Pollen Foraging Response to Brood Pheromone by Africanized and European Honey Bees (*Apis mellifera* L.)

TANYA PANKIW[1] AND WILLIAM L. RUBINK

Beneficial Insects Research Unit, Honey Bee Group, USDA-ARS, Weslaco, TX 78596

Ann. Entomol. Soc. Am. 95(6): 761–767 (2002)

ABSTRACT We examined the pollen foraging responses of Africanized and European honey bee colonies to hexane extractable compounds of Africanized and European larvae (brood pheromone). Brood pheromone was presented to broodless Africanized and European colonies equalized for numbers of bees, food stores and, empty comb space. The pheromone significantly increased the ratio of pollen to nonpollen foragers returning to colonies. There was no differential pollen foraging response to pheromone racial origin. European colonies in this study had a significantly higher proportion of pollen to nonpollen foragers entering colonies than did Africanized colonies for pheromone and control treatments. The proboscis extension response to sucrose was used to test the sensitivity to sucrose of eight Africanized (most similar to *Apis mellifera scutellata* Lepeletier) and six European colonies (most similar to *Apis mellifera ligustica* L.). Individual sensitivity to sucrose has been demonstrated as a neuro-sensory correlate of foraging behavior in European bees such that individuals that forage for pollen have lower response thresholds to sucrose than bees that forage for nectar. Africanized bees were significantly more likely to respond to lower concentrations of sucrose than European bees. We concluded that sucrose response threshold was a poor predictor for comparative foraging behavior of these races because the neuro-sensory systems of the two races may be differentially "tuned" by thresholds to defensive cues.

KEY WORDS Africanized honey bee, sucrose response threshold, pollen foraging behavior, brood pheromone

MOST PARENTS PROVIDE food for their young through mass or progressive provisioning. Sterile or semisterile daughters of the queen (called workers) assume all the tasks of provisioning. The regulatory mechanisms by which colonies allocate the foraging force are a dominant feature in the study of social organization (Seeley 1995, Dreller and Page 1999, Beshers and Fewell 2001). Honey bee (*Apis mellifera* L.) workers are differentiated into behavioral groups, a consequence of age-related change called temporal polyethism (Winston 1987). In general, workers in their third week of adult life progress from performing tasks inside the nest to those outside the nest, such as foraging. A division of labor also exists among foragers. Some foragers collect pollen and others collect nectar (reviewed by Winston 1987). Factors affect foraging behaviors include worker genotype, forage resource abundance and quality, and colony environment.

Genotype affects the probability that a forager will collect pollen or nectar. Hellmich et al. (1985) conducted two-way selection for a single colony-level trait, the amount of pollen stored in the comb. After four generations, the high and low pollen hoarding strains differed by fivefold in the amount of stored pollen. Calderone and Page (1988) demonstrated that the cause of these differences in the strains was a result of differences in the individual foraging behavior of workers; high strain bees were more likely to collect pollen. Page and Fondrk (1995) repeated the selection program of Hellmich et al. (1985) and got the same result. Hunt et al. (1995) mapped quantitative trait loci of pollen foraging behavior in the high and low pollen hoarding strains. The putative trait locus two (*pln* 2) was demonstrated to affect the amount of pollen stored in colonies (Hunt et al. 1995).

Calderone (1993) provides results that suggest the high pollen hoarding strain is more sensitive to the effects of larvae on pollen foraging behavior. Eight colonies, each headed by an unrelated, caged queen comprised of 1,000 same aged (single-cohort) high strain bees, and eight single-cohort colonies of 1,000 low strain bees were subjected to one of two treatments: 1) empty comb or, 2) comb with 500 eggs when workers were 12-d-old. The number of cells provisioned with pollen were counted daily for 9 d while the brood was in egg (3 d) and larval stages (5 d). There was a significant strain by brood treatment interaction demonstrating high strain bees filled significantly more cells with pollen in response to the brood treat-

[1] Department of Entomology, Texas A&M University, College Station, Texas 77843–2475 (e-mail: t-pankiw@tamu.edu).

0013-8746/02/0761–0767$02.00/0 © 2002 Entomological Society of America

ment than did the low strain (Calderone 1993). High strain colonies with brood filled 57% more cells per day with pollen than did high strain colonies with no brood. Alternatively, low strain colonies with brood filled 0.3 fewer cells per day than low strain colonies with no brood (not significantly different). These results were not a consequence of greater pollen use by the low strain because the number of larvae in the colonies was controlled and the caged queens were prevented from adding brood. The results could also not be explained by differential larval cannibalism (Calderone 1993). The results suggested that the high pollen hoarding strain is more sensitive to the effects of brood on pollen foraging.

