# Effects of comb age on honey bee colony growth and brood survivorship

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## SUMMARY

This research examined the effects of comb age on honey bee colony growth and brood survivorship. Experimental old combs were of an unknown age, but were dark and heavy as typical of combs one or more years old. New combs were produced just prior to the beginning of the experiment and had never had brood previously reared in them. Either old or new combs were installed into each of 21–24 nucleus colonies each year over a three-year period. On average, colonies with new comb produced a greater area ($cm^2$) of brood, a greater area ($cm^2$) of sealed brood, and a higher weight of individual young bees (mg). Brood survivorship was the only variable significantly higher in old comb.

**Keywords:** honey bees, *Apis mellifera*, beeswax, comb age, old comb, new comb

## INTRODUCTION

Honey bees (*Apis mellifera*) use structures like trees, hollows and man-made hives for shelter, but it is beeswax that provides the basic building material for the interior nest substrate. When comb is first constructed it is pliable and near-white in colour. Comb used for food storage takes on a yellowish hue over time due to the accumulation of pollen (Free & Williams, 1974). As comb used for brood rearing ages it becomes darker, almost black, and more brittle (Hepburn, 1998) because of accumulated faecal material (Jay, 1963), propolis and pollen (Free & Williams, 1974). The darker colour may also be a result of numerous undefined contaminants that are collected and absorbed in the wax over time. Wax comb consists primarily of hydrocarbons and ester components (Tulloch, 1980) which easily absorb many types of materials. Unfortunately, some materials including fungal and bacterial spores, pesticides and heavy metals may be detrimental to a colony's welfare. As materials accumulate in wax comb, the diameter of the cells becomes smaller (Hepburn & Kurstjens, 1988) and each time a larva pupates it spins a silken cocoon that remains in the cell after the adult emerges (Jay, 1963). Over time the mass ratio of silk to wax increases, and thereby wax comb goes from a single-phase material to a fibre-reinforced composite product (Hepburn & Kurstjens, 1988). Studies have suggested that smaller cell diameters result in smaller bees in old comb because of the lack of space and a relative shortage of food. Bees reared in old comb may weigh up to 19% less than bees reared in new comb (Buchner, 1955). Diminishing space may force larvae to moult to the non-feeding prepupal phase prematurely, causing nurse bees to cap the cells before larvae have developed maximally (Abdellatif, 1965).

Pheromones also are absorbed and transferred in the wax comb and, depending on their volatility, may remain for a considerable time (Naumann *et al.* 1991). One pheromone group relevant in the current context is brood pheromones. These contact pheromones are emitted by brood and communicate the presence, age and nutritional needs of immatures to nurse bees (Free, 1987).

In the wild, honey bee colonies are known to survive for about six years (Seeley, 1978). Once the colony dies, wax moths, mice and other nest scavengers remove the wax comb, leaving an empty cavity for the next colony to inhabit (Gilliam & Taber, 1991). Modern beekeeping practices disrupt this natural recycling process by housing bees on semi-artificial comb that may be years or even decades old. Advances in beekeeping equipment, like the Langstroth hive and wire-reinforced foundation, have added years to the life of wax comb.

Many beekeepers believe that it is not economically feasible to regularly remove and replace old comb with new foundation. Moreover, there is an energetic cost for the bees that must draw out the foundation into a functional comb using metabolically-derived beeswax. A typical nest contains around 100 000 cells (Seeley & Morse, 1976) which takes about 1200 g of wax to construct. The amount of sugar required to secrete the wax is energetically equivalent to 7.5 kg of honey, about one-third of the honey stores consumed by a colony over winter (Seeley, 1985).

However, it is possible that the economic savings of using long-lasting comb may be offset by deleterious effects of old comb acting as a biological sink for toxins and pathogens or as a physical constraint on larval development. This question led us to hypothesize that comb age affects honey bee colony growth and brood survivorship.

## MATERIALS AND METHODS

In a three-year field study, we compared the quantity of brood produced, brood survivorship, average body weight of adult bees and population of adult bees in colonies housed on brood combs comprised of either old beeswax or new, first-year beeswax.

Experimental colonies were set up in standard four-frame Langstroth nucleus hives (21 colonies in 1997, 21 in 1998, and 24 in 1999). Colonies were housed on deep brood combs belonging to one of two age classes: old comb or new comb. Brood combs in the old class were collected from a variety of sources throughout the apiary. Old combs were of an unknown age, but were dark and heavy as typical of combs one or more years old. We placed the old combs into strong colonies to clean them of debris. New brood combs were produced by placing frames of wax foundation into existing colonies during the spring nectar flow. Honey in all combs, old and new, was removed by allowing robber bees access to the combs. Combs were used only if they were completely drawn out. If pollen was present in the cells, the frames were soaked in water overnight and flushed clean. We measured numerous characteristics of all combs used at the start of the experiment (table 1).

