Do Measurements of Worker Cell Size Reliably Distinguish Africanized from European Honey Bees (*Apis mellifera* L.)?[1]

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ABSTRACT

Two experiments were conducted to determine whether the size of the cells in which bees develop affects the size of the cells they subsequently construct. The results indicate that when the size of bees has been modified through the use of foundation with larger or smaller cell bases, bees will construct natural cells of a size consistent with their genetic origin. When 22 Africanized colonies were hived on commercial European foundation, they subsequently constructed natural cells which were not significantly different in size from those of 66 Africanized colonies hived on all natural comb. Also, eight European colonies reared on noncommercial foundation with small cell bases subsequently constructed natural cells which were significantly larger than the cells from which they emerged. Therefore, averaging the width of 10 linear cells in three diagonal rows on naturally built comb is a relatively reliable and accurate method to distinguish between Africanized and European bees in the field. However, colonies with intermediate cell sizes, which may include some feral European colonies and "hybrids" between Africanized and European colonies, may not be evaluated with certainty.

Introduction

Is there an easy and reliable method to distinguish between Africanized and European bees in the field? One method consists of measuring the width of worker cells on naturally built comb. However, the reliability of this method has not been tested. The aim of the present study was to determine whether the size of the cells in which worker bees develop affects the size of the cells they subsequently construct. If bees construct the same size cells as those from which they emerge, then cell size may not be a reliable character to distinguish between Africanized and European bees, particularly when Africanized colonies are hived on commercial European size foundation.

Cell size measurements have been used by some researchers to distinguish between European and African derived subspecies in the field (Rinaldi et al., 1972; Cosenza and Batista, 1974 and references therein; Rinderer et al., 1982; 1986; Spivak et al., 1988). Table 1 summarizes previous reports of cell size measurements for European bees in the United States and Canada and for Africanized bees in the neotropics.

The recommended method of measuring cell size, obtained by combining the methods of various researchers, is as follows.

1) All measurements must be made on worker comb that is not constructed on commercial foundation. If such comb is unavailable, an empty frame or a top bar on which the bees can draw out natural comb is placed in the center of the brood nest. If the bees construct drone comb, another empty frame or top bar is inserted adjacent to the first. Cells containing nectar or honey toward the edges of the brood nest should not be measured since they tend to be larger (Taber & Owens, 1970; Seeley & Morse, 1976).

2) When the new worker comb contains eggs and larvae, the width of the worker cells is determined by measuring the distance spanned by ten linear cells from the outer wall of the first cell to the inner wall of the last with a centimeter ruler or caliper (Rinderer et al., 1986).

3) Ten cells are measured in three diagonal rows set 60° to each other on the comb (e.g., 0°, 60°, and 120°; or 30°, 90°, and 150°) (Figure 1) to reduce error due to cell shape irregularities and comb orientation (Spivak et al., 1988; Daly, 1990).

4) The distance spanned by 10 cells in each of the three directions is then averaged to give the final measurement of cell size for the colony.

As Africanized bees migrate into the United States, many swarms will be hived on commercial foundation in which the average distance spanned by 10 cell bases measures 5.3–5.4 cm (Dadant Medium Brood® or Duragilt®), or even larger (Perma-Dent® or Perma Comb®: 5.56–5.64 cm; reviewed in Erickson et al., 1990). When bees are reared in larger cells (through the use of foundation with enlarged cell bases), significantly larger workers emerge (Grout, 1937; Jagannadham & Goyal, 1983). If Africanized bees are hived on foundation in which 10 cell bases measure 5.3–5.4 cm, do succeeding generations consist of larger adults that draw out natural comb whose cells are within the size range of European bees, or do they construct smaller cells characteristic of Africanized bees?

Methods I

Twenty-two colonies of Africanized bees in Costa Rica were hived on combs based on commercial foundation at least three months prior to taking measurements. The colonies were part of a larger study conducted in Costa Rica between June 1984 and July 1986 (Spivak, 1992). The commercial foundation cell

[1]Mention of proprietary product does not constitute endorsement by the USDA-ARS

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bases measured 5.3–5.4 cm for 10 linear cells; however, the combs in all colonies had been drawn out by other colonies, and the wax was darkened from previous use. An empty frame was inserted in the center of the brood nest of each of the colonies to allow the bees to construct natural comb not based on foundation. Cell withs of 10 linear cells on the natural comb were measured in three diagonal rows as described above.

