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Adaptive tuning of an extended phenotype: honeybees seasonally shift their honey storage to optimize male production

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Organisms face the challenge of optimally allocating limited resources among investments that promote survival, growth or reproduction. In species whose members build complex nests, this resource allocation problem also applies to the building and use of the nest structure, a critical part of an individual's extended phenotype. Honeybee colonies face an acute problem of properly allocating one nest resource in particular, large cells of drone comb built for rearing drones, between reproduction (rearing drones) and survival (storing honey). Here the trade-off is inescapable, because a drone cell cannot be used simultaneously for drone production and honey storage. We predicted that the workers in a honeybee colony would solve this problem by preferentially using drone comb for producing drones when their mating opportunities are good (spring and early summer) and for honey storage when the drones' mating opportunities are poor (late summer and autumn). To test our prediction, we experimentally tested how drone comb and worker comb were used for honey storage from April to September. In spring and early summer, workers preferentially removed honey from drone comb, making it available for producing drones. In late summer and autumn, workers did not preferentially remove honey from drone comb. This study shows that a honeybee colony is able to fine-tune its extended phenotype by adaptively allocating a key nest resource, its drone comb, between survival and reproduction.

To maximize its lifetime reproductive success, an organism must optimally allocate its resources among survival, growth and reproduction (Stearns, 1992, page 72). This problem of optimal resource allocation pertains not only to the organism itself, but also to its extended phenotype if it is an organism that builds an external structure (Dawkins, 1982, page 195). For example, a female golden-silk orb weaver spider, *Nephila clavipes*, must allocate her silk and her time spent building between her web, which enhances her survival and growth, and her eggsac, which is key to her reproduction (Rainer, 2010, page 252). Likewise, a colony of yellow jacket wasps (*Vespula* spp.), which builds a paper nest containing combs enclosed in a protective envelope, must allocate its building materials and building efforts between small-cell combs for rearing workers, to boost colony survival and growth, and large-cell combs for rearing reproductives, for colony reproduction (Greene, 1991; Spradbery, 1973, page 97). Similarly, a male white-headed buffalo weaver bird, *Dinemellia dinemelli*, must allocate his time between gathering different nest materials for different purposes: soft grasses for the egg chamber, an investment in reproduction, and hard thorns for defending the nest's top and sides against predators, an investment in survival (Collias & Collias, 1964). So, just as all organisms must fine-tune their physiology to adaptively allocate resources among survival, growth and reproduction, organisms that also build external structures must also fine-tune how they build and use these structures so that their extended phenotypes are adaptively allocated among survival, growth and reproduction.

A honeybee colony illustrates especially clearly how trade-offs among survival, growth and reproduction can arise when the survival machinery of a living system includes an architectural structure. Unlike in the structures just mentioned, the beeswax combs that honeybees build can be used to boost survival or reproduction, depending on how the combs are used. A honeybee colony builds two types of comb: drone comb and worker comb (reviewed by Pratt, 2004). The hexagonal cells in drone comb are larger than those in worker comb (wall-to-wall dimension: 6.4 mm versus 5.2 mm) (Martin & Lindauer, 1966; Taber & Owens, 1970). The cells in both types of comb can be used for honey storage as well as brood rearing, but these two uses of a cell are mutually exclusive, so a colony faces a trade-off between survival (honey storage) and reproduction (brood rearing) in using its combs (Fig. 1). How does a

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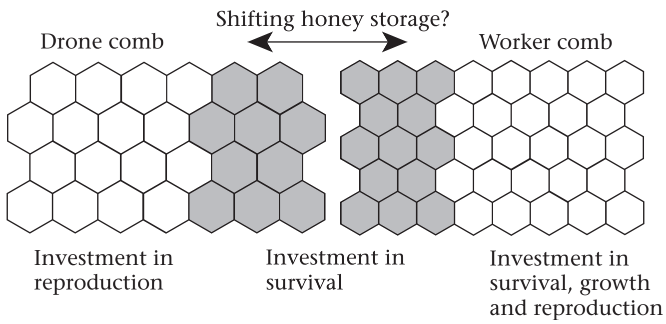

Figure 1. When honeybees store honey in their combs, they face a trade-off between survival and reproduction. Honey can be stored in both drone comb and worker comb. The shaded hexagons represent cells filled with honey, which cannot be used for brood production. This study focuses on whether workers shift the locus of honey storage between the two types of comb depending on the season. In natural nests, drone comb makes up only 17% of the nest (Seeley & Morse, 1976).

colony make the best use of its limited comb area, given this inescapable trade-off?

