurable change occurs in any of the characters over two generations.

Materials and Methods

Selection approach and procedures

Efficiency in selection is compromised with every additional target trait. Nevertheless, the traits noted above are minimally necessary to demonstrate a bee stock that would be widely acceptable to U.S. beekeepers. Aside from the benefits discussed for mite resistance and hygienic behavior, it is clear that high brood production, honey production, and gentleness are necessary for industry acceptance. Heritability for most is reasonably high, and ranges between 0.46-1.24 for Varroa-resistance (Harbo and Harris 1999a), 0.65 for hygienic behavior (Harbo and Harris 1999a), 0.16-1.0 for seasonal honey production (Collins 1986), and 0.57 for defense behavior

(Collins et al. 1984). Brood solidness is a measure of brood viability, a good indicator of inbreeding, responds to selection (Kubasek 1980), and is optimized if the investigator selects within a population of at least 50 breeder colonies (queens) and mates the daughters to drones representing the whole population (Page and Laidlaw 1982). The selection methods employed for each target trait (described below) were chosen on the bases of practicality and demonstrated efficacy.

In a single-character (honey production) selection program using maternal selection and open-mating, Calderone and Fondrk (1991) achieved significant improvements in three generations. Maternal selection is the conventional choice of the beekeeping industry, and drone saturation – a practice for optimizing desirable mating (Hellmich and Waller 1990) – is intuitively understood by most beekeepers but probably under-utilized.

This study approximated the closed population-breeding model developed by Robert E. Page, Jr. and co-workers (reviewed by Page and Laidlaw 1985, Laidlaw and Page 1986). The closed population method permits a breeder to select for targeted traits in a delimited queen population "closed" from uncontrolled introgression of new genes. Thus, selection can proceed rapidly. However, the breeder must also minimize the inbreeding that occurs simultaneously at the sex locus. In honey bees this inbreeding has the undesirable consequence of poor brood viability, expressed as a brood pattern that is "spotty." Hence the closed population model seeks to progressively improve a stock on some pre-selected criteria while at the same time minimizing the effects of homozygosity at the sex locus. In the so-called mass-selection / random mating approach, superior queens in the closed population are selected to produce all the queens for the next generation (Page and Laidlaw 1982). Although the expected rate of sex allele loss is high with such intense maternal-side selection, this disadvantage is partially offset by inseminating

daughters with a homogenized mixture of semen representing the whole closed population. Since the intention was to work without instrumental insemination, this study achieved this population-wide representation of drones via drone saturation with drones produced by every queen in the population (Hellmich and Waller 1990). Admittedly this open-mating scheme compromises the 'closed' aspect of the theoretic ideal. Any cost will be expressed as slower achievement of targeted goals. However this liability is easily offset by reduced risk of inbreeding at sex loci, elimination of queen performance problems associated with instrumental insemination (II), and greater adoption by industry.

Large numbers of breeder colonies in a closed population program help guarantee a long life to the project. Based on the theoretic work of Laidlaw and Page (1986), each closed population was conservatively estimated to have >90% probability of maintaining at least 85% brood viability for 20 generations.

The first round of selection commenced in spring 2003, once colony populations expressed progeny of test queens, and measurements on the second generation were finished by summer of 2004. In 2003 five colonies (21, 25, 26, 33, 35) were selected out of 23 colonies that survived a rainy season and consequently a very poor nectar flow.

Colonies and incipient queens

A dedicated apiary of fifty nucleus colonies was established in 2002 to house queens, perform selections, and propagate naturally mated daughters. Each colony was composed of five deep Langstroth combs, a food-storage super above a queen excluder, and a syrup feeder. Each year every colony was provided with *ca*. 2 pounds of worker bees, brood of all stages, stored honey, and a clipped and marked queen. Initial queens, purchased or donated, were chosen with the

intention of maximizing genetic variation and sex allele number in the incipient closed population. Some queens were used that had already been selected for suppressed mite reproduction or hygienic behavior. Sources of queens included: Robert Binnie, Rabun Co., GA; Glenn Apiaries, Fallbrook, CA; Heitkams' Honey Bees, Orland, CA; Jesse McCurdy, Perry, GA; Dann Purvis, Blairsville, GA; Rossman Apiaries, Moultrie, GA; Shumans Apiaries, Baxley, GA; B. Weaver Apiaries, Navasota, TX; Carl Webb, Clarkesville, GA; and Wilbanks Apiaries, Claxton, GA. Each year, queens for propagation were selected based on the methods below.