Africanized colonies have been shown to allocate a greater proportion of foragers to collecting pollen (Pesante et al. 1987). Page et al. (2000) produced colonies of bees derived from mating males from Africanized X European hybrid queens to sister European queens. As a consequence the genetic variation between colonies of the European by hybrid crosses was primarily because of variation in the genomes of the drone fathers derived from the hybrid queens. The high allele of *pln* 2, known to be associated with high pollen hoarding behavior (Hunt et al. 1995), was inherited from the Africanized bee population in these crosses (Page et al. 2000). Combined, these results suggest gene-based differential sensitivity to cues for pollen foraging on a population wide scale.

Colony environment also affects foraging behavior. Numbers of foragers collecting pollen and nectar are influenced by quantities of young larvae and the amount of pollen stored in combs in a colony. Quantities of stored pollen are regulated by colonies through negative feedback. Pollen foraging activity decreases when excess pollen is added to a colony, until the excess pollen has been depleted by the nurse bees and the quantity of stored pollen returns to near its previous level (Barker 1971, Free and Williams 1971, Moeller 1972, Fewell and Winston 1992). When pollen is removed from a colony, the number of pollen foragers remains high until the stored pollen is restored to the previous balance between foraging intake and nurse bee consumption (Lindauer 1952, Laere 1971, Fewell and Winston 1992). Increasing quantities of larvae stimulates more pollen foraging activity. Therefore, stored pollen acts like an inhibiting stimulus for pollen foraging while the presence of larvae act like a positive stimulus. The behavioral mechanisms by which larvae and stored pollen regulate foraging behavior are controversial (Camazine 1993, Camazine et al. 1998, Pankiw et al. 1998, Dreller et al. 1999, Dreller and Tarpy 2000, Pankiw and Page 2001a). However, it is clear that increasing amounts of pollen decreases pollen foraging and increasing amounts of larvae increases pollen foraging. Because the amount of comb space used by larvae and pollen are in dynamic interaction changing one without affecting the other is difficult. The use of larval pheromones by passes this difficulty.

Hexane extractable compounds from larvae and a synthetic blend have been demonstrated to release pollen foraging (Pankiw et al. 1998, Pankiw and Page 2001a). The number of pollen foragers increased by 2.5 times in colonies where brood pheromone was applied as a supplement to larvae (Pankiw et al. 1998). The pheromone had no effect on the number of nectar foragers. The ratio of pollen to nonpollen foragers increased significantly using synthetic brood pheromone in commercial almond pollinating colonies (Pankiw and Page 2001a). The pollen foraging releasing effect of the pheromone lasts for more than three but less than 4 h and then the number of foragers entering colonies returns to control levels. Here we asked whether the racial source of brood pheromone affected the pollen foraging behavior of Africanized and European colonies.

Recently, it has been demonstrated that response threshold to sucrose is associated with foraging behavior (Page et al. 1998, Pankiw and Page 1999, 2000). Honey bees respond with a reflexive extension of the proboscis to a sufficiently concentrated sucrose solution touched to the antennae (Bitterman et al. 1983). Pollen foragers have lower sucrose response thresholds than nectar foragers (Page et al. 1998). Genotype, independent of foraging experience, affects sucrose response thresholds. Strains of bees have been selected to store high and low amounts of pollen (Page et al. 1995). When fostered at the same time in the same colony high strain bees are more likely to forage for pollen and low strain bees to forage for nectar (Page et al. 1995). High strain preforaging aged bees have lower response thresholds to sucrose than low strain bees when fostered at the same time in the same unrelated colony (Pankiw and Page 1999). There is a causal link between sucrose response thresholds measured in preforaging aged bees and forage choice (Pankiw and Page 2000). Pollen foragers have lower response thresholds to sucrose as preforagers than nectar foragers (Pankiw and Page 2000). To date sucrose response thresholds have been examined in European honey bees (Page et al. 1998). Here we measured the sucrose response threshold, and pollen foraging behaviors of European and Africanized honey bee colonies treated with larval extracts of European and Africanized colonies.

Materials and Methods

Bees. We selected eight highly defensive (unpublished data) Africanized colonies maintained at the USDA Africanized apiary at Mission, TX, to include in this study. These colonies were established from locally collected feral swarms. Africanized bees have colonized this region since 1990 (Rubink et al. 1996). Six European colonies were derived from nondefensive (unpublished data), domesticated, commercial honey bee stock.

