

DEFENSIVE BEHAVIOUR OF AFRICANIZED AND EUROPEAN HONEYBEES AT TWO ELEVATIONS IN COLOMBIA¹

JOSÉ D. VILLA

U.S. Department of Agriculture, Agricultural Research Service, Honey-Bee Breeding, Genetics & Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, LA 70820, USA

Revised typescript received for publication 15 October 1987

Summary

The defensive behaviour of Africanized (10) and European (8) honeybee colonies was compared at 520 and 2450 m above sea level (34° and 17°C, respectively) by measuring the time to the first sting on a moving leather patch and the total number of stings received by the patch 30 seconds after the first sting. Considering results from both locations, Africanized colonies were, on average, twice as fast to sting and left eight times more stings ($P < 0.01$). Considering the responses of both geographical types, the average time to first sting was doubled and average number of stings was halved at the higher site ($0.01 < P < 0.05$). Interactions between site and geographical type were not significant.

Introduction

The intense 'aggressive' behaviour of Africanized bees has led to new studies on honeybee defence in the past 15 years. Most of the experimental work has centered on the genetics of defence, while less attention has been given to the influence and interaction of environmental factors on defensive behaviour of colonies.

The genetic components of defensive behaviour have been investigated using laboratory and field tests. Lines of European bees selected for high and low defence have shown differences in both hoarding cage tests (Collins, 1979) and in field tests (Boch & Rothenbuhler, 1974). The more dramatic differences between European and Africanized bees have been shown in laboratory tests (Collins, 1982), and in field tests in subtropical Brazil (Stort, 1975*a, b*) and in tropical Venezuela (Collins et al., 1982).

Few of the many environmental factors reported to influence defence in honeybees have been investigated experimentally (Collins et al., 1980). Collins (1980) found that 4-week-old European bees in hoarding cages reacted faster to alarm pheromones than newly emerged or 6-week-old bees. In similar tests, the speed, duration, strength and frequency of reactions to pheromone were greater at 35°C than at lower temperatures, but higher humidities only increased the speed of reaction (Collins, 1981). Higher temperatures also increased the attacks by healthy bees on intruders infected with paralysis virus, but the pattern was not consistent with healthy intruders or healthy hivemates (Drum & Rothenbuhler, 1984).

Field tests have shown that Africanized honeybees are more defensive in northern tropical areas of Brazil than in southern, subtropical areas (Michener, 1975). Although these differences may possibly be attributed to greater hybridization with gentle European bees in more temperate climates, there are indications that environmental conditions might be influencing defensive behaviour as well. Rothenbuhler (1974) reported that the same 3 colonies of Africanized bees left more stings on a leather square at 29°C than at 23°C. The results obtained by Brandeburgo et al. (1977) by testing colonies from subtropical Sao Paulo and from tropical Pernambuco at both locations, suggest that higher temperatures and humidities increase defensiveness in Africanized bees. However, the temperature ranges tested, were very similar and correlations between weather and defence parameters gave contradictory results at the two locations.

The present test represents the first quantitative side-by-side comparison of defence by Africanized and European colonies in two distinct environmental conditions. It permits comparisons between Africanized and European honeybees at different locations. It also allows testing for the effects of location on the defensive behaviour of bees and permits the detection of interactions between genotype and environment.

¹Based on part of a thesis submitted in partial fulfillment of the requirements for an M.A. degree to the Department of Entomology, University of Kansas, Lawrence.

Materials and Methods

The same Africanized (10) and European (8) colonies were tested for defensiveness after being established for 6 weeks at 2450 m above sea level (a.s.l.), then moved and tested again after 6 weeks at 520 m a.s.l. Four Africanized colonies were formed from captured swarms; the other 6 colonies were headed by queens reared from a feral Africanized colony and mated in an area of intense Africanization. Four of the European colonies were headed by queens from a mixed population of European races of bees in Kansas; the rest had queens reared and mated, prior to the arrival of Africanized bees, at a plateau where a feral population of black 'native' Colombian bees (*Apis mellifera mellifera*) had existed for decades. Comb measurements (Rinderer et al., 1986) and/or morphometric analyses (based on forewing, hind wing, femur, and tibia lengths; Daly & Balling, 1978) confirmed the origins of locally produced Africanized and European queens.

