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Manipulating nest architecture reveals three-dimensional building strategies and colony resilience in honeybees

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Form follows function throughout the development of an organism. This principle should apply beyond the organism to the nests they build, but empirical studies are lacking. Honeybees provide a uniquely suited system to study nest form and function throughout development because we can image the three-dimensional structure repeatedly and non-destructively. Here, we tracked nest-wide comb growth in six colonies over 45 days (control colonies) and found that colonies have a stereotypical process of development that maintains a spheroid nest shape. To experimentally test if nest structure is important for colony function, we shuffled the nests of an additional six colonies, weekly rearranging the comb positions and orientations (shuffled colonies). Surprisingly, we found no differences between control and shuffled colonies in multiple colony performance metrics— worker population, comb area, hive weight and nest temperature. However, using predictive modelling to examine how workers allocate comb to expand their nests, we show that shuffled colonies compensate for these disruptions by accounting for the three-dimensional structure to reconnect their nest. This suggests that nest architecture is more flexible than previously thought, and that superorganisms have mechanisms to compensate for drastic architectural perturbations and maintain colony function.

1. Introduction

Form and function share an intimate connection—the physical shape of cells, appendages and whole organisms evolves to serve a specific set of functions that aid survival and reproduction [1]. Selection shapes not only the final mature form, but also the entire progression of development [2]. The principal that function drives morphology throughout development should apply beyond the organism, which includes the extended phenotype and the structures organisms build [3]. However, we tend to focus on form and function of the organism, while overlooking their built environment.

Across the animal kingdom, organisms build nests to protect themselves and their offspring from unpredictable, unfavourable and dangerous environments [4]. These functional requirements inform all aspects of nest construction: site selection, building materials and the development of physical structure [5]. The physical form of how nests are spatially structured should be especially important when nests are critical for the organism's growth, survival and reproduction across generations, as in the case of superorganisms [6].

Social insect nests are among the largest, longest lasting and most fortified, relative to the individual nest builders [7]. The nest is part of the superorganism's extended phenotype, and to achieve a cohesive and functional structure, hundreds to millions of workers must coordinate their construction [3,8]. The nest's physical structure is inherently tied to the individual builders and their environment. Nests are influenced by environmental conditions [9] and worker

morphology [10], and their spatial organization can influence foraging recruitment [11]. While nests are known to be critically important, most studies of nest architecture involve single-timepoint destructive sampling (e.g. nest casting; [12–14]), so it is unknown how nest shape develops over time.

The western honeybee, *Apis mellifera*, is an ideal system for understanding nest development over time. Colonies will readily build combs upon movable wooden bee-frames in boxes, which mimic natural nests and allow for non-destructive repeated sampling. Furthermore, in contrast to other social insect nests which are initiated by a single individual and can take years to reach full size [15,16], honeybee colonies are founded by swarms containing thousands of workers, so the bulk of the nest structure is built in weeks [17,18]. Finally, unlike nests which are built from low-cost materials, such as mud pellets [19], honeybees build their nests from secreted wax, which is metabolically expensive [20,21]. Workers must therefore make careful decisions about how and where to add wax to the developing architecture to optimize nest function.

While *A. mellifera* is among the best-studied social insects [22–27], our understanding of the colony's nest development over time is limited. Honeybees build their nests within a confined space, such as a tree cavity, with multiple parallel combs that create a three-dimensional structure [28]. Previous work has shown that colonies grow quickly after moving into a new nest site [17,18,29,30], and that the orientation of the parallel combs is defined according to gravity and their previous nest [31,32]. However, the three-dimensional progression of nest establishment, growth and functional consequences are not well documented. For example, where within the empty cavity do workers initiate the nest, and when do workers begin new combs to create parallel nest structure? Additionally, while the three-dimensional arrangement of combs in honeybee nests has been assumed to serve an adaptive function [33–36], it has not been explicitly tested. Indeed, we lack even a basic description of how the nest-wide shape arises.

This study addresses two complementary gaps. The first gap is a detailed description of the three-dimensional honeybee nest, from colony founding, through the rapid nest growth phase. Using field photography and automated image analysis, we track three-dimensional comb growth in six colonies over 45 days (control colonies). The second gap is an experimental test of whether three-dimensional nest structure impacts colony performance and survival. We hypothesize that the parallel arrangement of combs in the nest is important for colony function, and predict that disrupting this stereotypical structure will reduce colony performance. To test this, we shuffled the position and orientation of each wooden frame in six additional colonies each week (shuffled colonies). Contrary to our expectations, colony-level performance metrics (worker population, total comb area, hive weight and nest temperature) were no different between the control colonies and the shuffled colonies. Using predictive modelling to examine how bees allocate comb as they expand their nests, we show that colonies account for the three-dimensional structure and prioritize building a tightly connected nest, which may help them compensate for the experimentally induced nest disruptions.

2. Methods

(a) Study site and experimental design

To track nest initiation and growth in three dimensions, we installed artificial swarms of *Apis mellifera* into empty nest-boxes at Auburn University (32°40′26.62″ N, 85°30′43.55″ W) and allowed the bees to build their nests naturally. Each colony began with 1.4 kg of bees (*ca* 10 700 workers) and a naturally mated queen (Gardner Apiaries, Baxley, GA). To simulate swarming conditions before installation, we fed the bees sucrose solution (1 : 1, by volume) for 3 days. On 4 April 2021, we installed the bees into 10-frame nest-boxes (Langstroth 'deep' boxes: 47 × 31 × 25 cm). The 10 wooden frames were oriented perpendicular to the southern-facing entrance, with no foundation or stabilizing wires. Each bee frame had a thin strip of beeswax (less than 2 mm) to encourage in-plane comb growth. Each colony received a 2 l sucrose solution feeder (1 : 1, by volume) on 5 April 2021, which was removed after 1 week. This was the only feed we provided to colonies throughout the entire experiment. Colonies were free to forage naturally.