Varroa mite levels

Colony Varroa levels (Szabo and Szabo 2002) were determined with standard sticky sheets modified to fit five frame nucleus hives; Delaplane and Hood (1997) established that 24-hour natural mite drop linearly predicts colony mite populations. The sticky sheets (Fig. 2) were placed underneath the colony on the bottom board and the number of mites counted after 24 hours. In 2003 one count per colony was made in August, and Varroa mite numbers were very low. In 2004 three counts were taken after leaving the sticky sheet for 72 hours under the colony. The data were converted to a 24-hour basis and averaged.

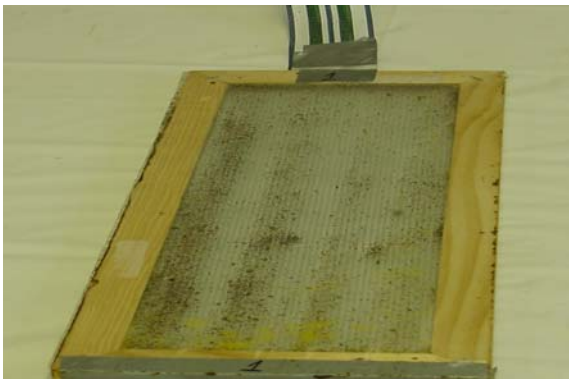

Figure 2: Sticky sheet for five-frame hives

Brood solidness

Brood solidness was determined by placing a grid that delimits 100 cells (Fig. 3) over a section of randomly chosen sealed brood and subtracting empty cells to determine the percentage brood solidness (R.E. Page, personal communication). In 2003 this measurement was taken on one occasion, and in 2004 twice. Each colony measure consisted of the mean of ten observations.

Figure 3: Grid that delimits 100 cells over a section of brood

Hygienic Behavior

Hygienic behavior was appraised in the field by freezing a section of brood with liquid nitrogen and determining the percentage of freeze-killed brood removed by bees after 72 hours (Spivak 1998, Figs. 4, 5, 6). The hygienic behavior of the 2003 generation was evaluated once in August. The 2004 generation was evaluated twice that year, once in June and again in July in every colony and the average response was used in the analysis.

Figure 4: Freezing a section of brood with liquid nitrogen.

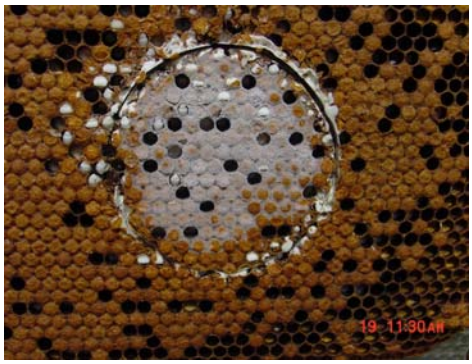

Figure 5: Freeze-killed brood.

Figure 6: Freeze-killed brood after removal by bees.

Honey Production

Seasonal honey production is predicted by 7-day colony net weight gains (Oldroyd et al. 1985) and total weight gain at the end of the season (Fig. 7). Honey production was measured in 2003 on a cotton field during August; colony net weight gain was recorded for one month until the nectar flow was over. In 2004 the net colony weight gain was measured during a sourwood nectar flow at the UGA Blairsville Experimental Station; the weight gain was recorded weekly, and the overall weight gain for one month summed.

Brood production

Brood production was measured as the area (cm²) of open brood (eggs and larvae) and sealed brood (prepupae and pupae) (Fig. 8). The area of brood was measured by placing a grid or transparent plastic sheet marked in cm² on the combs (Berry and Delaplane 2001). In 2003 only one brood area measurement was possible (in August) because of persistent rain. In 2004 every colony was evaluated two times, once in June and once in July, and then the mean was calculated as the value for brood production of that year.