The colonies were placed randomly in a 0.1 ha pasture at the higher site and along a 200 m fence row at the lower location. At the higher site, the colonies were in the open, but at the second site, they were in partial shade after midday. Colonies were separated from their nearest neighbour by at least 5 m. The populations of the colonies were equalized several times before the tests by removing sealed brood from the strongest colonies. This operation was last performed at both sites 1 week prior to the tests, so that all colonies had brood on 4 Langstroth frames and adult bees covering from 4.5 to 5.5 frames. Observations at each site during the 6 weeks prior to each test showed that consumption of resources exceeded collection.

Defensiveness was tested at 2450 m a.s.l. from 11.30 to 12.30 h. min on 9 August, 1983, and at 520 m from 15.00 to 16.30 h. min on 6 October, 1983. Air temperatures during the tests averaged 17°C at the first site and 34°C at the second. To initiate a test, a colony was approached from behind and a black suede rectangle (4 × 5 cm) attached to a 1-m wire was presented 10 cm from the entrance and moved in an arc as wide as the entrance at the rate of one pass per second. The time until the first bee stung the leather was recorded, and from that instant bees were allowed to sting the leather for 30 s. At the completion of a test on each colony, the suede was placed immediately in a plastic bag, which was then carried a 'safe' distance away so that the number of stings on both sides of the suede patch could be counted. New patches were used for each new colony. Colonies were tested only once at each location. The data for time to the first sting and for number of stings were analysed by a two-way ANOVA (with race as treatment and location as block).

Results

The time to the first sting as well as the number of stings the suede patch received during 30 s showed a highly significant effect of bee type and a significant effect of site (Tables 1, 2). There was clearly no interaction between type and site on time to the first sting (Table 1), but the probability was much lower (yet not statistically significant) for the interaction on the number of stings (Table 2).

Although no quantitative data were taken on distance the bees followed observers, there was an obvious difference between the two sites in the 'safe distances' for observers. At the higher

TABLE 1. Time to first sting (s) on a 20-cm² suede patch moved in front of 10 Africanized and 8 European colonies at 520 and 2450 m above sea level (means ± SE).

Site (a.s.l.)	Temp	Race		Site mean
		Africanized	European	
520 m	34°C	20.8 ± 6.84	83.1 ± 29.11	52.0 ± 14.79 <i>F</i> = 4.94*
2450 m	17°C	71.9 ± 22.31	125.0 ± 21.01	98.4 ± 14.79
Race mean		46.3 ± 13.95	104.1 ± 15.59	Race × Site <i>F</i> = 0.5 ns

ns *P* > 0.05

* 0.01 < *P* < 0.05

** *P* < 0.01

TABLE 2. Number of stings on a 20-cm² suede patch after 30 s from the first sting by 10 Africanized and 8 European colonies at 520 and 2450 m above sea level (means \pm SE).

Site (a.s.l.)	Temp	Race		Site mean
		Africanized	European	
520 m	34°C	31.5 \pm 5.95	5.1 \pm 1.87	18.3 \pm 2.90 <i>F</i> = 6.03*
2450 m	17°C	15.2 \pm 3.94	1.2 \pm 0.49	8.2 \pm 2.90
Race mean		23.3 \pm 2.74	3.2 \pm 3.06	Race \times Site <i>F</i> = 2.29 <i>ns</i>

F = 24.10***ns* *P* > 0.05* 0.01 < *P* < 0.05** *P* < 0.01

site, a person recording information was able to sit at the perimeter of the 0.1 ha pasture during the duration of the test, and the person testing walked away from the colony and counted stings within 10–20 m of the colonies. At the lower site, the recorder had to hide in the fence row at least 20 m from some test colonies while the tester had to run up to 250 m before he could safely count the number of stings in the leather.

Discussion

The influence of genetic and environmental factors on colony defensive behaviour is clearly demonstrated in this experiment. The genetic component matches other experiments that have compared Africanized and European bees (Stort, 1975*a, b*; Collins et al., 1982). The number of stings received during 30 s from Africanized and European colonies related to area of the leather patch (20 cm² on each side) give an average number of stings per cm² at the lower site of 0.78 and 0.12, respectively, matching the 0.85 and 0.10 obtained at a similar location in Venezuela by Collins et al. (1982). These measurements on small targets might be overestimating the response of Africanized colonies, given that differential response to differing sizes of targets exists between Africanized and European bees (Koeninger, 1979). On the other hand, the small targets could be underestimating the maximum number of stings possible from the most defensive colonies because of stinging bees covering the patch.