After the first week, we assigned half of the colonies to the *shuffle treatment* ($n = 6$) and half to the *control treatment* ($n = 6$). Treatments were balanced for colony size and hive weight. We inspected colonies in both the shuffle and control treatment groups each week (see below). For colonies in the shuffle treatment, however, we randomly rearranged the frame position (1–10) and orientation (forward- or backward-facing) at the end of each inspection, whereas colonies in the control treatment had their frames placed back in the same position/orientation (figure 1). The randomly determined shuffle order was the same for each colony in the shuffle treatment, but unique each week.

(b) Data collection

We inspected colonies in both treatment groups once per week, to measure number of workers (Liebefeld method: [37,38]) and comb area (5 × 5 cm mesh grid). BroodMinder scales located under each hive recorded the weight each hour, and BroodMinder thermometers located under the inner cover of each hive recorded temperature every 10 min (Broodminder, WI, Stoughton, USA). During each inspection, we ensured the thermometer was placed over the wooden frame with the most brood in the colony. To get uniform images of the comb built upon each wooden frame, we brushed the bees off and photographed both sides of the frame with a field-ready, PVC-framed, plastic shade-covered photography rig (348 × 66 × 44 cm), with controlled lighting, focal distance, and camera settings (Nikon Z50; aperture = f7.1, shutter speed = 1/80 s, focal length = 135 mm).

(c) Data analysis

To define the period of rapid nest growth, we performed a segmented regression of total nest area per colony over time for the control colonies. We then used the breaking point of the regression (45 days) as a cut-off for all subsequent analysis. To analyse comb shapes and growth patterns during the rapid initial growth phase, we trained a neural network to classify each pixel in every image as either comb, wooden frame or background (see electronic supplementary material for a description of this method). We cropped the output image so that only the interior of each frame (the area where bees build comb) was retained and downsampled the result so that each labelled pixel was approximately the size of a hexagonal worker cell (5.4 ± 0.2 mm; [39]). This produced a two-dimensional description of the presence or absence of comb cells across the interior of each frame. We stacked the information about each frame in the order they occurred in the nest to create a three-dimensional description (array) of the comb across the entire nest for each colony each week (figure 1).

To quantify where the bees initiated their nest within the initial empty nest-box, we calculated the centroids of the total comb built by each colony after the first week (before we assigned colonies to the shuffled versus control treatment groups). We divided the nest-box into four quadrants according to their cardinal directions (NE, NW, SE, SW) to discretize the
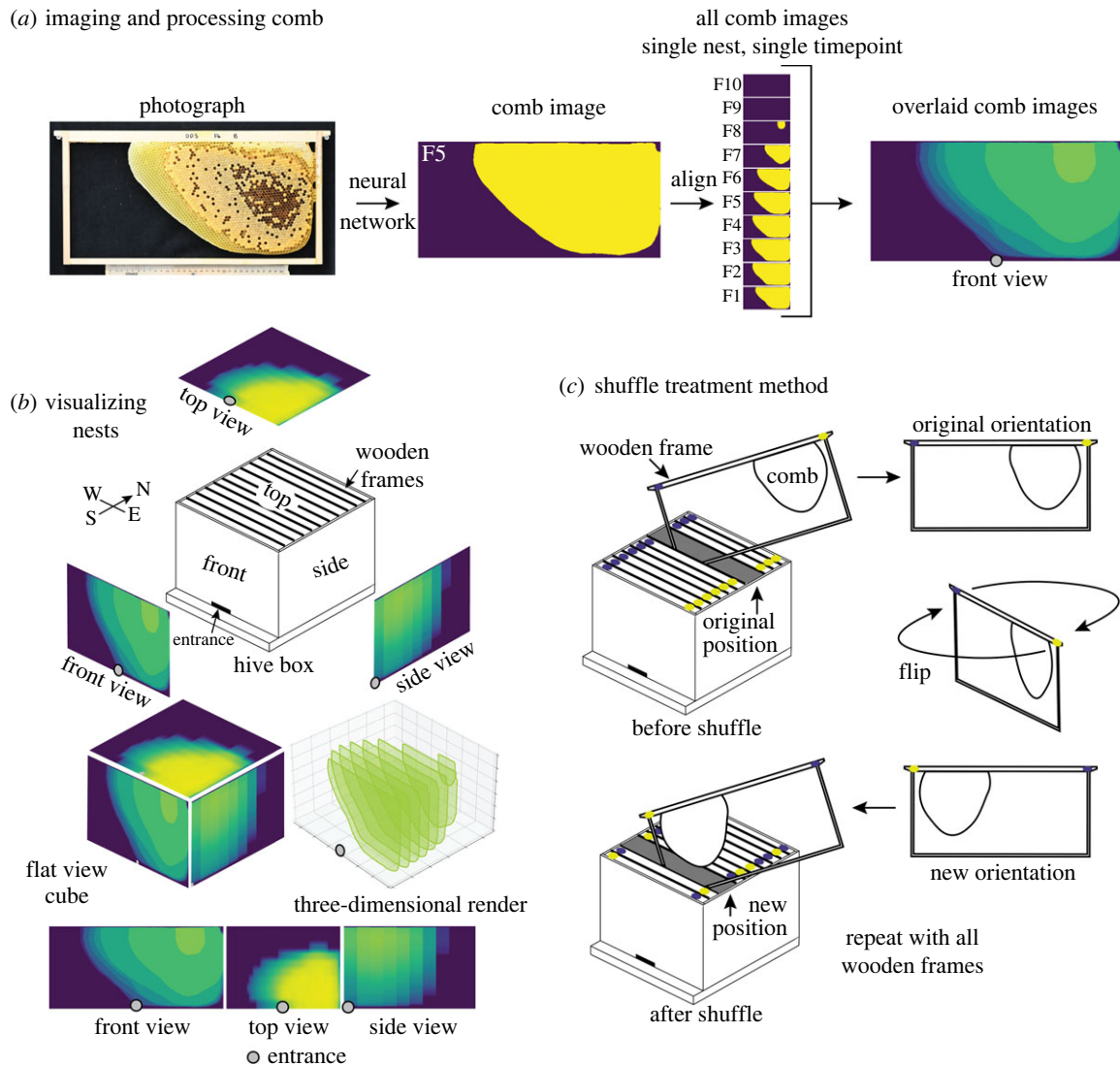
Figure 1. Overview of image processing, visualization and experimental methods. (*a*) Photographs of wooden frames with comb taken with the photography rig were processed with a trained neural network to generate a simplified comb image. These images can be overlaid to create a visual of the overall nest form. (*b*) Diagram showing how to visualize overlaid comb images from different perspectives. (*c*) Visual overview of the methods we used to shuffle the wooden frames in the shuffle treatment. Every week, we randomly determined the new position and orientation of each wooden frame in the nests of colonies in the shuffle treatment. Coloured dots denote comb orientation.

results, and assigned colonies to the quadrant in which their nest centroid was positioned.