Gentleness

Gentleness, also known as defense behavior, was measured by dragging a leather patch across the tops of exposed combs for a total of 120 seconds and counting the number of stings received by the patch (Delaplane and Harbo, 1987) (Fig. 9). Twenty passes were made on the exposed tops; each pass took approximately 10 seconds. No smoke was used to open or handle the colony during the test to allow natural defensive behavior to be expressed.

Figure 7: Measuring weight gain in the colonies.

Figure 8: Transparent plastic sheet marked in cm² on the combs.

Figure 9: Dragging a leather patch across the tops of exposed combs.

Selection Index

Proportional variables (% hygiene, % brood solidness) were first arcsine-transformed (Proc Standard, SAS 1992), and then each parameter value was converted to a *z* score, which standardizes measures into the same scale of standard deviation units. This permits the measures to be developed into a single selection index value in spite of differences in units of measure (Rinderer, 1986).

Characters were weighted in the following manner: brood solidness (0.3), colony Varroa levels (0.2), hygienic behavior (0.2), honey production (0.1), brood production (0.1), and gentleness (0.1). The highest weight value (0.3) was given to brood solidness because inbreeding is the greatest risk in a closed population breeding program (Laidlaw and Page 1986) and good brood viability is considered foundational to the entire project. Colony Varroa levels and hygienic behavior were weighted equally (0.2) since these traits are directly related to disease and pest resistance. Honey production, brood production and gentleness received the lowest weight values as these traits already have a long history of selection pressure by beekeepers. Negative signs were assigned to colony Varroa levels and defense behavior (gentleness) because they are undesired traits.

The top 20% (n=5) of 2003 queens were selected out of 24 colonies that successfully overwintered. Daughters were reared from these queens and these virgins used to requeen every colony in the population in 2004. Providing each colony a comb of drone cells derived from drone-sized foundation encouraged drone saturation within the mating area. Thus, the compromise inherent in the closed population model is approximated; maternally selected daughters are out-crossed with drones produced by all (including non-selected) queens in the population.

Analysis of variance (ANOVA) was used to compare average values of the selected characters between generations. The SAS system for Windows V8 was used to perform the ANOVA and means separated by Duncan's test ($\alpha \leq 0.05$). The Pearson correlation coefficients between all traits were estimated using the Microsoft Excel statistical function.

Results and Discussion

Varroa mite levels

There was no difference in average number of Varroa mites in 24 hours between generations (F=3.4; df=1,72; P=0.07). Varroa levels were very low in both generations, with an average of 0.5 ± 0.16 (mean ± standard error) mites per 24 hours for the first generation and 0.92 ± 0.14 for the second, suggesting that not enough variation existed in this character to permit directional change by breeding.

Brood solidness

Brood solidness was not statistically different between the two generations (F=1.5; df=1,71; P=0.2), but as expected there was a numeric reduction between the first (88.4 ± 1.5%) and the second generations (86.2 ± 1.2%). A selection program that approximates a closed population will suffer the reduction of sex allele variability, and consequently reduced brood solidness or viability (Page and Laidlaw 1985). Wolke (1980) demonstrated that 75% or better brood viability is necessary to ensure maximum honey production for progeny of multiply mated queens. Kubasek (1980) demonstrated that brood solidness and viability would respond to selection within a population of at least 50 breeder colonies (queens) where mating occurs with drones

representing the whole population (Page and Laidlaw 1985). During this study fewer than fifty colonies were evaluated in both years because not all the colonies produced enough brood or did not survive to perform the test.

Hygienic behavior

Percent hygienic expression was significantly improved (F=27.01; df=1,68; P<0.0001) from the first (56.3 ± 7.4%) to the second generation (88.8 ± 1.8%), implying a good sensitivity to selection. These results are consistent with other breeding programs (Spivak 1992) and the high heritability of this trait (h^2 = 0.65 [Harbo and Harris 1999a]).

