SHORT COMMUNICATION

Gravity-Independent Orientation of Honeycomb Cells

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Abstract Honey bees have long been assumed to build their comb with the cells in either of two preferred orientations with respect to gravity ("vertical" or "horizontal"). I show here that these typical cell orientations in fact derive from substrate orientation and a simple building rule, rather than the influence of gravity itself. When bees were induced to build comb on substrates at four different orientations with respect to gravity, they always made cells with one vertex pointing directly toward the substrate. This produced horizontal and vertical cells on vertical and horizontal substrates, respectively, but yielded intermediate orientations on oblique substrates. The apparent preference for vertical and horizontal cells may simply reflect substrate orientation in the rectilinear hives from which cell measurements have been taken.

The comb of the honey bee is one of the most celebrated examples of pattern formation in nature, and much research has been directed at the behavioral and sensory mechanisms underlying its construction (von Frisch 1974; Hepburn 1986). Bees have long been thought to rely on a gravitational sense not only to achieve the vertical orientation of whole combs but also to orient the cells within combs (Martin and Lindauer 1966). Cells are most often found in either a "vertical" orientation with one vertex pointing directly upward, or a "horizontal" orientation with horizontal walls at the top and bottom of each cell (Fig. 1) (Hepburn 1986). They are less often reported in intermediate orientations between these extremes. A simple explanation for this pattern is that the bees actively regulate their construction to produce the desired orientations with respect to gravity (Gontarski 1949; Martin and Lindauer 1966; von Oelsen and Rademacher 1979). Bees can detect the direction of gravity through sensillae on the thorax and abdomen (Lindauer and Nedel 1959) and could plausibly use this information to guide their construction.

An alternative mechanism, proposed 70 years ago but never adequately tested (Lau 1959; Wedmore 1929), attributes the typical orientations to the bees' habit of beginning each comb with a row of cells whose side walls are perpendicular to the substrate. On a horizontal substrate these cells would by default have a vertical orientation, on a vertical substrate a horizontal orientation, and on an oblique substrate an intermediate orientation. Because the first row serves as a template for the subsequent row (and so on for the following rows), a cascade of propagating orderliness maintains the original cell orientation over a broad expanse of comb.

No experiment to distinguish these hypotheses has yet been reported, although the cells of colonies built aboard the space shuttle were oriented with a vertex pointing toward the substrate (Vandenberg et al. 1985). This is consistent with the substrate-dependent mechanism, but the bees' behavior when completely deprived of gravitational cues can say little about the way in which they normally orient their cells.

The present study gave bees the option to use either gravity or substrate cues in order to determine which mechanism they actually employ. Colonies were induced to build comb on substrates at four different orientations with respect to gravity. The orientation of cells in the resulting combs was then measured to determine whether they were consistently horizontal or vertical, as predicted under gravity-dependent orientation, or consistently positioned with one vertex toward the substrate, as predicted under substrate-dependent orientation.

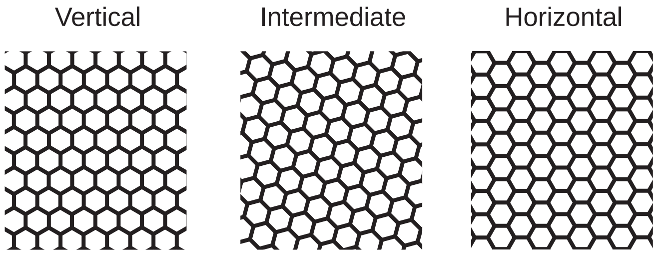

Fig. 1. Three orientations of cells in honeycomb

Experiments were performed on two small colonies (4000–6000 workers) of largely Italian stock (*Apis mellifera ligustica*), housed in observation hives. In addition to two frames of fully drawn comb, each hive contained two specially constructed frames without any comb. Each of the modified frames was bisected vertically by a double layer of wire mesh, and each half was fitted with a wooden slat positioned at one of four angles from horizontal: 0°, 10°, 20°, or 30°. This range of inclinations was used because a 30° rotation is sufficient to encompass the entire range of cell orientations from horizontal to vertical. To ensure that the bees built in the plane of the frame, a thin strip of unembossed beeswax was attached to the midline of each slat's lower surface. After the modified frames were added to the hive, the bees were given several days in which to build, and the orientation of the resulting comb cells was measured. Ten cells were selected from each comb by means of a sampling grid and pseudorandom number generator. For each cell a line passing through its highest vertex and the opposite vertex was traced onto the glass front of the hive. The angle between this line and a plumb line was measured with a protractor. The angle between the substrate and a horizontal line was also measured, because small variations in frame placement sometimes caused them to deviate by 1–2° from the intended inclinations. The experiment was repeated four times on colony 1 at Cornell University's Liddell Field Station near Ithaca, N.Y. (August–September 1993) and three times on colony 2 at the Archbold Biological Station near Lake Placid, Fla. (December 1993–January 1994). The combined data for all replicates were analyzed by regressing mean cell orientation for each comb against the measured substrate angle.