We developed a *nest connectedness metric* to quantify how comb grows in relation to the comb in neighbouring wooden frames. For each comb cell in a nest, we calculated if either or both adjacent frames also had comb at the same location. Each comb pixel can therefore have zero, one or two 'comb neighbours' (adjacent pieces of comb). We average the total number of comb neighbours across the whole nest and divide by 2 (each frame has two sides), to summarize the overall proximity of the comb in the nest. The resulting nest connectedness score ranges from 0 to 1, where a low score indicates a nest of isolated comb fragments, and a high score indicates a nest of tightly grouped parallel combs.

(d) Predictive comb growth models

To understand how bees allocate new comb within their nests, we built three simulation-based models to predict where new comb would be built during each week. Based on our overarching hypothesis that the three-dimensional arrangement of combs is important for colony function, we designed two predictive models: one that considers the surrounding comb structure when placing new comb (*neighbour model*), and one that does not (*dilation model*). The dilation model expands all existing comb edges equally, while the

neighbour model preferentially expands combs that fill gaps in the three-dimensional structure. We also built a control model (*random placement model*) to test the null hypothesis that comb is added randomly throughout the nest. See the electronic supplementary material for a detailed description of the models along with the associated code. For each colony, we calculated a 'wax budget' by subtracting the total nest area at the end of the week by the total nest area at the beginning of the week (i.e. the actual comb growth that week). Each model predicts the location of new comb growth constrained by the wax budget such that the predicted and observed nest growth is equal. To evaluate the comb predictions made by each model, we measured how accurately the shape of the predicted nest growth matched the observed nest growth at the end of the week. We calculated the accuracy of predicted nest growth by summing the overlapping area between model-predicted growth and observed growth for across the nest and divided by that week's growth (the wax budget). Values could range from 0% to 100% (0%: no overlapping area between predicted and observed growth; 100%: perfect overlap).

(e) Statistical analyses

We performed all statistical tests with the SciPy [40], Statsmodels [41] and Pingouin [42] packages in Python 3.10 (Python Software

Foundation). Values are reported as mean ± standard deviation unless otherwise noted.

For nest initiation, we used a chi-squared test (χ^2) to determine if there was a bias in which quadrant of the hive box colonies chose to begin building their nest in the first week. To determine the point at which colonies initiate a new comb on a previously empty wooden frame to expand their nest, we focused on the pairs of combs at the nest edge of control colonies. We defined a nest-edge comb pair as two adjacent combs that had empty wooden frames on one side—this excluded combs abutted to the wall. One comb in the pair is the actual nest edge, and the other is one comb in from the edge. With these combs, we performed a logistic regression with the response variable as whether the comb was the actual nest edge, and comb area as the dependent variable. We then determined the threshold comb size for initiating a new comb by calculating the comb size at which the probability of being the nest edge comb was 50% according to the logistic regression.

To assess whether there was a difference in colony-level performance between control and shuffle treatments, we used a two-way mixed ANOVA for each performance metric (nest area, worker population, hive weight, nest temperature). In each test, we assigned the performance metric as the response variable, the week as the within-subject factor, and the treatment as the between-subject factor.

To compare our comb building models, we used a two-way mixed ANOVA with a Tukey pairwise comparison *post hoc* test. We assigned the nest shape accuracy as the response variable, the model as the within-subject factor and the treatment as the between-subject factor. We compared predicted and observed nest connectedness for shuffled colonies and control colonies separately with a two-way repeated measures ANOVA and a Tukey pairwise comparison *post hoc* test. We assigned the nest connectedness metric as the response variable and the model and week as the within-subject factors.

3. Results

(a) Three-dimensional nest initiation and development

We first determined where colonies initiate their nest within an empty cavity, and the size and shape of that nest. Eight out of 12 colonies (67%) initiated their nests in the SE quadrant of the nest-box (10 of 12, 83%, on the east half; 9 of 12, 75%, on the south half (entrance side); $n = 12$ colonies, control and shuffled colonies combined, as they had not yet been shuffled; χ^2 test $p = 0.010$, figure 2a). Colonies built comb on 6.1 ± 0.8 wooden frames after the first week, showing that they develop multiple parallel combs simultaneously. Nests were spheroid to slightly ellipsoid (figure 2b), with ellipsoid nests being longer on the east–west axis than the north–south axis. All combs were built on adjacent wooden frames (i.e. no empty wooden frames between combs) with comb size gradually decreasing on either side of the largest comb, until reaching the wall of the nest-box. Colonies built comb rapidly—in the first 8 days, they constructed 20.6 ± 2.4% of the total nest area they would build over the entire season.

The rapid growth phase continued for 45 days (19 May 2021), until the increase in total nest area tapered (piecewise linear regression, $p < 0.0001$, breaking point = 45.8 days, electronic supplementary material, figure S1). Total comb growth was linear during the rapid growth phase, with 1107 ± 431 cm² of comb added to the nest each week (equivalent to 1.3 full wooden frames). By day 45, colonies built 85.6 ± 9.0% of the total nest area they would build over the entire season (74.0 ± 9.0% of the total available space in the nest-box; by day 200, the end of the field season, colonies had filled 87.7 ± 12.4% of the total available space in the nest-box). All subsequent analyses are performed within these first 45 days to focus on the rapid growth phase.