Proboscis Extension Response to Sucrose Assay (PER assay). Newly emerged bees were tested for their proboscis extension response to an increasing concentration series of sucrose. Combs containing emerging bees from the above sources were placed in an incubator maintained at 33°C and ≈55% RH. At

maximum intervals of 15 min newly emerged bees were removed from the combs and paint-marked to identify colony source and placed in a common cage and held at incubator conditions above. Bees were collected this way for 4 h. Two hours after collection was complete bees were placed in small plastic holders. The holders restrained body movement and allowed the head, mouthparts, and antennae to move freely. At least 1 h lapsed before testing began after placing bees in the holders. The tester was blind to the source of bees. The number of bees tested from eight Africanized colonies was 100, 63, 96, 87, 61, 72, and 59, respectively. The number of bees tested from six European colonies was 107, 77, 59, 123, 61, 133, and 60, respectively. Bees were tested over seven days. The number of bees tested per day from the different sources depended on the rate of emergence from selected combs.

Extension of the proboscis is a reflex response to antennal stimulation with sucrose solution (Bitterman et al. 1983). The lowest concentration of sucrose that elicits a response is interpreted as an individual's response threshold. All bees were first tested for their response to water. Any bees responding to water were allowed to imbibe water until they no longer responded to water stimulation. In this way we controlled for the confounding effect of thirst on sucrose sensitivity (Edgecomb et al. 1989, Pankiw et al. 2001). The ascending concentrations of sucrose solution were based on a $\log_{10}$ series of -1.0, -0.5, 0.0, 0.5, 1.0, and 1.5 corresponding to sucrose concentrations of 0.1, 0.3, 1, 3, 10, and 30% (wt/vol). Sucrose solutions were prepared using distilled and Milli-pore filtered water as the solvent for Sigma sucrose (99.5% purity). A droplet of solution was expressed from the tip of a 27-gauge needle and touched to each antenna. Positive or negative proboscis extension response to each sucrose concentration was recorded.

Brood Pheromone Extract. Brood pheromone was prepared by extracting 40 g of 2 to 4 day old larvae from multiple African and European colony sources in 20 ml of HPLC grade n-hexane (Sigma, St. Louis, MO) for 1 h at 20°C (after Pankiw et al. 1998). We used a standard method to control for size differences between the races by preparing pheromone extracts by weight (Simon and Nicholelis 2001). Forty-gram larval equivalents (LEq) of extract were presented on glass plates (164 cm^2) trussed with wire for suspension in the hive. The extract was poured onto the glass plates and the hexane was allowed to evaporate. The plates were immediately placed in the treatment colonies suspended between two wax combs in the center of the hive. Control plates were treated with 20 ml of hexane and presented in the same way.

Experimental Design. Eight Africanized colonies and six European colonies were prepared by moving a subset of bees and the queen from previously identified sources into five-frame Langstroth-deep nucleus colonies. Estimates of the number of bees in each hive were performed using a metered template. Six European and eight Africanized colonies were alternately subjected to three treatments: 1) 40 g LEq of Africanized brood pheromone, 2) 40 g LEq of European brood pheromone, and 3) control of 20 ml of hexane. Two Africanized colonies absconded during the course of the experiment and were replaced by two additional Africanized colonies.

Colonies were manipulated the evening before the pheromone treatments ($\approx$12 h) such that all immature bees were removed. Each colony contained two frames of honey, one frame with 30 cm^2 of pollen, and one frame of empty comb space. Treatments were placed in colonies at 7:00 AM. We conducted five minute entrance counts of returning pollen and nonpollen foragers at 8:00 AM. Each colony was measured two times by two people for a total of four 5-min counts per colony. The people performing the counts were blind to the treatments. Colonies contained their own brood for 1 to 2 days between treatments.

Statistical Analyses. All data were subjected to diagnostic tests for the assumptions of analysis of variance (ANOVA) and nonparametric statistics. The lowest concentration that elicited proboscis extension is interpreted as an individual's response threshold. A mean response threshold or each colony was calculated. Colony means were used in the Mann-Whitney *U* test to examine differences in sucrose response thresholds between test days and within races for pooling validation (Sokal and Rohlf 1995, SAS 2000).