The environmental component of the results is not as easy to interpret. Many of the direct and indirect environmental factors possibly influencing defence were similar at the two test sites: stored resources, available forage, and number of bees in each colony. The effect of altitude, which was the intended treatment, is not readily determined because changes in elevation are correlated with partial gas pressures, ultraviolet radiation, sun, shade and air temperatures (Mani & Giddings, 1980).

During the tests, the most obvious difference between the two sites was temperature (17° and 34°C). Because the results of the tests correspond to the expected increase of defence with increasing temperature (Brandeburgo et al., 1977; Collins, 1981), it is tempting to consider it as the most important component of altitude in affecting the defensive behaviour of both Africanized and European bees. The time to first sting quantifies the speed with which the first bee in the colony goes through the sequence of 'alerting, activating, orienting and culminating' (Collins et al., 1980). If this is done by guard bees, lower temperatures could increase the time it took them to enact the sequence. In the first place, lower temperatures increase aggregation (Kronenberg & Heller, 1982) and there might be a smaller proportion of bees at the entrance available for the visual stimulus of the moving target. Secondly, if guard bees separated from the cluster maintain thoracic temperatures closer to ambient, they might require a longer time to warm up at the higher site to the 27°C required for flight (Heinrich, 1979). The number of stings after 30 s quantifies the response of the group of bees to the pheromone emission from the first and following stings. Lower temperatures would decrease the diffusion rate of the pheromone and therefore the number of stings induced by the first sting.

The results presented here could lend themselves to hasty conclusions if the variance in defensive behaviour and the influence of multiple environmental factors are not considered

carefully. The use of this defence test to discriminate between Africanized and European bees would be of little value; although the average values were widely separated, there was overlap in both attributes that would have led to misidentifications if used as the sole discriminator. There was also no clear correlation between the defensive response of individual colonies under the two environmental conditions so that colonies that would have been misidentified at the highest site would have been different from the misidentified colonies at the lower location. Similar defence tests on European and Africanized bees in Argentina (Krell et al., 1985) showed a marked discrepancy with regard to the results of the Daly et al. (1982) morphometric analysis.

The average measures of defensive behaviour suggest that it might be easier for beekeepers to keep aggressive Africanized bees in the highlands of the tropics. This would not necessarily mean a problem-free situation. The experiment measured only initial reaction time and initial strength of reaction, and therefore only quantified reactions to minor disturbances. Once colonies have been strongly disturbed beyond a 'point of no return,' where pheromone emissions are high and bees have warmed up to attack-temperatures (Heinrich, 1979), defensive behaviour might be as strong at high as at low elevations.

If the effect of temperature on the defensiveness of Africanized bees proves to be as strong as suggested by these preliminary results, beekeepers in 'cooler' temperate climates will not be protected from the stinging problems encountered by beekeepers in the 'warmer' tropics. Even though average yearly temperatures are higher in the tropics than in many temperate climates, summer temperatures in many temperate regions surpass even the highest temperatures in the tropics. In the southern USA, where the impact of Africanized bees is predicted to be most serious (McDowell, 1984; Taylor, 1985), beekeeping activities coincide with these warmest periods. The higher defensive behaviour that could be expected during these months might add to the already predicted economic difficulties of beekeeping in an Africanized area.

Acknowledgements

Financial support was provided by Proyecto Abejas Africanas, Colombia (Universidad Nacional de Colombia, International Development Research Centre). The assistance with data collection by Carl Lowenberger and Maurice V. Smith, and with its morphometric analyses by Adolfo Molina and John A. Quiroz, is appreciated. Centros de Produccion (Universidad Nacional de Colombia) and Alvaro Villa offered apiary sites. The USDA, ARS Honey-Bee Breeding, Genetics and Physiology Research Laboratory, in cooperation with the Louisiana Agricultural Experiment Station, provided support for the preparation of this report.