In addition, measurements were made of the cell widths of 62 Africanzied colonies captured from feral swarms that constructed all natural combs. A Student's t-test was used to compare the cell size of Africanized colonies hived on commercial foundation with the cell size of Africanized colonies on all natural comb.

Old, darkened comb contains cast off pupal skins, cocoons, and feces from previous brood cycles and thus may have smaller cell volumes that reduce the size of the emerging bees (Grout, 1937). Because there was no control for the age of the combs in the 22 Africanized colonies, a new experiment was devised to test with more precision whether the size of the cells from which bees emerge affects the size of the cells they subsequently build.

Methods II

Eight colonies of European-derived honey bees were hived in 5-frame nucleus colonies in the summer of 1988 in Tuscon, Arizona. The combs were constructed on commercial foundation in which 10 cell bases measured 5.3–5.4 cm. Each queen was marked on the thorax with enamel paint for identification. In May 1989, the width of 10 linear cells was measured in each of three diagonals on the center comb ("Commercial Foundation"), and a sample of 50 bees was collected from brood combs of each colony. Subsequently, the center comb was replaced with a top bar ("Top Bar-1"). When the bees had drawn out comb from Top Bar-1, the cell widths of 10 linear cells in three diagonal rows on the natural comb were measured for each colony.

Over the course of two months (August and September), the five frames (including Top Bar-1) were replaced with frames containing noncommercial foundation ("Small Foundation") in which 10 cell bases measured 5.0–5.1 cm. One frame was removed at a time from each colony and replaced with Small Foundation to maintain adequate brood and food reserves while the new foundation was being drawn. Each colony was provided a continuous supply of sugar syrup while building comb. The new combs were inspected to ensure that the cells were drawn out regularly and were replaced with a new sheet of

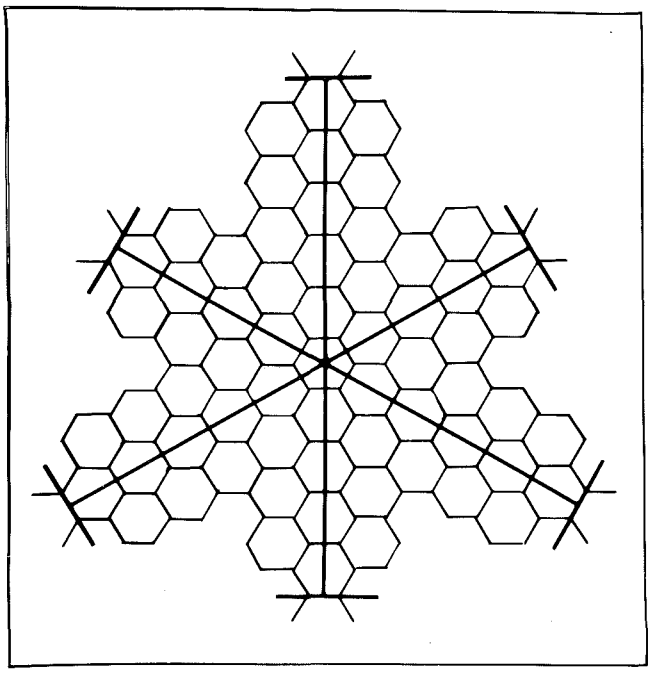

Figure 1. The three diagonals (in this case, 0°, 60° and 120°) of ten linear worker cells used in measuring the average cell size for a colony.

Table 1. Measurements of worker cell widths for European bees in the United States and Canada and Africanized bees in South America.