To understand how the shape of the nest develops over time, we measured the point at which bees add new combs to the nest, the shape of individual combs within the three-dimensional parallel structure, and their relationship to the available space within the nest cavity. Nests generally maintained a spheroid form, with all leading comb edges advancing equally up to the wooden frame's border (figure 2b, electronic supplementary material, figures S2 and S3). Colonies initiated a new comb when the nest's outermost comb surpassed 180.8 ± 3.7 cm² (logistic regression, $r^2 = 0.59$, $p < 0.0001$, figure 2c), suggesting that workers have a defined threshold for adding new comb to their parallel nest structure. After the first week, new combs were initiated approximately every three weeks (0.36 ± 1.88 new combs per week). The rate of comb growth increased the farther away from the nest initiation point, indicating that the growth 'force' moved toward the nest edges as the available space was filled (electronic supplementary material, figure S4). Comb height and width grew equally until the comb approached the bottom of the wooden frame, and then expanded with a lateral bias (figure 2b.iii,d, electronic supplementary material, figure S3). There was, however, a slight interaction between comb growth and the shape of the nest cavity; as the whole nest conformed to the shape of the nest-box, comb that had not yet reached the maximum available height grew with a slight lateral bias (blue hues in figure 2d, electronic supplementary material, figure S3). As the entire nest becomes laterally biased, new comb growth also expands with a lateral bias, instead of following an equal aspect ratio as did the initial combs, showing that overall nest shape can influence future growth of individual combs. Given these stereotypical patterns of nest growth (nests initiated in the same locations: figure 1a; spheroid nest shape: figure 1a.ii,b; similar size threshold for adding combs: figure 1c), we next examined the functional consequences of disrupting this three-dimensional structure.

(b) Impact of disrupting three-dimensional nest organization

To determine whether three-dimensional nest structure is important for colony function, we shuffled the order and orientation of combs in the 'shuffle' treatment group and measured its impact on colony performance. This shuffling disrupted the three-dimensional spheroid nest structures observed in the control colonies (figure 3a). Therefore, while control nests had high and slightly increasing nest connectedness across weeks, shuffled nests had a dramatic reduction in nest connectedness after each weekly shuffle event (figure 3b). The workers' building behaviour, however, increased nest connectedness by 55% by the end of each week (repeated measures ANOVA, $p < 0.0001$), showing that workers consistently reconnect the nest after each shuffle event. Compared with control colonies, shuffled colonies had a less stereotypical pattern of nest expansion. Unlike control colonies, which only initiated a new comb once per wooden frame, shuffled colonies initiated new comb on frames where comb was already present (43% of the wooden frames had more than one comb-initiation site;
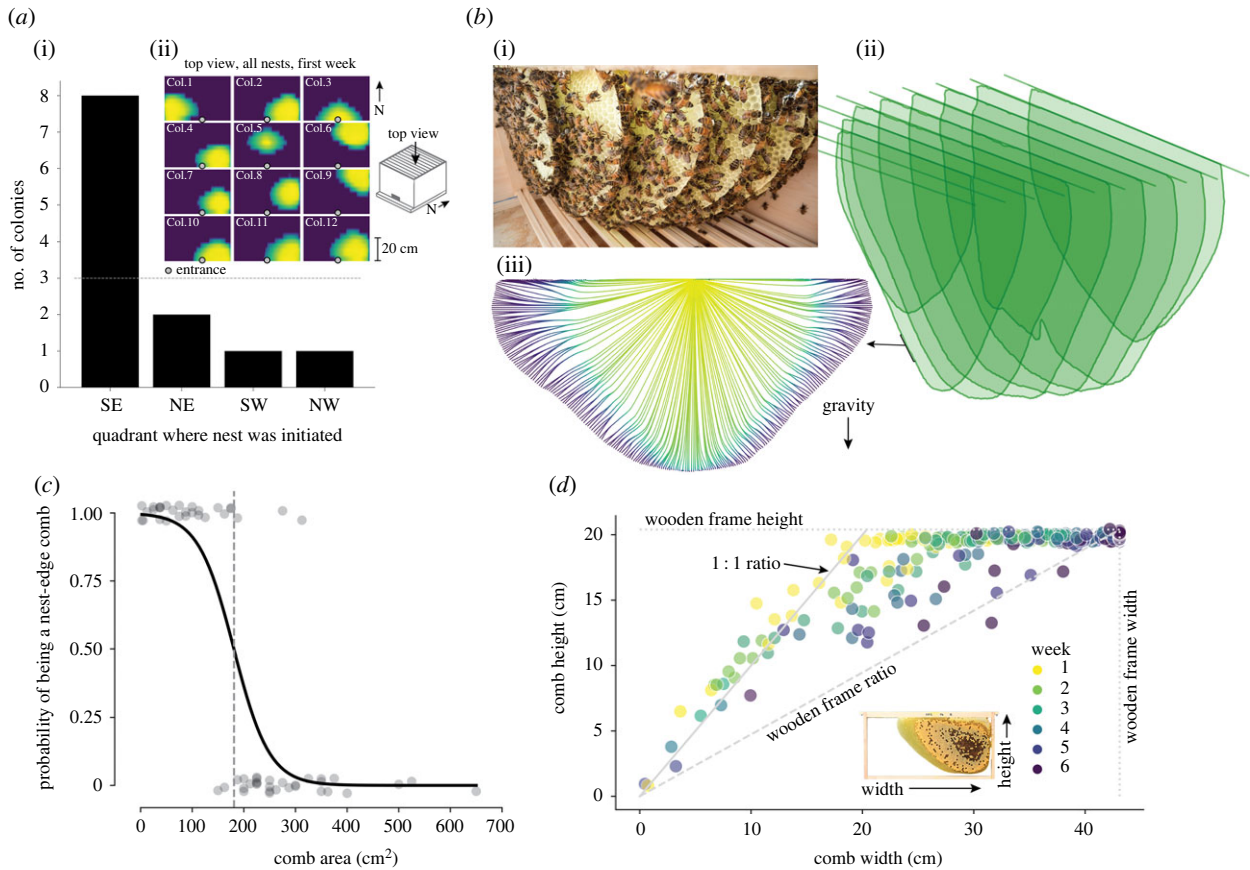
Figure 2. How colonies initiated and grew their nest over the first six weeks. (*a*) In the first week, colonies initiated their nests within an empty hive box with a bias toward the southeastern quadrant, seen as a (*a*.i) histogram where the dashed line represents the expected value if nest initiation was evenly distributed among quadrants and (*a*.ii) from a top view of nests where the colour map indicates the depth of comb (yellow: high; purple: low). (*b*.i) Photograph showing the smooth, parallel comb structure of a nest and (*b*.ii) a three-dimensional snapshot view of an example nest composed of translucent comb masks to show structure. (*b*.iii) A single frame of comb from the nest in (*b*.ii) over six weeks, where lines represent direction of growth and colour represents time (see (*d*) for colour legend). (*c*) Logistic regression for whether the combs on the edge of the nest had two neighbouring combs with respect to their comb area. The vertical line represents the comb area threshold above which workers were more likely than not to initiate a new comb on a previously empty frame (i.e. the size threshold for adding a new comb to the nest). (*d*) Comb height with respect to comb width, where each point is an individual frame at one timepoint. The solid line represents equal growth rate of height and width; the dashed line represents height-width growth in equal proportion to available space; the dotted lines represent the limits of available empty space within the frames.