Honey production

Honey production, measured as the total net weight gain of the colony during a month of available nectar flow, was statistically (F=7.3; df=1,72; P=0.0085) higher in the second generation (4.2 ± 0.4 kg) than in the first generation (2.4 ± 0.4). Our results are comparable and consistent with Calderone and Fondrk (1991) who showed moderate success for selection for high colony weight gain using selected mothers and unselected drones. Heritability of honey production ranges from 0.23 to 0.9 (el Bandy 1967, Pirchner et al. 1962, cited by Collins 1986) and even 1.0 for honey production on cotton (el Bandy 1967, cited by Collins 1986). Although there are always differences between years in nectar flows, this variation is unlikely to compromise the integrity of the selection program. Regardless of year effects, the protocol is always selecting the top producers within year, and there is no reason to presume that a stock selected under comparatively poor conditions will perform differently under good conditions.

Brood production

Brood production was significantly (F=29.8; df=1,72; P<0.0001) higher in the second generation (6587 ± 230.3 cm²) than the first (4360.8 ± 341.6 cm²). Heritability for this trait is relatively high (h^2=0.35 el Bandy 1967 cited by Collins 1986) and this could explain the difference after selection. On the other hand the amount of brood reared in a colony is controlled by environmental factors as well as genetics, fluctuating from no brood at all during winter to much brood during spring and summer. Our measurements were taken during the summer in both years (2003 and 2004) in an attempt to avoid an effect of environment.

Gentleness

There was variation in colony response to the gentleness test, probably as a result of environmental cues like temperature. The mean number of stings for the 2003 generation was 26.0 ± 5.7 compared with the 2004 generation at 18.5 ± 2.37. These differences were not significant (F=2.1; df=1,72; P=0.1). Even though number of stings on a target leather patch is the most documented defense character to discriminate colonies, the variation on this response is large. One way to improve the accuracy of the test could be to use 3.0 ml 1% isopentyl acetate in paraffin oil (Collins et al. 1994) as a standard stimulation to the colony. One could also reduce the time of exposure of the colony to 60 seconds instead of the 120 seconds, causing less stress to colonies and the person performing the test. Furthermore stings are the last of a long behavioral cascade, so alternative lower levels of defense, such as number of guard bees at the hive entrance after isopentyl acetate stimulation, could be considered to quantify colony gentleness.

Correlations

The strongest positive correlation was between honey production and brood production (R=0.6385). This agrees with the results of Rinderer and Collins (1986) (R=0.51) even though the value present work is higher. Brood solidness was positively correlated with honey production (R=0.4379) and brood production (R=0.3145) but the correlation was not high in either case. Hygienic behavior had a positive correlation with brood production (R=0.4421) and honey production (R=0.3455), but the correlation in either case could not be categorized as strong. As additional generations are added to this data set, it would be useful to include some of these dependent characters as covariates for one another in order to more rigorously isolate the effects of additive genetic selection.