Logistic regression model analysis was used to render visual displays and perform statistical analysis of PER assay data by Africanized and European honey bee race. Individuals were repeatedly tested for their responses to an ascending series of sucrose concentrations, therefore, responses were not independent after the first solution in the assay. The repeated measures and dependent options were used in the PROC GENMOD procedure of SAS (Ben-Shahar et al. 2000, SAS 2000, Hartz et al. 2001). PROC GENMOD produced generalized estimating equations (GEE) for the main effect, race of bee. PROC GENMOD also calculated a logistic regression line that best fitted each response curve and tested differences between slopes. Results for slope difference is presented as the Δ (delta) regression coefficient ($\pm$SE). Where the Δ regression coefficient was significant, slopes were significantly different.

ANOVA was used to examine colony size in Africanized and European colonies (Sokal and Rohlf 1995, SAS 2000). We used a saturated categorical model to examine the main effects of treatment, race of honey bee, and their interaction effect on the number of pollen and nonpollen foragers entering colonies (Stokes et al. 1997, Le 1998, SAS 2000).

Results

Proboscis Extension Response to Sucrose. Sucrose response thresholds fit the assumption for homogeneity of variances (Levene's Statistic = 0.6, df = 1, $P >$ 0.05), allowing the use of Mann-Whitney *U* and Kruskal-Wallis tests to compare sucrose response threshold differences (Siegel and Castellan 1988, Ka-

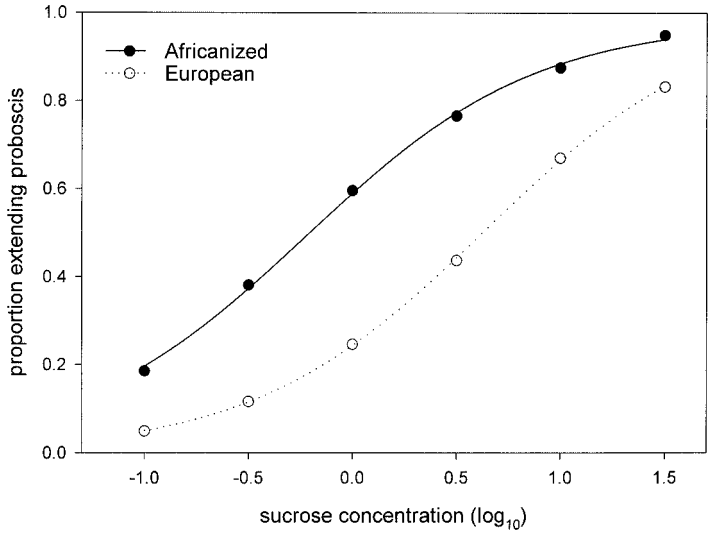

Fig. 1. Logistic regression curves of Africanized and European bee proboscis extension responses to sucrose. Individuals were tested for their sucrose response threshold by repeatedly touching each antenna with an ascending concentration series of sucrose. Africanized bees were significantly more responsive to sucrose solutions than European bees.

suya 2001). There was a significant day effect on response threshold (Kruskal-Wallis $\chi^2 = 87.1$, df = 6, $P <$ 0.0001). However, within each day the response thresholds ($\log_{10}$ of sucrose concentration) of Africanized (AHB) was consistently significantly lower than European (EHB) sources; Day 1: AHB, $-0.3 \pm$ 0.07; EHB, 0.3 ± 0.07, Mann-Whitney $U = 2106$, $P <$ 0.0001; Day 2: AHB, $-0.4 \pm .12$; EHB, 0.6 ± 0.06, $U =$ 882, $P < 0.0001$; Day 3: AHB, $0.02 \pm 0.05+$ EHB, $0.5 \pm$ .09, $U = 4236$, $P < 0.0001$; Day 4: AHB, 0.0 ± 0.1; EHB, 0.8 ± 0.1, $U = 450$, $P < 0.0001$; Day 5: AHB, 0.05 ± 0.06; EHB, 0.6 ± 0.08, $U = 2692$, $P < 0.0001$; Day 6: 0.3 ± 0.08; EHB, 0.8 ± 0.06, $U = 2268$, $P < 0.0001$; Day 7: AHB, 0.1 ± 0.06; EHB, 0.9 ± 0.06, $U = 1886$, $P < 0.0001$). Therefore, we pooled days to perform logistic regression analysis. Africanized bees were significantly more responsive to sucrose than European bees (GEE $\chi^2 =$ 135, df = 1, $P < 0.0001$). Sucrose response curves were significantly different such that Africanized bees had a significantly lower response threshold to the ascending sucrose concentration series expressed as a greater proportion of bees responding to each concentration than European bees (Δ regression coefficient = -0.6 ± 0.05, $P < 0.0001$; Fig. 1).