electronic supplementary material, figure S5). Compared with control colonies, shuffled colonies were more likely to build new comb far from a frame's existing comb, which contributed to their nest reconnection efforts (electronic supplementary material, figure S6). Overall, the nests of shuffled colonies deviated from the stereotypical growth and connectedness we observed in the nests of control colonies.

We next compared metrics of colony performance between the control and shuffle treatments, to determine the functional impact of disrupting three-dimensional nest organization. Surprisingly, the two groups did not differ in any of our metrics, which included: worker population (two-way mixed ANOVA; $p = 0.83$), nest area ($p = 0.47$), hive weight ($p = 0.90$), or nest temperature ($p = 0.69$; figure 3*c*). We had predicted that colonies in the shuffle treatment group would have reduced colony performance compared with the control colonies, given the disruptive nature of the shuffle events, but these results show no observed differences in colony-level performance between the two groups (figure 3*c*). However, given that workers actively improved nest connectedness after each weekly shuffle event (figure 3*b*), this could point to the colony's resilience to architectural challenges. Therefore, we next examined how workers allocate their comb building efforts in a growing

nest, and whether this allocation differed between the treatment groups.

(c) Predicting three-dimensional comb growth

To understand the decisions workers make when depositing wax to expand their nest, we generated three different models that predict where new comb will be added, given the current nest structure. The *dilation model* simply expands all comb edges equally; the *neighbour model* prioritizes filling gaps between combs in the three-dimensional structure; the *random placement model* distributes wax randomly throughout the nest to serve as a control. Each model predicts where comb will be added by the end of the week, which is then compared with the observed nest growth. Figure 4*a,b* illustrates the predictions from these comb growth models, using an example frame from a control nest and a shuffled nest.

We evaluated the accuracy of our models by calculating how well the predicted nest shape matched the observed nest shape. The best-performing model depended on the treatment group: the dilation model best predicted nest growth in the control colonies, whereas the neighbour model best predicted nest growth in the shuffled colonies (two-way mixed ANOVA with Tukey pairwise comparisons, $p < 0.0001$,
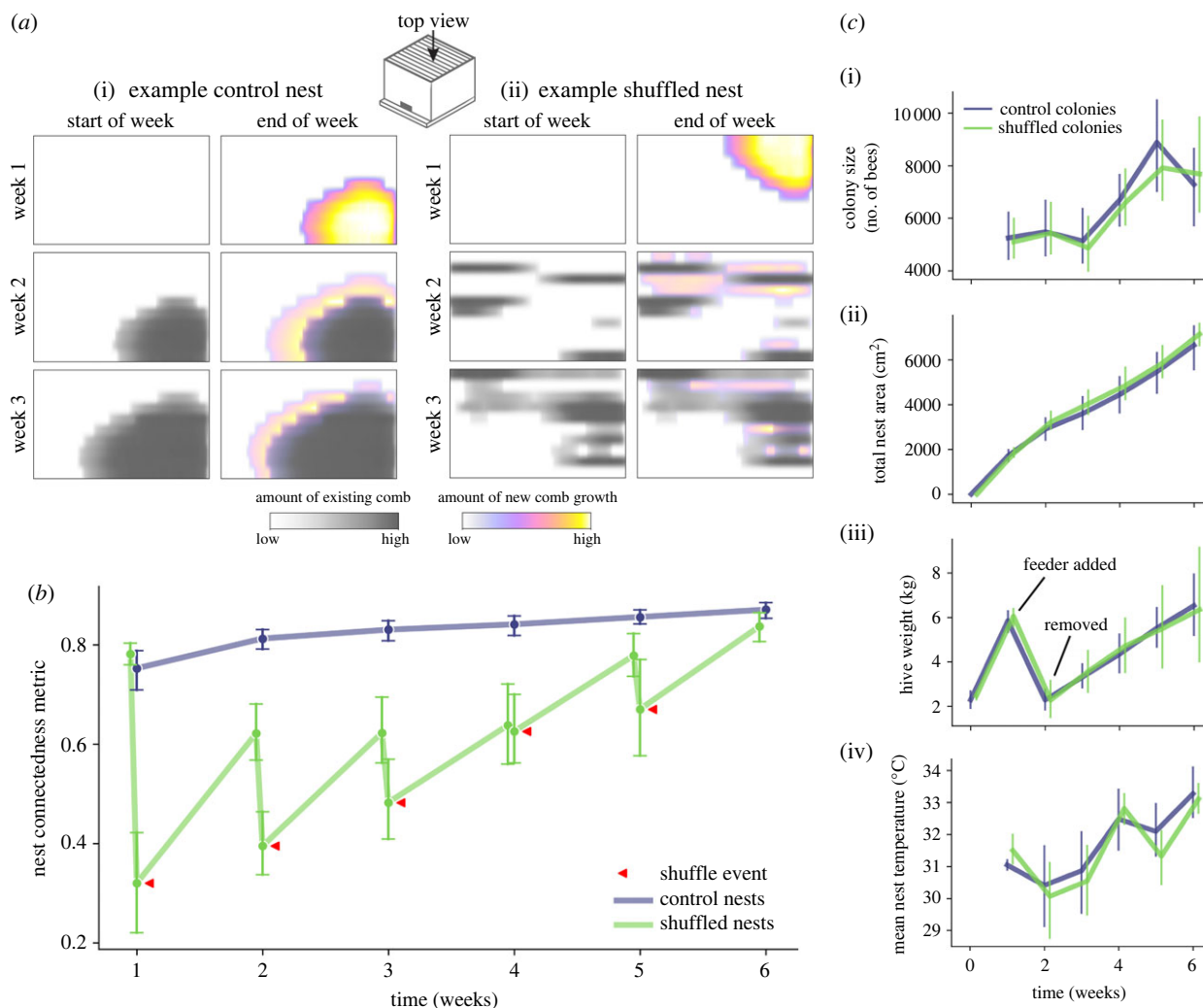
Figure 3. Impact and response to nest shuffling. (a) Comb growth viewed from above from the start of the week on the left, to the end of the week on the right for (a.i) an example control nest and (a.ii) an example shuffled nest. New comb growth is represented by a coloured heat map while comb existing at the start of the week is in greyscale. (b) Nest connectedness measured by the proportion of cells in the nest that directly face an across-frame neighbouring cell, comparing shuffled nests with control nests. Shuffling events occurred at the beginning of each week, indicated by red triangles. (c) Colony-level performance metrics did not differ between shuffled and control colonies in (c.i) colony size, (c.ii) nest area, (c.iii) hive weight or (c.iv) average nest temperature. In (b-c), error bars indicate 95% confidence intervals.

