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Notes and comments

PRELIMINARY OBSERVATIONS ON THE SUSCEPTIBILITY OF AFRICANIZED HONEY BEES TO AMERICAN FOULBROOD[1]

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Certain lines of European honey bees, *Apis mellifera*, have genetically determined resistance to American foulbrood (AFB). This resistance is caused mostly by the hygienic removal of infected brood by workers and by the physiological resistance of larvae to infection by *Bacillus larvae* (Bamrick & Rothenbuhler, 1961; Rothenbuhler, 1964; Newton & Ostasiewski, 1986 and references therein). It is unclear to what degree Africanized honey bees may be vulnerable to this disease. Honey bees in subsaharan Africa and the Africanized honey bee population of South America probably have not encountered serious challenges by AFB, but may have been exposed to it within the last decade as they advanced through Central America (Nixon, 1982; personal observation).

The issue of relative hygienic behaviour of Africanized honey bees remains unresolved following two small-scale studies in Brazil. Cosenza and Silva (1972) found complete removal of freeze-killed brood by all Africanized and most hybrid colonies within 86 h, while Caucasian colonies removed significantly less brood (86%). Lengler (1977) found no differences in dead brood removal between Africanized and European honey bees during a 4-day test. No investigations have probed the susceptibility of larval Africanized bees to *B. larvae*.

We reinvestigated hygienic behaviour and compared physiological susceptibility to *B. larvae* infection in Africanized and European honey bees. The studies

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TABLE 1. Percentages [$\bar{x}$ ± s.d. (minimum)] of cells with pin-killed brood that were uncapped, and of uncapped cells that had occupants completely removed, during three days by nine colonies of each bee type. Results of *t*-tests of transformed variables (see text) compare bee type means for each parameter for each day; no tests were performed on means derived from observations having no variability.

	Day 1	Day 2	Day 3
Cells uncapped			
Africanized	94 ± 8 (74)	97 ± 5 (84)	100 ± 1 (96)
European	98 ± 4 (88)	100 ± 0 (100)	100 ± 0 (100)
$P > t$	0.182	–	–
Brood removed from uncapped cells			
Africanized	64 ± 33 (20)	77 ± 31 (28)	86 ± 21 (48)
European	91 ± 14 (63)	100 ± 1 (98)	100 ± 0 (100)
$P > t$	0.056	0.042	–

took place in Coyolito, Guanacaste Province, Costa Rica, using Africanized colonies established from locally caught swarms, and European (Italian and Carniolan) colonies derived from queens imported from Hawaii, USA. All Africanized colonies used had probabilities of Africanization of ≥ 0.996 according to discriminant analysis of morphology (Rinderer *et al.*, 1993). Both hygienic and physiological data were collected from the same general pool of 10 Africanized and 10 European colonies.

Hygienic behaviour of workers was tested by puncturing, with a pin, a total of 50 prepupae or pupae (in groups of seven and eight cells) per colony (Newton & Ostasiewski, 1986). After one, two and three days, we counted the number of cells remaining capped and the number of uncapped cells having dead brood not wholly removed. The proportions, P, of cells uncapped and of uncapped cells having brood completely removed were transformed to $\sin^{-1}(P)^{0.5}$ for each observation. Daily means of transformed data were tested with two-tailed *t*-tests to determine if the proportions of cells uncapped and brood removed differed between bee types. Nine colonies of each bee type were tested. Uncapping of dead brood was similar for the bee types on day 1 (table 1). European colonies had fully uncapped brood by day 2, whereas uncapping in Africanized colonies was not complete until day 3. Removal of uncapped dead brood was greater among European colonies than Africanized colonies on days 1 and 2 (table 1). The European bees had virtually completed brood removal on day 2, but six of nine of the Africanized colonies had dead brood present on day 2, and three still had dead brood on day 3. Times until complete hygienic removal of pin-killed brood in European colonies (1–2 days) and Africanized colonies (2–3 or more days) corresponded to the times found by Newton and Ostasiewski (1986) for bees known to be resistant (1.95 days) and susceptible (2.80 days), respectively, to AFB.

Physiological susceptibility to infection was evaluated by the method of Bamrick and Rothenbuhler (1961). Larvae were treated with a quantity of locally obtained *B. larvae* spores expected to induce moderate mortality, and at a time for which susceptible and resistant lines of European honey bees show the greatest difference in susceptibility (Bamrick & Rothenbuhler, 1961). Larvae for treatment were obtained from eight colonies of each bee type by caging queens on patches of empty comb for 12 h, and then waiting until brood was on average 18 h old (range 12–24 h). Larvae in four out of every five rows of cells were treated with a dose of *c.* 1 000 spores in 0.23 µl of sterile water; every fifth row was treated with water only. Combs with treated (and control) larvae were distributed among eight nurse colonies. The numbers of treated and control larvae remaining after 24–36 h were counted to obtain a base count; final counts, taken 14 days later, were subtracted from base counts to give mortality of treated and control larvae from each test colony. Mortality of control larvae was subtracted from that of treated larvae to obtain a corrected mortality due to treatment. Mortality of treated larvae in Africanized colonies (25 ± 18% [$\bar{x}$ ± s.d.], range = 3–78%) was lower than that in European colonies (50 ± 24%, range = 18–72%) ($t = 2.24$, d.f. = 13, $P = 0.043$). Survival of control larvae did not differ between bee types (Africanized bees, 86 ± 11%; European bees, 72 ± 20%; $t = 1.60$, d.f. = 13, $P = 0.134$).

Our investigation of comparative disease resistance in Africanized bees yielded opposing results for the two resistance modes studied. We found that resistance was not ubiquitous in Africanized honey bees; AFB occurred in both bee types when larvae were inoculated with *B. larvae* spores. The comparatively low infection rate in Africanized bees, however, does suggest that these bees may possess some physiological resistance to this disease. Yet the difference between bee types may have been somewhat exaggerated by the slightly shorter development period for

Africanized eggs (Harbo *et al.*, 1981); Africanized larvae, if older when treated, could have been less susceptible (Bamrick & Rothenbuhler, 1961). The findings of comparatively reduced hygienic behaviour in Africanized bees could be a consequence of this population not having encountered serious larval diseases and of the common response of these bees absconding when confronted with unfavourable conditions. Given that some Africanized colonies removed brood as quickly as the fastest European colonies, the potential for improved nest cleaning appears to exist in the Africanized population and probably could be realized by natural or artificial selection. The great variability in behaviour and physiology among bees of both types indicates further work is warranted. Our observations hopefully will lead to studies that investigate potential differences in hygienic behaviour more thoroughly, that establish age and dose responses to *B. larvae* in broader populations of both bee types, and that define how the interaction of hygienic activities and physiological disease resistance is manifested in honey bee colonies.

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