Effect of Brood Pheromone on Pollen Foraging. Africanized colonies contained a mean of 4531 ± 723 bees and European colonies 5312 ± 538 bees. There was no significant difference in colony size (ANOVA $F_{1,12} = 0.6$, $P > 0.05$). Numbers of foragers observed entering colonies cannot be explained by differential colony size. Treatments significantly affected foraging behavior ($\chi^2 = 691$, df = 2, $P < 0.0001$; Fig. 2). Colonies treated with brood pheromone were significantly more likely to forage for pollen than control colonies without pheromone. There was no significant differential response by the two races to the pheromone treatments (race*treatment interaction $\chi^2 = 3.8$, df = 2, $P > 0.05$). This means that racial origin of the pheromone did not result in different pollen foraging responses by Africanized and European colonies (Fig. 2). There was a significant effect of race on the ratio of pollen to nonpollen foragers entering colonies such that European colonies foraged more for pollen than did Africanized colonies ($\chi^2 = 692$, df = 1, $P < 0.0001$; Fig. 2).

Discussion

The mean proboscis extension response to sucrose by Africanized colonies was lower than European colonies. Africanized and European bees appear to be differentially constrained in their proboscis extension response to sucrose. The proboscis extension response was used to sample sensitivity to varying concentrations of sucrose. Sugar has been used frequently as a reward stimulus in many studies of foraging behavioral decisions. For example, the recruitment dance of the honey bee (Frisch 1967) has been used as a quantitative window into the perception of sugar and has suggested functional relationships between sucrose concentration and its perception (Waddington 1982, Waddington and Gottlieb 1990, Waddington and Kirchner 1992, Raveret-Richter and Waddington 1993, Waddington 1997). Page et al. (1998) determined another potential analysis of honey bees' perception of sugar using the proboscis extension response. A series of studies followed using wild-type and selected strains of European bees. Collectively these studies demonstrate that genotype places constraints on sucrose response threshold plasticity (Pankiw and Page 1999; Pankiw et al. 2001). The race-based lower response threshold of African bees was not associated with a higher probability to collect pollen. A more definitive test for differential responses to brood pher-

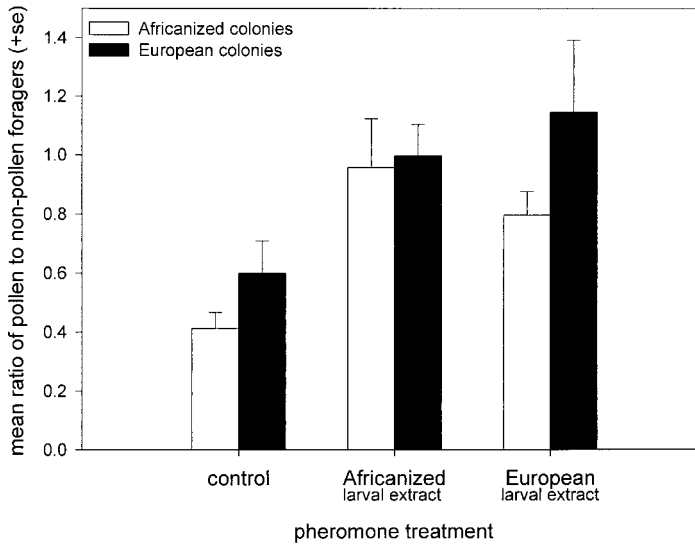

Fig. 2. Mean ratio of pollen to nonpollen foragers entering brood pheromone treated and control Africanized and European colonies. Africanized and European colonies were significantly more responsive to the stimulatory effects of larval extracts on pollen foraging. The racial origin of larval extracts did not differentially affect pollen foraging response.

omone and forage choice bias among these races should be conducted as a common garden experiment measuring the ratio of pollen to nonpollen foragers of cross- and co-fostered Africanized and European bees in pheromone and control colonies.

We speculate that differences in sucrose response thresholds in the two races may be related to their differential defensive behaviors. Neuro-chemicals within the central nervous system are associated with defensive behavior. Oral doses of tryptophan produce hyperactive states in European honey bees (Harris and Woodring 1999). Injection with octopamine potentiates the sting extension response in European honey bees (Burrell and Smith 1995). The opioid system in honey bees has also been implicated with stinging response to the major alarm pheromone, isopentyl acetate (Nuñez et al. 1998). Sucrose response thresholds may reflect a different state of arousal in Africanized bees as a consequence of natural selection for defensive behavior. If so, then, we may not expect to see an equivalent association of response threshold to sucrose and foraging behavior across the two races.