We collected bees from existing colonies or from standard mail-order 0.9 kg (2 lb) packages, and combined them into large cages to achieve a homogeneous mixture. Each year we set up 21–24 test colonies each with 0.62–1.03 kg of bees. For each colony, we collected a sample of bees and determined average weight per bee. Using average weight per bee and the starting net weight of each test colony we calculated starting bee populations. Each colony was provided with a caged, open-mated queen. After each colony was stocked with bees, it was placed inside a chilled building to reduce the threat of overheating. Bees were kept inside until after dark, then transported to a test apiary site in Oconee County (Georgia, USA) and released. Entrances to colonies were faced in various directions to discourage drift. Colonies were fed a 1 : 1 sugar : water solution *ad libitum* for the duration of the study. Colonies were treated with Terramycin antibiotic to

**TABLE 1. Physical characteristics of combs used at the beginning of the experiment. Values are mean ± s.e. (*n*).**

| Comb measurements | New comb | Old comb |
|---|---|---|
| Inner cell diameter (mm) | 4.9 ± 0.02 (100) | 4.6 ± 0.02 (100) |
| Comb weight (g) w/wood frame | 368.2 ± 4.2 (96) | 591.5 ± 18.4 (96) |
| Cells per 10 cm | 18.3 ± 0.02 (96) | 18.5 ± 0.05 (96) |
| Cells per 4 $cm^2$ | 16.3 ± 0.2 (96) | 16.0 ± 0.2 (96) |
| Total available comb space ($cm^2$) on both sides | 6182.0 ± 66.1 (96) | 6371.5 ± 39.0 (96) |

prevent brood diseases and a 0.1 kg vegetable oil patty to control tracheal mites (*Acarapis woodi*) (Delaplane, 1992). Five to seven days later, we released the queens. This marked day zero of the experiment. Colonies were removed from the experiment if their queens failed or if colony populations dwindled to non-viable levels.

On days 7 (1998, 1999), 14 (1997–1999), and 21 (1997, 1999) we measured for each colony the area ($cm^2$) of all brood including eggs, larvae and sealed brood, using a measuring grid marked in $cm^2$. Brood survivorship was measured on day 7 (1997), and 7 and 14 (1998, 1999) by placing a sheet of transparent acetate onto a comb, marking on it the location of 10–40 cells of live, uncapped larvae, excluding eggs and drone brood. Three days later, we placed the same sheet of acetate onto the coordinating frame and counted the surviving capped and uncapped cells in order to determine percentage brood survivorship.

The experiment was dismantled on day 21 (1998, 1999) or day 28 (1997). Before dawn on the day of dismantling we screened the entrances to capture all bees. We determined net weight of bees by weighing each hive with bees, brushing out bees, then reweighing the hive empty. We then calculated bee populations as before. For all years we measured area ($cm^2$) of sealed brood for each colony. Average weight of newly emerged bees was determined by bagging combs of emerging bees and collecting and weighing bees the next day.

### Analyses

A completely randomized design analysis of variance, blocked on year (Proc GLM; SAS Institute, 1992) was used to test the effects of comb age class on area of total brood for days 7, 14 and 21, area of sealed brood, brood survivorship on two sampling dates, ending weight of young bees, ending bee population and change in bee population. There were no interactions of year with treatment; therefore, residual error was used as the error term. Differences were deemed significant at $\alpha \leq 0.05$.

## RESULTS

There were no interactions of treatment by year for any of the variables measured. On average, colonies maintained on new comb had a greater area of total brood, area of sealed brood and higher young bee weight (tables 2 and 3). Brood survivorship was either unaffected by treatment or higher in the old comb class.

Total area ($cm^2$) of brood was significantly higher in the new comb colonies on days 14 and 21. Area of sealed brood was also significantly higher in new comb (table 3). There were year effects for all of the brood area variables (table 2). Survivorship of brood tended to be higher in the old comb than in the new comb, but was significantly so only for week 2 (table 3); there were year effects for both weeks 1 and 2 (table 2). Newly emerged bees weighed significantly more if they were reared in new comb than in old comb (tables 2 and 3). Comb age produced no statistically significant treatment effects in ending adult bee population or change in adult bee population; however, there were year effects. The trend was for higher ending bee populations in new comb and, correspondingly, a greater loss of bees in old comb. It is noteworthy that the analysis of variance showed near-significant treatment effects ($P \leq 0.0858$, table 2).