Measurement in cm/10 cells	Original unit of measurement	Reference
	European bees in the U.S. and Canada	
5.21	0.521 cm/1 cell ± 0.0457 (s.d.) n=32	Taber & Owens, 1970 (Tucson, Arizona)
5.4	13.5cm/25 cells 12.9–13.6 (range) n=41	Anonymous, 1972 (Guelph, Canada)
5.2	5.2 mm/1 cell (ave.) n=21	Seeley & Morse, 1976 (Ithaca, New York)
5.2–5.3	825–850 cells/dm^2	Root, 1974 (and references therein, U.S.)
5.2–5.3	5.2cm–5.3cm/10 cells in 3 diagonal rows ±0.02 (s.e.) n=22	Rinderer et al 1982; 1986 (Baton Rouge, Louisiana)
5.27	52.7mm/10 cells in 3 diagonal rows ±0.0281 (s.e.) n=900 cells	Daly, 1990 (Berkeley, California)
	Africanized Bees in Neotropics	
4.85	909 cells/dm^2 4.85 cm/10 cells in 3 diagonal rows	Rinaldi et al., 1972 (Tucumán, Argentina)
5.0	12.5cm/25 cells 12.1–13.1 (range) n=33	Anonymous, 1972 (northern Brazil)
5.12	12.8cm/25 cells 12.1–13.6 (range) n=22	Anonymous, 1972 (southern Brazil)
4.82	4.82mm/1 cell ±0.09 (s.e.) n=4	Cosenza & Batista, 1974 (Minas Gerais, Brazil)
4.8–4.9	4.8–4.9cm/10 cells in 3 diagonal rows ± 0.02 (s.e.) n=20	Rinderer et al 1982; 1986 (Acarigua, Venezuela)

foundation if the cells were not drawn to match the cell bases.

The colonies were provided sugar syrup and pollen supplements through the winter to stimulate continuous brood rearing and replacement of the worker population through the colder months. In May 1990, the marked queens were located to ensure that they had not been superseded, and another sample of 50 young bees was collected from each colony. A new top bar ("Top Bar-2") was inserted in the center of the brood nest. Measurements were made of the new comb drawn from Top Bar-2 for each colony.

As a final control for the possibility that bees use the cells on adjacent combs as a template to construct new cells, in June 1990 all combs were removed simultaneously from the colonies and were replaced with top bars ("All Top Bars"). Each colony was again provided sugar syrup, and measurements were made of the new cells.

Differences between the cell widths among the colonies for the five treatments (Commercial Foundation, Top Bar-1, Small Foundation, Top Bar-2, and All Top Bars) were analyzed by a two-way analysis of variance without replication due to the testing of the same colonies over time (Sokal & Rohlf, 1981; pp. 344–348).

The width of the thorax and area of the left forewing were measured on ten randomly chosen bees per sample of 50 bees. Thorax width measurements were made using a digital caliper. Forewing area was measured by drawing the wing with a camera lucida and then digitizing the area using a Houston Instrument Hi-Pad™® Digitizer 114-DT. The area of the wing was calculated by the "Digitize" program, Version 3.0, developed by R. E. Strauss at the University of Arizona. The differences in size between the bees hived on commercial foundation and smaller, noncommercial foundation were analyzed using separate two-way analyses of variance for thorax widths and forewing areas.

Table 2. Mean cell size (± s.d.) of Africanized colonies in Costa Rica when the bees developed in cells based on commercial foundation or in all naturally built comb.

Type of comb in which bees developed	no. colonies	cell size[1] (cm)	range (cm)
commercial foundation	22	4.98 ± 0.123	4.70–5.15
natural comb	62	4.94 ± 0.107	4.70–5.10

[1] Cell size = average of 10 linear cells measured in each of three diagonal rows. All measurements were made on naturally built comb in center of brood nest. Means not significantly different; P ≥ 0.05.

Table 3. Mean size of cells on the center comb of the colony when bees developed in comb based on commercial foundation or smaller, noncommercial foundation. Measurements on Commercial and Small Foundation were made of cells constructed from foundation. Measurements on Top Bars were made of cells on naturally built comb.

Type of center comb in the colony	no. colonies	Mean cell size[1] ± s.d.
Commercial Foundation	8	5.37 ± 0.033[a]
Top Bar-1	8	5.35 ± 0.046[a]
Small Foundation	8	5.08 ± 0.019[b]
Top Bar-2	8	5.26 ± 0.042[c]
All Top Bars	4	5.28 ± 0.043[c]

[1] Means followed by different letters are significantly different at $P \leq 0.05$; Tukey's Studentized Range Test.

Results I

The mean width of 10 linear cells in the comb naturally built by 22 Africanized colonies hived on commercial foundation did not differ significantly from that of 62 Africanized colonies which contained only naturally built comb ($t = -1.62$; $df = 82$, $P \geq 0.05$) (Table 2).