Africanized and European colonies clearly responded to the brood pheromone stimuli for pollen foraging. There was no differential response to the racial origin of the brood pheromone. Interestingly, Africanized colonies foraged less for pollen than did European colonies for all treatments. This is in contrast to Pesante et al. (1987) that report Africanized colonies collected more pollen that European colonies. The primary difference between Pesante et al. (1987) and this study is that here we controlled intra-colony factors such as number of bees, amount of comb area used by honey, pollen, and empty space. Amount of larvae, pollen and empty space have clearly been demonstrated to affect the number of pollen and nonpollen foragers (Free 1967, Barker 1971, Free and Williams 1971, AL-Tikrity et al. 1972, Fewell and Winston 1992, Camazine 1993, Eckert et al. 1994, Pankiw et al. 1998, Dreller and Tarpy 2000, Pankiw and Page 2001a, 2001b). Previous studies likely confounded the effects of variation in intra-colony stimuli and foraging behavior. Here we controlled intra-colony stimuli and varied the origin of the larval chemical cues.

European colonies had a significantly higher ratio of pollen to nonpollen foragers than Africanized colonies. This is interesting and potentially attributable to a number of factors. An individual's perception of the foraging environment affects foraging behavior at the individual and colony-level. Foraging and recruitment behavior changes as a result of pollen and nectar quality and quantity. Probability to dance and the rate of dance turns is positively related to pollen quality (Waddington et al. 1998). Bees perceive the value of nectar and change their foraging behavior accordingly. Increasing nectar volume and concentration, increases the probability of recruitment dances and the number of bees collecting nectar (Waddington 1982, Waddington and Gottlieb 1990, Waddington and Kirchner 1992). Individual colonies may have recruited to different resources and thus resource quality and quantity might have driven individual colony foraging behavior. Colony-level perception of the foraging environment would also affect recruitment. Our results demonstrate how colonies foraging from the same apiary with the same internal colony conditions respond to unknown cues that significantly affected the ratio of pollen and nonpollen foragers.

Colony phenotype is a consequence of natural selection acting on genetic variability affecting response threshold distributions of colonies. The introgression of African genes into the feral bee population of North

America has undoubtedly affected more than the response threshold distribution of defensive behavior traits. In addition, to colony-level foraging behavior differences we observed individual differences in sucrose response thresholds. Further comparative studies of natural and controlled populations of Africanized and European honey bees should provide additional insight into honey bee ecology and adaptation. Such future research may help to further explain not only the successful radiation of Africanized honey bees through most of the Americas, but also contribute to improved apicultural technologies.

Acknowledgments

We thank Roy Medrano and Art Cavazos for their assistance. This research was funded by a contribution from Biagro Western, Visalia, CA, to TPankiw. USDA/APHIS is gratefully acknowledged for their collaboration in the establishment and maintenance of Africanized honey bee colonies in secluded areas of the Moore Air Base Facility, Mission, TX.

References Cited

Al-Tikrity, W. S., A. W. Benton, R. C. Hillman, and W. W. Clarke, Jr. 1972. The relationship between the amount of unsealed brood in honeybee colonies and their pollen collection. J. Apic. Res. 11: 9–12.

Barker, R. J. 1971. The influence of food inside the hive on pollen collection. J. Apic. Res. 10: 23–26.

Ben-Shahar, Y., C. K. Thompson, S. M. Hartz, B. H. Smith, and G. E. Robinson. 2000. Performance on a reversal learning test is associated with division of labor in the honey bee, *Apis mellifera.* Anim. Cog. 3: 119–125.

Beshers, S. N., and J. H. Fewell. 2001. Models of division of labor in social insects. Ann. Rev. Entomol. 46: 413–440.

Bitterman, M. E., R. Menzel, A. Feitz, and S. Schäfer. 1983. Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). J. Comp. Psychol. 97: 107–119.

Burrell, B. D., and B. H. Smith. 1995. Modulation of the honey bee (*Apis mellifera*) sting response by octopamine. J. Insect Behav. 41: 671–680.

Calderone, N. W. 1993. Genotypic effects on the response of worker honey bees, *Apis mellifera,* to the colony environment. Anim. Behav. 46: 403–404.