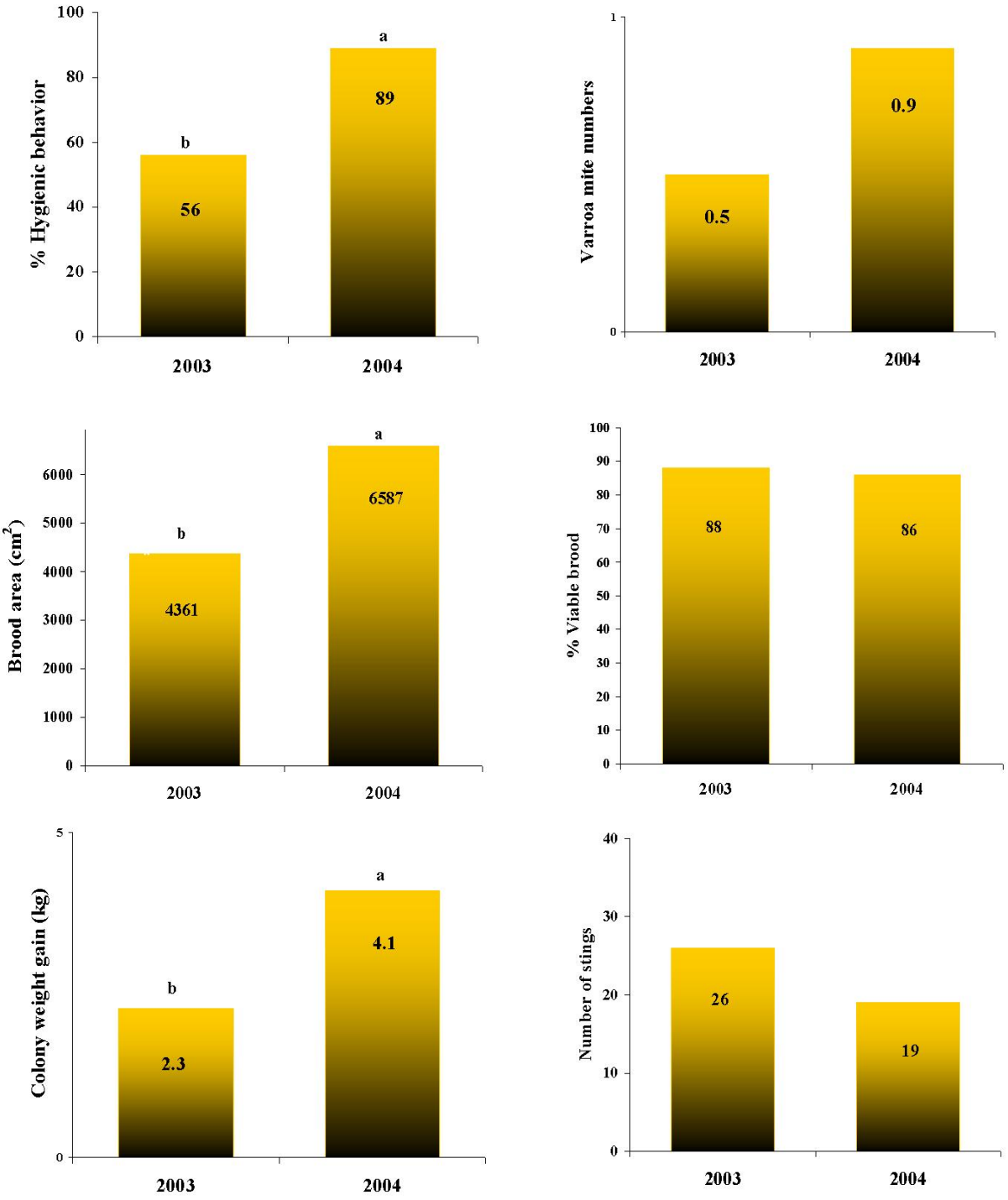

Figure 10: Graphic comparison between generations for all traits. **a** and **b** denote statistically significant differences.

CHAPTER 3

CONCLUSIONS

Hygienic behavior, brood production and honey production seem to be responsive to selection using this selection index. All three of these traits were significantly higher in the second generation. Gentleness, Varroa levels and brood viability did not change significantly after selection.

The results show some evidence that a multi-trait selection program using open mating and drone saturation (probably the most minimal conditions for ensuring desirable matings) could work on honey bees (*Apis mellifera* L.) for the traits described in this thesis. However, it remains to determine if such an ambitious multiple-objective scheme as herein proposed can be achieved in subsequent generations without the mating control afforded by instrumentally insemination. The matter should be settled by research because the potential benefits – an economically viable pest-resistant bee derived by conventional methods – are large and national in their impact.

REFERENCES

Anderson, D. L., and Trueman, J.W.H. 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Experimental and Applied Acarology 24: 165-189

Berry, J.A. and Delaplane K. S. 2001. Effects of comb age on honey bee colony growth and brood survivorship. Journal of Apicultural Research 40(1): 3-8

Büchler, R. 1997. Aktuelle Ergebnisse zur Selecktion auf *Varroa*toleranz. Allg. Deut. Emkerzeitung 31: 10-15

Calderone, N.W. and M.K. Fondrk. 1991. Selection for high and low colony weight gain in the honey bee, *Apis mellifera*, using selected queens and random males. Apidologie 22: 49-60

Collins, A.M. 1986. Quantitative genetics. *In* Bee genetics and breeding [T.E. Rinderer, ed.] Academic, pp. 283-304

Collins, A.M., Rinderer, T.E., Harbo, J.R., and Brown, M.A. 1984. Heritabilities and correlations for several characters in the honey bee. Journal of Heredity 75: 135-140