References

- BOCH, R.; ROTHENBUHLER, W. C. (1974) Defensive behaviour and production of alarm pheromone in honeybees. *J. apic. Res.* 13 : 217-221
- BRANDEBURGO, M. A. M.; GONÇALVES L. S.; KERR, W. E. (1977) Estudo du correlacao entro caracteres comportamentais (agressividade) de abelhas Africanizadas e condicoes climaticas. *Ciênc. Cult., S Paulo.* 29 : 750
- COLLINS, A. M. (1979) Genetics of the response of the honeybee to an alarm chemical, isopentyl acetate. *J. apic. Res.* 18 : 285-291
- (1980) Effect of age on the response to alarm pheromones by caged honey bees. *Ann. ent. Soc. Am.* 73 : 307-309
- (1981) Effects of temperature and humidity on honeybee response to alarm pheromones. *J. apic. Res.* 20 : 13-18
- (1982) Behaviour genetics of honey bee alarm communication. pp. 307-311 from Proceedings IX Congress of the IUSSI. ed. M. D. Breed. Boulder, Colorado: Westview
- COLLINS, A. M.; RINDERER, T. E.; HARBO, J. R.; BOLTEN, A. B. (1982) Colony defence by Africanized and European honey bees. *Science.* 218 : 72-74
- COLLINS, A. M.; RINDERER, T. E.; TUCKER, K. W.; SYLVESTER, H. A.; LACKETT J. A. (1980) A model of honeybee defensive behaviour. *J. apic. Res.* 19 : 224-231
- DALY, H. V.; Balling, S. S. (1978) Identification of Africanized honeybees in the western hemisphere by discriminant analysis. *J. Kans. ent. Soc.* 51 : 857-869
- DALY, H. V.; HOELMER, K.; NORMAN, P.; ALLEN, T. (1982) Computer-assisted measurement and identification of honey bees. *Ann. ent. Soc. Am.* 75 : 591-594

- DRUM, N. H.; ROTHENBUHLER, W. C. (1984) Effect of temperature on non-stinging aggressive responses of worker honeybees to diseased and healthy bees. *J. apic. Res.* 23 : 82-87
- HEINRICH, B. (1979) Thermoregulation of African and European honeybees during foraging, attack, and hive exits and returns. *J. exp. Biol.* 85 : 217-229
- KOENIGER, N. (1979) Differences in optical releasers of attack flight between *Apis mellifera carnica* and *Apis mellifera adansonii*. p. 56 from *Apiculture in Hot Climates*. ed. V. Harnaj. Bucharest: Apimondia
- KRELL, R.; DIETZ, A.; EISCHEN, F. A. (1985) A preliminary study on winter survival of Africanized and European honey bees in Cordoba, Argentina. *Apidologie*. 16 : 109-118
- KRONENBERG, F.; HELLER, H. C. (1982) Colonial thermoregulation in honey bees (*Apis mellifera*). *J. comp. Physiol.* 148 : 65-76
- MANI, M. S.; GIDDINGS, L. E. (1980) Ecology of the highlands from *Monographie Biologicae*. ed. J. Illies. The Hague: Jung
- MCDOWELL, R. (1984) The Africanized honey bee in the United States. *Agr. Economic Report No. 519*. Washington: US Government Printing Office
- MICHENER, C. D. (1975) The Brazilian bee problem. *A. Rev. Ent.* 20 : 390-416
- RINDERER, T. E.; SYLVESTER, H. A.; BROWN, M. A.; VILLA, J.; PESANTE, D.; COLLINS, A. M. (1986) Field and simplified techniques for identifying Africanized and European honey bees. *Apidologie*. 17 : 33-48
- ROTHENBUHLER, W. C. (1974) Further analysis of Committee's data on the Brazilian bee. *Am. Bee J.* 114 : 128
- STORT, A. C. (1975a) Genetic study of aggressiveness of two subspecies of *Apis mellifera* in Brazil. 2. Time at which the first sting reached the leather ball. *J. apic. Res.* 14 : 171-175
- (1975b) Genetic study of aggressiveness of two subspecies of *Apis mellifera* in Brazil. V. Number of stings in the leather ball. *J. Kans. ent. Soc.* 48 : 381-387
- TAYLOR, O. R. (1985) The potential impact of African bees in the United States. *Bull. ent. Soc. Am.* 31 : 14-24