Calderone, N. W., and R. E. Page. 1988. Genotypic variability in age polyethism and task specialization in the honey bee, *Apis mellifera* (Hymenoptera: Apidae). Behav. Ecol. Sociobiol. 22: 17–25.

Camazine, S. 1993. The regulation of pollen foraging by honey bees: how foragers assess the colony's need for pollen. Behav. Ecol. Sociobiol. 32: 265–272.

Camazine, S., K. Crailsheim, N. Hrassnigg, G. E. Robinson, B. Loenhard, and H. Kropiunigg. 1998. Protein trophallaxis and the regulation of pollen foraging by honey bees (*Apis mellifera* L.). Apidologie 29: 159–170.

Dreller, C., and R. E. Page. 1999. Genetic, developmental and environmental determinants of honey bee foraging behavior, pp. 187–202. *In* C. Detrain, J. L. Deneubourg and J. M. Pasteels [Eds.], Information Processing in Social Insects. Birkhäuser Verlag, Basel, Switzerland.

Dreller, C., and D. R. Tarpy. 2000. Perception of the pollen need by foragers in a honeybee colony. Anim. Behav. 59: 91–96.

Dreller, C., R. E. Page, and M. K. Fondrk. 1999. Regulation of pollen foraging in honeybee colonies: effects of young brood, stored pollen, and empty space. Behav. Ecol. Sociobiol. 45: 227–233.

Eckert, C. D., M. L. Winston, and R. C. Ydenberg. 1994. The relationship between population size, amount of brood, and individual foraging behaviour in the honey bee, *Apis mellifera* L. Oecologia 97: 248–255.

Edgecomb, R. S., A. R. Pyle, and L. L. Murdock. 1989. The role of water in tarsal thresholds to sugar in the blowfly *Phormia regina.* J. Exper. Biol. 142: 245–255.

Fewell, J. H., and M. L. Winston. 1992. Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. Behav. Ecol. Sociobiol. 30: 387–393.

Free, J. B. 1967. Factors determining the collection of pollen by honeybee foragers. Anim. Behav. 15: 134–144.

Free, J. B., and I. H. Williams. 1971. The effect of giving pollen and pollen supplement to honeybee colonies on the amount of pollen collected. J. Apic. Res. 10: 87–90.

Frisch, K., von. 1967. The dance language and orientation of bees. Belknap/Harvard University Press, Cambridge, MA.

Guzmán-Novoa, E., and R. E. Page. 2000. Pollen collection and foraging force by European and European X Africanized hybrid honey bees (Hymenoptera: Apidae) in mixed genotype colonies are similar. Ann. Entomol. Soc. Am. 93: 141–144.

Harris, J. W., and J. Woodring. 1999. Effects of dietary precursors to biogenic amines on the behavioral response from groups of caged worker honey bees (*Apis mellifera*) to alarm pheromone component isopentyl acetate. Physiol. Entomol. 24: 285–291.

Hartz, S. M., Y. Ben-Shahar, and M. Tyler. 2001. Logistic growth curve analysis in associative learning data. Anim. Cog. 4: 185–189.

Hellmich, R. L., and W. C. Rothenbuhler. 1986. Relationship between different amounts of brood and the collection and use of pollen by honey bees (*Apis mellifera* L.). Apidologie 17: 13–20.

Hunt, G. E., R. E. Page, M. K. Fondrk, and C. J. Dullum. 1995. Major quantitative trait loci affecting honey bee foraging behavior. Genetics 141: 1537–1545.

Kasuya, E. 2001. Mann-Whitney U tests when variances are unequal. Anim. Behav. 61:1247- 1249.

Laere, O. v., Martens N. 1971. Influence d'une diminution artificielle de la provision de proteines sur l'activite de collete de la colonie d'abeilles. Apidologie 2: 197–204.

Le, C. T. 1998. Applied Categorical Data Analysis. Wiley-Interscience Publication, New York.

Lindauer, M. 1952. Ein Beitrag zur Frage der Arbeitsteilung im Bienenstaat. Z. Vergl. Physiol. 34: 299–345.

Moeller, F. E. 1972. Honey bee collection of corn pollen reduced by feeding pollen in the hive. Am. Bee J. 112: 210–212.

Nuñez, J., L. Almeida, N. Balderrama, and M. Giurfa. 1998. Alarm pheromone induces stress analgesia via an opioid system in the honeybee. Physiol. Behav. 63: 75–80.