## DISCUSSION

### Brood production

Increased brood production in new comb may arise from differences in the survivorship of brood (but see next section), quality of brood care given by nurse bees, and the queen's egg production. The literature does not report explicit studies on the effects of comb age on nurse bee behaviour or queen egg-laying performance. Thus, we believe that differences in a queen's egg-laying behaviour are the best explanation for our observed results.

Queens are able to distinguish between worker cells and drone cells by appraising the width of the cell with their forelegs (Koeniger, 1970). The cell diameters in

**TABLE 2. Analysis of variance of the effects of year (yr), comb age (new or old = treatment [tmt]) and interactions (yr × tmt) on nine dependent variables. Treatment and year effects were tested against residual error because treatment and year never interacted significantly. Differences were accepted at the $\alpha \leq 0.05$.**

| Variable | Source of variation | d.f. | *F* | *P > F* |
|---|---|---|---|---|
| Area (cm²) of total brood for day 7 (1998, 1999) | yr | 1 | 6.9 | 0.0126 * |
| | tmt | 1 | 1.4 | 0.2523 |
| | yr × tmt | 1 | 0.1 | 0.7310 |
| Area (cm²) of total brood for day 14 (1997-1999) | yr | 2 | 50.2 | 0.0001 ** |
| | tmt | 1 | 13.5 | 0.0005 ** |
| | yr × tmt | 2 | 1.1 | 0.3378 |
| Area (cm²) of total brood for day 21 (1997, 1999) | yr | 1 | 29.0 | 0.0001** |
| | tmt | 1 | 7.3 | 0.0102 * |
| | yr × tmt | 1 | 0.06 | 0.8074 |
| Area (cm²) of sealed brood (1997-1999) | yr | 2 | 26.8 | 0.0001 ** |
| | tmt | 1 | 5.3 | 0.0257 * |
| | yr × tmt | 2 | 0.04 | 0.9643 |
| Brood survivorship for week 1 (%) | yr | 2 | 10.8 | 0.0001 ** |
| | tmt | 1 | 1.4 | 0.2369 |
| | yr × tmt | 2 | 0.3 | 0.7832 |
| Brood survivorship for week 2 (%) | yr | 1 | 6.0 | 0.0194 * |
| | tmt | 1 | 7.3 | 0.0104 * |
| | yr × tmt | 1 | 2.7 | 0.1060 |
| Weight (mg) of young bee | yr | 2 | 1.8 | 0.1784 |
| | tmt | 1 | 5.2 | 0.0262 * |
| | yr × tmt | 2 | 0.71 | 0.4980 |
| Ending adult bee population | yr | 2 | 14.7 | 0.0001 ** |
| | tmt | 1 | 3.1 | 0.0858 |
| | yr × tmt | 2 | 0.5 | 0.6003 |
| Change in adult bee population | yr | 2 | 11.1 | 0.0001 ** |
| | tmt | 1 | 3.7 | 0.0580 |

**TABLE 3. Effects of comb age on brood production, brood survivorship, weight of young bees, and adult bee populations. Values are means ± s.e. (*n*). A * indicates significant differences within row ($\alpha \leq 0.05$).**

| Dependent variables | New comb | Old comb |
|---|---|---|
| Area (cm²) of total brood for day 7 (1998, 1999) | 1193.6 ± 71.39 (21) | 1071.7 ± 87.2 (21) |
| Area (cm²) of total brood for day 14 (1997-1999) | 2040.2 ± 140.6 (32) | 1600.8 ± 138.6 (31) * |
| Area (cm²) of total brood for day 21 (1997, 1999) | 2356.6 ± 154.6 (22) | 1865.7 ± 191.9 (21) * |
| Area (cm2) of sealed brood (1997-1999) | 1115.3 ± 86.2 (32) | 907.0 ± 93.0 (31) * |
| Brood survivorship for week 1 (%) (1997-1999) | 79.3 ± 4.2 (31) | 86.9 ± 4.7 (31) |
| Brood survivorship for week 2 (%) (1998, 1999) | 88.1 ± 2.4 (21) | 94.8 ± 1.1 (21) * |
| Weight (mg) of young bee (1997-1999) | 106.3 ± 1.0 (31) | 98.2 ± 3.6 (31) * |
| Ending adult bee population (1997-1999) | 3978.2 ± 241.7 (32) | 3398.7 ± 296.3 (31) |
| Change in adult bee population (1997-1999) | -2986.2 ± 234.7 (32) | –3648.0 ± 287.4 (31) |

old comb are comparatively small (table 1 and Abdellatif, 1965); thus, an average reduction of cell diameter in old comb may have a negative effect on a queen's egg-laying productivity.