figure 4c). As discussed above, shuffled colonies increased their nest connectedness by the end of the week (figure 3b), which was best captured by the neighbour model. Nest connectedness was significantly higher in the predicted nests generated by the neighbour model than the dilation model (repeated measures ANOVA with Tukey pairwise comparisons, $p < 0.0001$, figure 4d), and nest connectedness in the neighbour model matched with measures of nest connectedness in the observed nests (Tukey pairwise comparisons, $p = 0.61$, figure 4d). By simply adding comb to the nest, the nest connectedness metric increased slightly over time (figure 3b). However, our model comparisons show that shuffled colonies increase nest connectedness by more than would be expected if they were simply expanding all comb edges equally (dilation model), and instead matched the predictions of the gap-filling neighbour model (figure 4d), which takes into account the three-dimensional nest structure. For control colonies, nests predicted by both the dilation model and the neighbour model did not differ in connectedness from the observed nests (Tukey pairwise comparisons, dilation-observed: $p = 0.85$, neighbour-observed: $p = 0.10$, electronic supplementary material, figure S7).

These results highlight that colonies work to promote a key feature of overall nest form across conditions—nest connectedness. While the dilation model performs well for control colonies that can expand their nest without disturbance, the dilation model performs poorly for shuffled colonies that face a disrupted nest. The neighbour model, however, performs equally well for both control and shuffled colonies. The shuffle treatment reveals that workers prioritize nest connectedness by accounting for the three-dimensional structure of their nest.