Page, R. E., J. Erber, and M. K. Fondrk. 1998. The effect of genotype on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). J. Comp. Physiol. A 182: 489–500.

Page, R. E., and M. K. Fondrk. 1995. The effects of colony-level selection on the social organization of honey bee (*Apis mellifera* L.): colony-level components of pollen hoarding. Behav. Ecol. Sociobiol. 36: 135–144.

Page, R. E., M. K. Fondrk, G. J. Hunt, E. Guzmán-Novoa, M. A. Humphries, K. Nguyen, and A. S. Green. 2000. Genetic dissection of honeybee (*Apis mellifera* L.) foraging behavior. J. Hered. 91: 474–479.

Page, R. E., K. D. Waddington, G. J. Hunt, and M. K. Fondrk. 1995. Genetic determinants of honey bee foraging behavior. Anim. Behav. 50: 1617–1625.

Pankiw, T., and R. E. Page. 1999. The effects of genotype, age, and caste on response thresholds to sucrose and foraging behavior of honey bees. J. Comp. Physiol. A185: 207- 213.

Pankiw, T., and R. E. Page. 2000. Response thresholds to sucrose predict foraging division of labor in honey bees. Behav. Ecol. Sociobiol. 47: 265–267.

Pankiw, T., and R. E. Page. 2001a. Brood pheromone modulates sucrose response thresholds in honeybees (*Apis mellifera* L.). Behav. Ecol. Sociobiol. 49: 206–213.

Pankiw, T., and R. E. Page. 2001b. Genotype and colony environment affect honey bee (*Apis mellifera* L.) development and foraging behavior. Behav. Ecol. Sociobiol. 51: 87–94.

Pankiw, T., R. E. Page, and M. K. Fondrk. 1998. Brood pheromone stimulates pollen foraging in honey bees (*Apis mellifera*). Behav. Ecol. Sociobiol. 44: 193–198.

Pankiw, T., K. D. Waddington., and R. E. Page. 2001. Modulation of sucrose response thresholds in honey bees (*Apis mellifera*): influence of genotype, feeding and foraging experience. J. Comp. Physiol. A 187: 293–301.

Pesante, D., T. E. Rinderer, and A. M. Collins. 1987. Differential pollen collection by Africanized and European honeybees in the neotropics. J. Apic. Res. 26: 24–29.

Raveret-Richter, M., and K. D. Waddington. 1993. Past foraging experience influences honey bee dance behaviour. Anim. Behav. 46: 123–128.

Rubink, W. L., P. Luévano-Martínez, E. A. Sugden, W. T. Wilson, and A. M. Collins. 1996. Subtropical Apis mellifera (Hymenoptera: Apidae) swarming in dynamics and Africanization in northeastern Mexico and southern Texas. Ann. Entomol. Soc. Am. 89: 243–251.

SAS. 2000. The SAS System Version 8.01 computer program, version By SAS, Cary, NC.

Seeley, T. D. 1995. The wisdom of the hive. The social physiology of honey bee colonies. Harvard University Press, Cambridge, MA.

Siegel, S., and N. J. Castellan, Jr. 1988. Nonparametric Statistics for Behavioral Sciences. McGraw Hill, New York.

Simon, S. A., and M. A. L Nicholelis [Eds.]. 2001. Methods in Chemosensory Research. CRC, New York.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. The principles and practice of statistics in biological research. W. H. Freeman and Company, New York

Stokes, M. E., C. S. Davis, and G. G. Koch. 1997. Categorical Data Analysis Using the SAS System. SAS Institute, Cary, NC.

Waddington, K. D. 1982. Honeybee foraging profitability and round dance correlates. J. Comp. Physiol. A 148: 279–301.

Waddington, K. D. 1997. Foraging behavior of nectarivores and pollen collectors. Acta Hortic. 437: 175–191.

Waddington, K. D., and N. Gottlieb. 1990. Actual versus perceived profitability: a study of floral-choice of honey bees. J. Insect Behav. 3: 429–441.

Waddington, K. D., and W. Kirchner. 1992. Correlates of profitability of food sources in honey bee round dances. Ethology 92: 1–6.

Waddington, K. D., C. M. Nelson, and R.E.J. Page. 1998. Effects of pollen quality and genotype on the dance of foraging honey bees. Anim. Behav. 56: 35–39.

Winston, M. L. 1987. The biology of the honey bee. Harvard University Press, Cambridge, MA.

Received for publication 12 December 2001; accepted 9 August 2002.