Older comb is known to harbour numerous contaminants that may be detrimental to the brood's health. Old comb has been associated with increased incidence of chalkbrood (Koenig *et al.*, 1986), and diseases like nosema (Bailey & Ball, 1991) and American foulbrood (Gilliam, 1985) which are spread from colony to colony by infectious wax. The queen may be sensitive to these contaminants and not lay eggs in particular cells. Also, the old comb may harbour brood pheromones (Free & Winder, 1983) that act as egglaying inhibitors to the queen because she perceives the cell to be already occupied.

Another phenomenon relevant to this study is the observation that bees prefer to store honey and pollen in cells that have been previously used for brood rearing. In the wild, as a colony grows and continues to add new comb, brood rearing gradually shifts into this new comb and the honey is stored in the old brood comb (Free & Williams, 1974). In unmanaged colonies this behaviour may serve to avoid the negative effects of old comb on brood production. However, modern beekeeping practices inhibit this natural process by forcing bees to reuse old brood comb for brood rearing and to store honey in comb never used for brood rearing.

### Brood survivorship

Brood communicate to the worker bees their presence in the cell, caste, age and hunger levels through mechanical and chemical signals (Free, 1987). The chemical signals are the brood pheromones that may be the causative agent responsible for the increased survivorship found in old comb in this study. Wax comb acts as a reservoir for absorbing and transmitting pheromones which may explain why honey bee swarms are more attracted to older comb (Naumann *et al.*, 1991). The presence of brood pheromones stimulates pollen foraging (Pankiw *et al.*, 1998), enhances brood recognition (Le Conte*et al.*, 1994) and stimulates nurse bees to feed larvae (Le Conte *et al.*, 1995), all of which are important factors in brood survivorship. Free & Winder (1983) determined that brood survival was greater in cells which had been used previously for brood rearing than in comb cells never used before. Taken together these studies demonstrate that pheromones incorporated in wax comb may improve brood survivorship. The differences in brood survivorship noted in our study may be partly explained by more optimal concentrations of brood pheromones in older comb.

In our study we found the seemingly paradoxical results of higher brood production in new comb but higher brood survivorship in old comb. We believe that this is best reconciled, internally and with the literature, by positing that the egg-laying rate of queens is highest in new comb, but once placed in a cell the chances of a larva's survival are best in old comb. Nevertheless, overall brood production is highest in new comb (table 3); apparently the benefits of maximized egg production exceed the benefits of maximized brood survival.

### Weight of emerging young bees

Higher weight of emerging young bees in new comb is best explained by differences between the two comb age classes in the average diameter of cells. As brood comb ages, the diameter of the cells decreases due to accumulated cocoons and faecal material that are deposited by the larval and pupal instars developing within the cell (Jay, 1963). The body weight of a worker bee is mediated by genetics (Ruttner & Mackensen, 1952) as well as by environmental effects such as the amount of food fed to larvae (Daly & Morse, 1991; Fyg, 1959) and the size of the natal cell (Jay, 1963; Abdellatif, 1965). Buchner (1955) determined that the mean weight of newly emerged bees from old comb in which 68 generations had emerged was about 19% smaller (96.1 mg) than the controls (118.3 mg). In our study bees reared in new comb weighed about 8.3% more than those reared in old comb, which is similar to Abdellitif's (1965) finding that worker bees reared in old comb in which 70 generations had been reared have an 8% reduction in body weight.

### Adult bee population

Lower bee populations in the old comb may result from an accumulation of foreign contaminants sequestered in the older comb causing higher mortality. Smith & Wilcox (1990) documented 35 toxic chemicals found in wax. Also, contaminants in the wax comb may mask hive signature and nestmate recognition cues, making it difficult for foraging bees to return to their own colony. Some nestmate recognition cues are obtained from the wax comb (Breed & Stiller, 1992), and Breed *et al.* (1988a) discovered that colony odour acquired from wax comb can mask the genetic differences between bees. Colony odour is transferred to the adult bees by exposure to the comb substrate and can alter the recognition phenotype in as little as five minutes (Breed *et al.*, 1988b).

### Conclusions

Over three years of field study, honey bee colonies housed on new comb had a greater area of total brood, a greater area of sealed brood, and higher weight of individual young bees. Brood survivorship was the only variable significantly higher in old comb. The bulk of the evidence suggests that new combs optimize overall honey bee colony health and reproduction. These findings suggest that beekeepers should eliminate very old brood combs from their operations.


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