4. Discussion

Honeybee colonies build a well-connected, spheroid nest of parallel combs that expands from the nest origin in all directions to fill the cavity. Contrary to our hypothesis, disrupting three-dimensional nest structure during the colony's rapid growth phase did not hinder colony-level performance: there was no difference in worker population, comb area, hive weight or nest temperature between the control and shuffle treatments. However, our comb building models
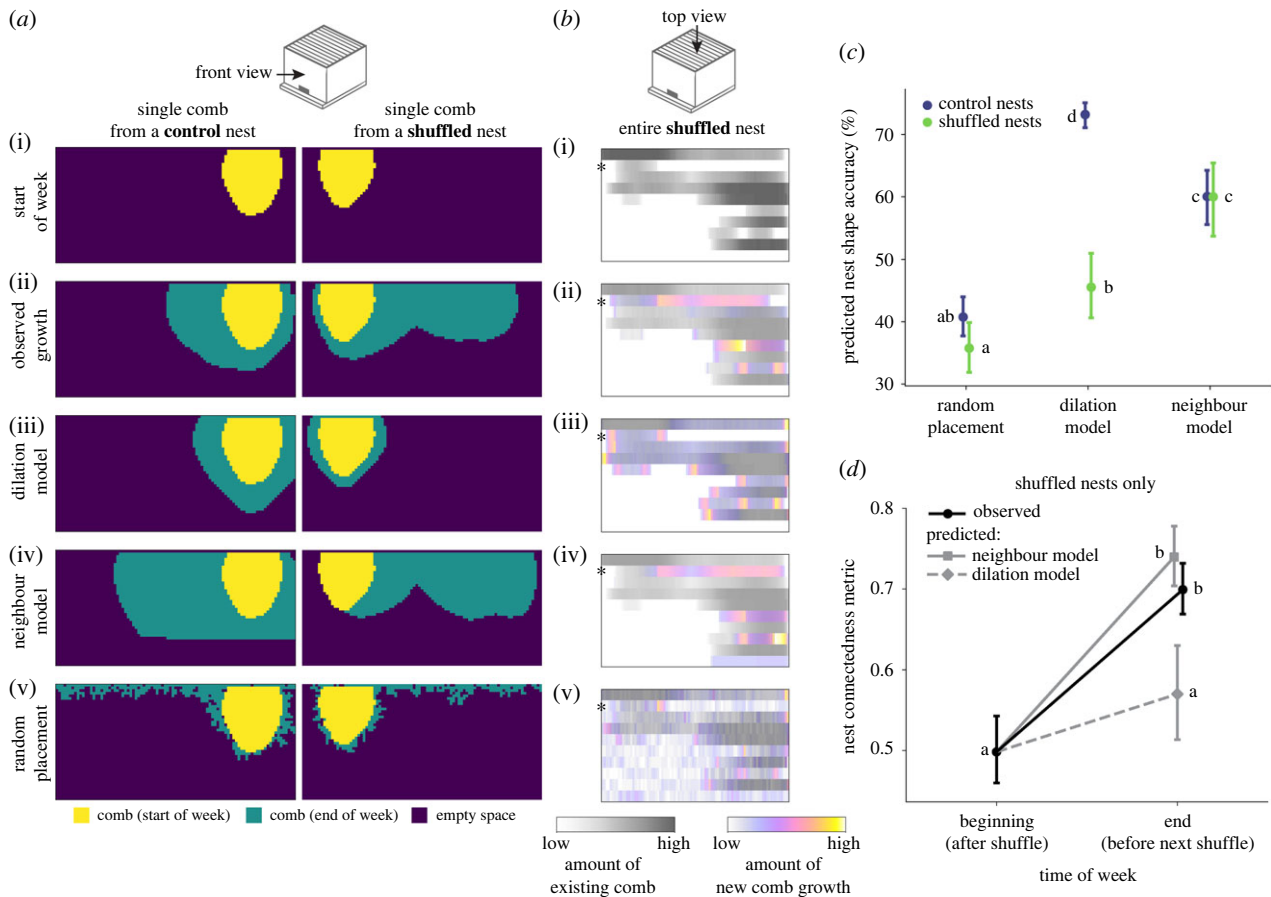
Figure 4. A comparison of comb growth models. (*a*) Front view of a single frame of comb from an example control nest (left column) and a shuffled nest (right column). (*a*.i) The comb at the start of the week, (*a*.ii) the observed comb growth by the end of the week, and the end-of-week comb growth predicted by (*a*.iii) the dilation model, (*a*.iv) the neighbour model and (*a*.v) random placement. (*b*) Top view of the example shuffled nest, now showing the whole nest structure, where asterisks indicate the example frame shown in panel A (shuffled nest). Observed and predicted comb growth is represented by a coloured heat map while comb existing at the start of the week is in greyscale. The same subgraph categories from panel (*a*.i–*a*.v) apply to (*b*.i–*b*.v). (*c*) Overall predicted nest shape accuracy across all three models for both control and shuffled nests. (*d*) Observed and predicted nest connectedness for shuffled nests across all six weeks. For (*c*,*d*), error bars indicate 95% confidence intervals and compact letter displays were generated from *post hoc* Tukey HSD pairwise comparisons.

show that colonies prioritize structural connectedness when expanding their nest, which led shuffled colonies to repair connections in their three-dimensional nest structure.

To fully understand the functional significance of the nest structure, we must first consider the development of its form. The western honeybee (*A. mellifera*) is a cavity-nester, but the common ancestor of *Apis* nested in the open, like the present-day giant honeybee *A. dorsata* and red dwarf honeybee *A. florea* [43]. Modifying a nest to be built within a cavity required some adaptations in their building behaviour, but also similarities with the ancestral state. In *A. florea*, the single circular comb grows evenly in height and width [44]. Similarly, we found that the parallel combs in *A. mellifera* nests initially grow with an equal aspect ratio, but once the ventral tip approaches the limit of available space, combs become horizontally biased to conform to the horizontally biased wooden frames (figure 2*d*). This shows that bees can easily adapt their comb shape to the available space, a trait that probably evolved with their transition to cavity nesting. Combs initiated after the nest has already become laterally biased incorporate this bias before reaching the limits of available space. Therefore, workers may consider the overall nest shape as they add subsequent combs to maintain a well-connected nest—potential evidence for cognition and planning during nest construction [45]. Workers consistently initiated a new comb when the comb on the outer edge of

the nest reached 180 cm^2, which is only 20% of the available frame space. This threshold shows that colonies are expanding several parallel combs simultaneously instead of building each comb to the edge of available space one at a time. Simultaneous comb construction promotes the well-connected spheroid nest not just as a final product, but throughout development. Comb growth slowed before physically touching the wooden frame (electronic supplementary material, figure S3, S6), so the workers in the building cluster can sense the upcoming barrier and reduce their comb expansion. This creates walking space on the sides and bottom of each comb [28]. The cavity-nesting bees are not simply duplicating the sheets of comb built by open-nesting bees—they actively adapt their building behaviour to a three-dimensional cavity.

Given that workers are actively building a characteristically stereotyped three-dimensional structure, we expected that this structure would be important for colony function, and that our disruption would impact colony performance. However, shuffling combs to disrupt nest structure did not appear to hinder the ability of colonies to rear brood, thermoregulate or forage (figure 3*c*). The rapid nest growth phase, weekly comb shuffling and colony performance metrics occurred over 45 days—enough time to complete two full brood cycles (21 days from egg to eclosion; [46]), and ample opportunity to impact brood rearing, but the colony sizes were no different between the two groups. We also

found no difference in the amount of comb built each week. The high metabolic cost of wax probably puts a physiological limit on the amount of wax a colony can produce [20], which may vary with colony size and available resources. While we found no difference in the *amount* of wax built between treatment groups, we did find a difference in *where* the workers chose to allocate the wax within their nest.