Presumably, a colony of honeybees makes use of the cells in its nest in a way that tends to maximize its genetic success (fitness). Of special interest is how a colony uses its drone comb, because the fitness return on using a drone cell for reproduction is seasonally variable. For example, there is a high return on using drone comb to produce drones in the spring when mating opportunities are common, but this return is low in the autumn when mating opportunities are rare. A colony has three control points for adaptively allocating its drone comb cells to reproduction (rearing drones) or survival (storing honey): (1) cleaning cells in the drone comb to receive drone brood, or filling them with honey, (2) laying drone eggs in the drone comb cells and (3) rearing or removing drone brood in the drone comb cells (reviewed by Boes, 2010).

This paper focuses on the first control point, because it is the clearest example of the workers in a honeybee colony adaptively fine-tuning the use of their extended phenotype. The second control point relies on the queen's egg-laying behaviour, which is influenced by the season and the colony's nutritional state (Sasaki & Obara, 2001), but the queen cannot single-handedly make drone comb available for drone brood. The third control point, like the first, relies on the workers' behaviour, but cannibalizing brood occurs only as a last resort during a food shortage (Moritz & Southwick, 1992, pp. 69, 211; Weiss, 1984). How workers use cells of drone comb for brood rearing or honey storage, on the other hand, is a powerful first step that workers can take to adaptively adjust their colony's investment in reproduction.

When should a colony invest in drones? In temperate regions, virgin queens are produced most intensively during the swarming season, hence in the spring and early summer (Winston, 1987, page 183). As expected, drone production also peaks in the spring, a few weeks before the emergence of virgin queens (Allen, 1958, 1963, 1965; Lee & Winston, 1987; Page, 1981). In tropical regions, the peak of drone production also coincides with the swarming season (Schneider & McNally, 1994). If a colony is going to produce drones that will have success in mating with virgin queens from other colonies, then it must produce a well-timed squadron of drones. And although drone production is time-sensitive, a colony must also complete other tasks, such as worker production, food collection and nest construction. In this frenzy of activities, do workers also adjust where they store their honey to keep the drone comb available for drone production? Previous research suggests not. When Sasaki, Satoh, and Obara (1996) looked at honey storage in cells of drone and worker comb, they found that the workers in their study colonies did not preferentially remove honey from drone comb relative to worker comb. Their study, however, was conducted in September, so their colonies were probably not producing drones, and hence probably not taking measures to foster drone production.

The aim of the present study was to test the hypothesis that worker honeybees adaptively adjust their use of drone comb for honey storage as a function of season. Based on this hypothesis, we predicted that colonies would tend to keep the cells in drone comb clear of honey in spring and early summer, when the returns on using drone cells for reproduction are high, but not in the late summer and autumn, when these returns are low, but the return on using drone cells for survival (honey storage) are high. We checked our prediction, and so tested our hypothesis, by tracking how colonies used cells of drone comb and worker comb for honey storage, from April to September.

METHODS

Experimental Set-up

Once a month, from April to September, we installed two test frames in the hive of each of six to eight colonies (six colonies in June; seven colonies in April, May and July; and eight colonies in August and September). One test frame held drone comb and the other held worker comb. We tracked the change in the area of honey-containing cells (Table 1) in the test frame of drone comb relative to the test frame of worker comb within each colony to determine whether drone comb was used differently than worker comb in each month. A comb cell cannot be used for brood rearing unless it is completely empty of honey. When workers empty cells of honey, they make them available for brood rearing, and, conversely, when workers deposit honey in cells, they render them unavailable for brood rearing.

Each colony had its two test frames for 14 days during each month of the experiment. Each test frame had no comb cells containing brood or pollen, but each test frame started out with a standard number of comb cells containing a sugar solution similar to nectar. This was achieved as follows. First, each test frame was placed above a colony not involved in the study for 4 days, so any cells containing honey were emptied. Second, we filled the now empty cells in each frame by sprinkling 500 ml of 50:50 (vol:vol) sucrose solution evenly among all the cells. Third, each test frame was placed in a study colony. Once a test frame was placed in a colony, the worker bees of this colony could either clean its cells or add honey to them, thereby changing the area of its honey-containing cells. None of the cells had pollen deposited in them during the experiment.

We measured the area of honey-containing cells on both sides of each colony's two test frames (one drone comb, one worker comb)

Table 1
Definitions

Term	Description
Frame	Wooden structure used in a movable-frame hive to hold a beeswax comb
Comb	Beeswax structure made up of hexagonal cells. The diameter of the cells determines whether it is drone comb (wall-to-wall distance ca. 6.4 mm) or worker comb (ca. 5.4 mm) (Martin & Lindauer, 1966; Taber & Owens, 1970)
Honey-containing cells	Comb cells that contain honey, partially ripened honey, nectar or sucrose solution (which mimics nectar). We did not differentiate among these liquids in the test frames because they all are forms of stored food
Brood	Collective term for the eggs, larvae and pupae in a colony
Queen excluder	A wire screen through which workers, but not queens, can pass