Collins, A.M., Daly, H.V., Rinderer, T. E., Harbo, J.R., and Hoelmer, K. 1994. Correlations between morphology and colony defence in *Apis mellifera* L. Journal of Apicultural Research 33(1): 3-10

Delaplane, K.S. 1997. Practical science-research helping beekeepers 3.Varroa. Bee World 78(4): 155-164

Delaplane, K.S. 2003. Toward delaying economic threshold for *Varroa*. American Bee Journal 143(4): 318-319

Delaplane, K.S. and J.R. Harbo. 1987. Effect of queenlessness on worker survival, honey gain, and defense behaviour in honeybees. Journal of Apicultural Research 26(1): 37-42

Delaplane, K.S. and W.M. Hood. 1997. Effects of delayed acaricide treatment in honey bee colonies parasitized by *Varroa jacobsoni* and a late-season treatment threshold for the southeastern USA. Journal of Apicultural Research 36(3/4): 125-132

Elzen, P.J., Eischen, F.A., Baxter, J.B., Pettis, J., Elzen, G.W., and Wilson, W.T. 1998. Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations. American Bee Journal 138(9): 674-676

Elzen, P.J., Baxter, J.R., Spivak, M., and Wilson, W.T. 1999. Amitraz resistance in varroa: new discovery in North America. American Bee Journal 139(5): 362

Harbo, J.R. 1986a. Oviposition rates of instrumentally inseminated and naturally mated queen honey bees (Hymenoptera: Apidae). Annals of the Entomological Society of America 79: 112-115

Harbo, J.R. 1986b. Propagation and instrumental insemination. *In* Bee genetics and breeding [T.E. Rinderer, ed.] Academic, pp. 361-389

Harbo, J.R. and T.I. Szabo. 1984. A comparison of instrumentally-inseminated and naturally mated queens. Journal of Apicultural Research 23(1): 31-36

Harbo, J.R. and R.A. Hoopingarner. 1997. Honey bees (Hymenoptera: Apidae) in the United States that express resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). Journal of Economic Entomology 90(4): 893-898

Harbo, J.R. and J.W. Harris. 1999a. Heritability in honey bees (Hymenoptera: Apidae) of characteristics associated with resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae). Journal of Economic Entomology 92(2): 261-265

Harbo, J.R. and J.W. Harris. 1999b. Selecting honey bees for resistance to *Varroa jacobsoni*. Apidologie 30: 183-196

Harbo, J.R. and J.W. Harris. 2001. Resistance to *Varroa destructor* (Mesostigmata: Varroidae) when mite-resistant queen honey bees (Hymenoptera: Apidae) were free-mated with unselected drones. Journal of Economic Entomology 94(6): 1319-1323

Hellmich, R.L. and G.D. Waller. 1990. Preparing for Africanized honey bees: evaluating control in mating apiaries. American Bee Journal 130(8): 537-542

Kubasek, K.J. 1980. Selection for increased number of sex alleles in closed populations of the honey bee: an investigation via computer simulation. MS thesis, Louisiana State University

Laidlaw, H.H., Jr. and Page, R.E., Jr. 1986. Mating designs. *In* Bee genetics and breeding (T.E. Rinderer, ed.) Academic, pp. 323-344

Laidlaw, H. H., Jr. and R.E. Page, Jr. 1997. Selective breeding. *In* Queen Rearing and Bee Breeding. Wicwas Press, Cheshire, Connecticut, pp.165-189

Mathieu, L., and Faucon, J. 2000. Changes in the response time for *Varroa jacobsoni* exposed to amitraz. Journal of Apicultural Research 39(3-4): 155-158

Milani, N. 1999. The resistance of *Varroa jacobsoni* Oud. to acaricides. Apidologie 30: 229-234

Miyagi T., Peng, C.Y.S., Chuang, R.Y., Mussen, E.C., Spivak, M., and Doi, R.H. 2000. Verification of oxytetracycline-resistant American foulbrood pathogen *Paenibacillus larvae* in the United States. Journal of Invertebrate Pathology 75: 95-96