The surprising lack of difference in colony performance between control and shuffled colonies raises the question: is nest structure unimportant for colony function, or were the bees able to compensate for the disruption by changing how they expanded their nest? Our comb building models can answer this question. The simple dilation model best predicted the shape of control nests—all comb edges of the nest expand at an equal rate with no consideration for three-dimensional structure. This shows that constructing a globally cohesive nest structure does not require workers to have global information. If nest structure were unimportant, we would expect shuffled colonies to build new comb no differently than control colonies, unbothered by the disjointed nest. However, nest growth in the shuffled colonies was predicted significantly better by the neighbour model, which accounts for the three-dimensional space to fill gaps, and thus prioritizes nest connectedness (figure 4). Additionally, shuffled colonies consistently increased nest connectedness following each shuffle event (figure 3b), even more than predicted by simply adding comb equally throughout the nest. Using the same amount of wax as control colonies, shuffled colonies abandoned some outlying combs and strategically filled gaps between others (electronic supplementary material, figure S8), enabling them to maintain a cohesive nest structure, which probably overcame the repeated shuffling of their nest and prevented any measurable losses of colony-level performance. This shows that honeybees do account for three-dimensional nest structure, and that they direct their building efforts to preserve nest connectedness. Honeybees are not alone in their ability to adapt their nest—some bird species alter their nest design to compensate for temperatures that differ with season, altitude and latitude [5]. While these examples show design compensation in nests built anew, the honeybees are adapting their nest throughout the building process.

Bees probably prioritize three-dimensional nest connectedness for several reasons. (i) A well-connected nest will minimize the surface area to volume ratio, which helps the efficiency of thermoregulation. Nest thermoregulation is essential for colony function; it allows larvae to develop normally and enables winter survival [47–49]. Minimizing surface area probably reduces the energy required to maintain target temperatures. Similarly, bird nests are built to enhance incubation and prevent heat loss, with nest surface area among the most important design factors [50–52]. (ii) A well-connected nest may improve information-sharing, to promote foraging efficiency and propagating alarm response. In harvester ants, for example, connectivity between nest chambers has a stronger effect on colony behaviour than chamber volume, increasing the speed of recruitment to food [11]. (iii) A well-connected nest may offer shorter travel distances to target cells, which increases efficiency (e.g. foragers depositing pollen, nurses feeding larvae, queen laying eggs). In polydomous ant species, travel costs are minimized by more connections between nests [53]; similar efficiencies may occur at a smaller scale within honeybee nests.

What mechanism are bees using to fill gaps and promote nest connectedness? Swarming behaviour may provide some insights. When bees move into a cavity, they do not distribute themselves evenly throughout the interior surface, but instead remain in a tight cluster. This physical bee-structure may help guide the shape of the nest during its development. Especially in cavities that are larger than the ideal nest [28], clustering may guide the initial arrangement of parallel combs. In our shuffled colonies, bees remained tightly linked regardless of comb distribution; where the bees were densest, the most comb grew (electronic supplementary material, figure S8). Bees are known to form festoons around the comb building area, linking their legs together and hanging from anchor points to create curtain-like structures [54]. How festoons contribute to comb construction is poorly understood, but they may play a role in how bees detect and fill gaps in the three-dimensional structure—a possible mechanism for collective sensing over spatial scales larger than individuals [55]. A semi-fluid bee cluster may also provide the flexibility to build a well-connected nest in unpredictable nest cavities, around obstacles, or after natural disturbances cause combs to break and fall.

The first decision colonies face after selecting a cavity is choosing the location within the cavity to start building comb. We found that colonies were biased towards initiating their nest in the southeastern quadrant of the nest-box. This may be due to warmth from the morning sun (which would aid in thermoregulation) or the proximity to the southern entrance (to reduce travel time and response time to threats). Teasing apart the influence of these variables, however, would require explicit testing. After deciding where to initiate the nest, workers enter the rapid growth phase. This study was performed in Auburn, AL, a subtropical region of the United States, but our results corroborate results from across the continent. The rapid growth phase in our colonies lasted 45 days, during which colonies built 85% of the total nest area they would build that year. Similarly, in Fort Langley, BC, Canada, after 44 days, colonies built 90% of the total nest area that would be built that year [56]; in Ithaca, NY, USA, after 46 days, colonies built $79 \pm 4\%$ [18]. The similarities between these values probably show the importance of quickly initiating a nest, regardless of longitude/latitude.

The stereotypical growth patterns show that the nest itself has an established process of development. Unlike the physical form of most organisms, however, this structure can be completely rearranged and it still maintains its function as a nest. Our experimental manipulations, akin to rearranging the organs within an organism, did not impact colony performance. In honeybees, this is accomplished at least partially by workers shifting their building behaviour, a mechanism that the superorganism can employ for addressing unprecedented developmental problems. Similar mechanisms can be seen at a smaller biological scale, like molecular processes having multiple backup pathways to overcome damaged DNA [57]. The honeybee nest is more flexible than previously thought, even during the earliest stages of nest development. Is this a universal feature of social insect nests, or is it restricted to taxa whose nests serve similar functions to honeybees (e.g. thermoregulation, cavity conformation)?

Nests provide a stable environment for raising offspring across the animal kingdom. Just as site selection and building material choices can help reduce the investment needed for

brooding behaviour [58], the physical structure of the nest plays an essential role in maintaining homeostasis. Here we show that nest connectedness—a physical property of nest structure—is probably under selection throughout nest development. How widespread is the importance of nest connectedness? It may depend on the builders' ecology and what functions the nest is required to serve. For example, nest connectedness may be important for species that thermoregulate, while other properties like entrance turrets may be important for species that require increased gas exchange [59]. The nest architecture of subterranean ants varies wildly between species, with differences in shaft and chamber features, proportions, sizes and numbers [6]. Researchers have only begun to test the functional significance of nest features on colony performance—which can lead to surprising results. A rare example study of manipulating nest architecture showed that subdividing chambers in artificial fire ant nests (*Solenopsis invicta*) to mimic natural nest architecture did not influence brood rearing efficiency as expected [60], a similar narrative to our study. Diving deeper into why certain features exist and how they interact with behaviour can show how defined functions drive specific forms of nest morphology across taxa.

Here we provide the first detailed description of three-dimensional nest structure during the rapid growth phase of honeybee colony development. We then experimentally disrupt this three-dimensional structure and, surprisingly, find no evidence for impact on colony-level performance. However, using models of nest growth, we show that the building strategy of the workers counteracted these manipulations, to ensure the nest is well-connected. This showcases the importance of accounting for three-dimensional nest structure during nest development and provides insight into the adaptive and resilient nature of the superorganism.

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