The results show a clear dependence of cell orientation on substrate orientation, and complete independence from the direction of gravity. Cells were consistently oriented with a vertex pointing toward the substrate, regardless of the orientation of this substrate with respect to gravity (Fig. 2). The regression of cell orientation on substrate orientation was highly significant (Fig. 3). The bees produced both horizontal and vertical cells, as well as cells with intermediate orientations. It should be noted that this finding does not depend on the bees' being able to measure the orientation of the substrate. The observed relationship will arise as long as builders consistently start the first row of cells with side walls perpendicular to the substrate.

These findings contradict an earlier observation that bees rotated in a centrifuge orient their comb cells with respect to the resultant of gravity and the centrifugal force rather than with respect to the sub-

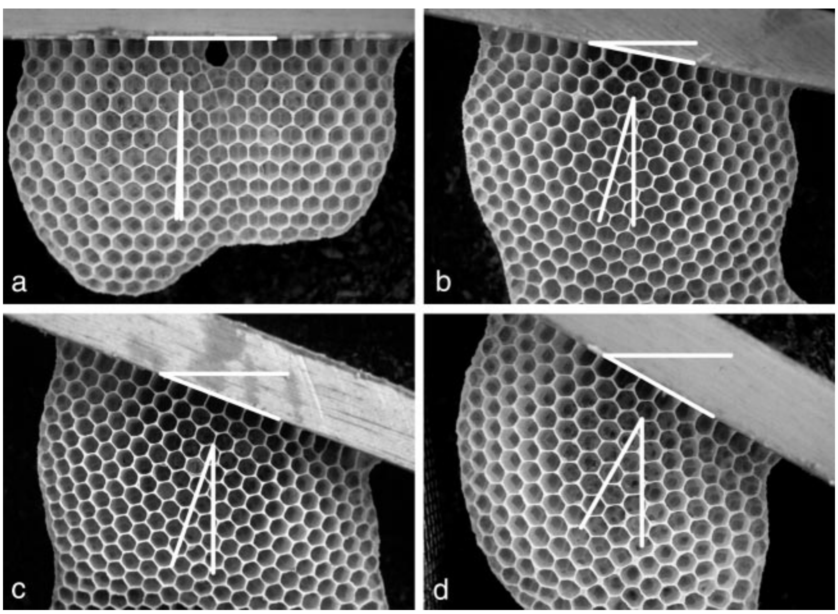

Fig. 2a–d. Comb built on wooden slats at four different inclinations from horizontal. a) 0°. b) 10°. c) 20°. d) 30°. On each comb are shown the angle between the horizontal and the substrate, and the angle between a plumb line and the cell axis passing through the highest cell vertex. The latter is shown for a single cell near the comb center, but was actually measured for ten randomly chosen cells

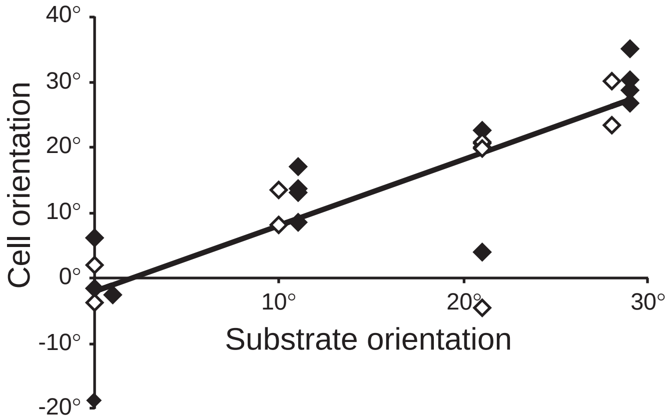

Fig. 3. Regression of cell orientation (CO) on substrate orientation (SO), showing that cells have a fixed orientation with respect to the substrate, regardless of the relative position of the gravity vector ($CO = -2.3 + 1.0 * SO$, $R^2 = 0.67$, $P < 0.000001$). *Closed symbols* colony 1; *open symbols* colony 2

strate (Gontarski 1949). This difference may reflect an abnormal influence of the hypergravitational forces (up to 1.2 *g*) generated by the centrifuge. Another attempt to examine the influence of gravity on building was undertaken by blocking the gravity sensillae of all workers in a colony (Martin and Lindauer 1966). The bees in this colony refrained from building altogether. In the present study the manipulations were completely noninvasive and could not have interfered with any natural activity of the animals.

The tree cavities in which colonies typically nest are likely to have irregularly oriented walls and ceilings, so that combs are frequently started on surfaces departing by several degrees from the horizontal or vertical. No data are available on the orientation of cells in natural nests, but from the present findings one would predict that they should be highly variable. The reported preference for vertical and horizontal cells may simply reflect substrate orientation in the rectilinear hives from which measurements of cell orientation have been taken.

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