Morse, R.A. 1997. Introduction. *In* Honey bee pests, predators, and diseases 3d ed. (R.A. Morse and K. Flottum, eds.) A.I. Root Co., Medina, Ohio, pp. 1-8

Morse, R.A., and Calderone, N.W. 2000. The value of honey bees as pollinators of U.S. crops in 2000. Bee Culture special insert, March 2000

NASS, National Agricultural Statistics Service, 2003. Georgia Farm Report, htt://www.nass.usda.gov/ga/pubs/farmrpts/04/frmrpt03.pdf

Oldroyd, B.P., C. Moran, and F.W. Nicholas. 1985. Diallele crosses of honeybees. I. A genetic analysis of honey production using a fixed effects model. Journal of Apicultural Research 24: 243-249

Page, R.E., Jr. and Laidlaw, H.H., Jr. 1982. Closed population honeybee breeding. 2. Comparative methods of stock maintenance and selective breeding. Journal of Apicultural Research 21(1): 38-44

Page, R.E., Jr. and Laidlaw, H.H., Jr. 1985. Closed population honeybee breeding. Journal of Apicultural Research 24(1): 63-74

Rinderer, T.E. and Collins, A.M. 1986. Behavioral Genetics. *In* Bee genetics and breeding [T.E. Rinderer, ed.] Academic, pp. 155-173

Rinderer, T.E. 1986. Selection. *In* Bee genetics and breeding [T.E. Rinderer, ed.] Academic, pp. 305-321

Rinderer, T.E., de Guzman, L.I., Kulincevic, J.M., Delatte, G.T., Beaman, L.D., and S.M. Buco. 1993. The breeding, importing, testing, and general characteristics of Yugoslavian honey bees bred for resistance to *Varroa jacobsoni*. American Bee Journal 133(3): 197-200

Rinderer, T.E., de Guzman, L.I., Harris, J., Kuznetsov, V., Delatte, G.T., Stelzer, J.A., and Beaman, L. 2000. The release of ARS Russian honey bees. American Bee Journal 140(4); 305-307

Rinderer, T.E., de Guzman, L.I., Delatte, G.T., Stelzer, J.A., Lancaster, V.A., Williams, J.L., Beaman, L.D., Kuznetsov, V., Bigalk, M., Bernard, S.J., and Tubbs, H. 2001. Multi-state field trials of ARS Russian honey bees. 2. Honey production 1999, 2000. American Bee Journal 141(10): 726-729

Sanford, M.T. 2001. Introduction, spread and economic impact of *varroa* mites in North America. *In* Mites of the honey bee. (T.C. Webster and K.S. Delaplane, eds.) Dadant, Hamilton, Illinois, pp. 149-162

Spivak, M. 1996. Honey bee hygienic behavior and defense against *Varroa jacobsoni*. Apidologie 27: 245-260

SAS Institute, 1992. SAS/STAT user's guide version 6. SAS Institute, 4th edition, Cary, NC, pp, 846

Spivak, M. 1998. Hygienic behavior of honey bees and its application for control of brood diseases and *varroa*, Part II. Bee World 79(4): 169-186

Spivak, M. and G. Reuter. 1998. Performance of hygienic honey bee colonies in a commercial apiary. Apidologie 29: 291-302

Spivak, M. and G. Reuter. 2001. *Varroa destructor* infestation in untreated honey bee (Hymenoptera: Apidae) colonies selected for hygienic behavior. Journal of Economic Entomology 94(2): 326-331

Szabo, T.I. 1998. Progress report on selective breeding of honey bees for resistance to parasitic mites. American Bee Journal 138(6): 464-466

Szabo, T.I. and D.C. Szabo. 2002. *Varroa* infestation levels of honey bee colonies during the fifth year of a breeding program: report for 2001. American Bee Journal 142(6): 423-427

Taber, S. 1995. Breeding super bees. A.I. Root, Medina, Ohio

Woodrow, A.W. and E.C. Holst. 1942. The mechanism of colony resistance to American foulbrood. Journal of Economic Entomology 35: 327-330

Wolke, J. 1980. Effect of sex allele homo-heterozygosity on honeybee colony populations and on their honey production. I. Favourable development conditions and unrestricted queens. Journal of Apicultural Research 19, 51-63