Results II

The results of the two-way ANOVA indicated that there were significant differences between the cell widths for the five treatments ($F = 81.72$; $df = 4,24$; $P \leq 0.0001$). A Tukey's comparison among means demonstrated a significant effect of the type of comb in which bees develop on the cells they subsequently construct (Table 3). The size of the cells the bees constructed from Top Bar-1 were not significantly different from the size of the cells drawn from the center comb of commercial foundation. After being hived for eight months on smaller foundation, the cells the bees constructed from Top Bar-2 were significantly larger than the cells from which they emerged; however, they were significantly smaller than the cells drawn from Top Bar-1. When the combs from the colonies were simultaneously replaced with all top bars, four of the eight colonies absconded. The remaining four colonies drew out cells which were not significantly different in size than cells drawn from Top Bar-2, but were significantly smaller than the cells drawn from both Commercial Foundation and Top Bar-1. There were no significant differences among the colonies for any of the treatments ($F = 1.47$; $df = 7,24$; $P \geq 0.05$).

Replacing the commercial foundation with smaller foundation resulted in significantly reduced thorax widths and forewing areas of the European bees (Table 4). The mean thorax width was significantly greater in bees that developed in cells based on commercial foundation ($F = 21.96$; $df = 1,7$; $P \leq 0.005$). There were no significant differences in thorax width among the eight colonies ($F = 0.769$; $df = 7,7$; $P \geq 0.05$). The mean forewing area was also greater for bees that developed in cells based on commercial foundation ($F = 27.82$; $df = 1,7$; $P \leq 0.005$), and there were no significant differences in forewing area among the eight colonies ($F = 1.71$; $df = 7,7$; $P \geq 0.05$).

Discussion

As Africanized bees continue their migration into the United States, hobbyist, part-time and commercial beekeepers (especially in the southern states) will eventually have to decide whether to maintain European bees, "hybrids" between European queens and Africanized drones, or Africanized bees. Many beekeepers may continue to purchase queens from commercial producers in an attempt to maintain European bees. These queens probably will be from certified stocks which have been identified by a morphometric or biochemical technique such as allozymes, DNA analysis, or gas chromatography. However, since many beekeepers do not requeen their colonies regularly and because of the cost and effort involved in having all of one's colonies identified morphometrically or bio-

Table 4. Mean thorax widths and forewing areas ± s.d. of samples of 10 European bees per 8 colonies, when bees developed in cells based on commercial foundation or smaller, noncommercial foundation[1].

	Thorax width mm	Forewing area mm^2
Commercial Foundation	3.02 ± 0.085	18.95 ± 0.929
Small Foundation	2.89 ± 0.096	17.29 ± 0.947

[1] Means within columns are significantly different, $P \leq 0.005$.

chemically, beekeepers will require quick and accurate techniques to evaluate their own captured swarms and hived colonies in the field. Beekeepers will need to monitor the behavior of their colonies and to watch for dramatic changes. Ultimately, the best field identification is based on behavior; all beekeepers should attempt to maintain the most gentle, manageable, and productive colonies possible, irrespective of the bees' origin. However, behavioral assessments made while a colony is being managed depend on various conditions (e.g., how the bees are manipulated, the population of the colony, the weather) and thus can not easily be standardized. The best indicator of change in the genetics of a colony may therefore be measurements of cell size.

These two experiments suggest that averaging the width of 10 linear cells in three diagonal rows on naturally built comb in the center of the brood nest is a relatively reliable and accurate method to distinguish between potentially Africanized and domestic European bees in the field. Even when the size of the bees was modified through the use of foundation with larger or smaller cell bases, the bees reverted to constructing cells of a size more consistent with their genetic origin when they were allowed to construct natural comb. Therefore, if beekeepers capture a swarm or have a European colony they suspect has been usurped by an invading Africanized swarm and allow the colony to construct at least one natural comb (not based on foundation) in the center of the brood nest, they will obtain a relatively good indicator of the identity of bees in question.

There is one drawback to the use of cell size which stems from a paucity of basic information about the feral European bees in the United States and hybrids between European and Africanized bees. Cell size measurements may distinguish between large domestic (European) bees and small Africanized bees, but they may not be reliable for small domestic or feral European bees (such as those in southern Arizona, E. Erickson, unpubl. data), or hybrids between European queens and Africanized drones (or vice versa). As pointed out by Rinderer et al. (1986), more studies need to be conducted to determine whether cell sizes of 5.0−5.2 cm are characteristic of feral European colonies in geographic areas other than Arizona or of hybrid colonies. Until these studies are done, bees with intermediate cell sizes should be considered suspect and analyzed with a more precise technique when exact identification of the colony is desired.

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