Report

Current Biology

Form, function, and evolutionary origins of architectural symmetry in honey bee nests

Highlights

- Honey bees stockpile nest contents symmetrically, creating a mirror image
- Independent colonies living on either side of a comb will mimic each other's nests
- Symmetry has adaptive benefits for colony growth and temperature stability
- Nest symmetry is conserved across multiple species of *Apis*

Authors

Michael L. Smith, Peter R. Marting, Claire S. Bailey, ..., Ethan B. Rowe, Maritza R. Spott, Benjamin Koger

Correspondence

msmith@ab.mpg.de

In brief

Smith et al. show that honey bees stockpile their nests symmetrically on either side of the double-sided comb, revealing architectural symmetry in the extended phenotype. This symmetry provides the colony with adaptive benefits, remains conserved in three-dimensional (3D) nest structures, and spans multiple species of *Apis*.

Smith et al., 2024, Current Biology *34*, 5813–5821
December 16, 2024
https://doi.org/10.1016/j.cub.2024.10.022

CellPress

Current Biology

CellPress
OPEN ACCESS

Report

Form, function, and evolutionary origins of architectural symmetry in honey bee nests

Michael L. Smith,[1,2,3,4,10,11,*] Peter R. Marting,[1] Claire S. Bailey,[1,5] Bajaree Chuttong,[6] Erica R. Maul,[1] Roberto Molinari,[7] P. Prathibha,[1] Ethan B. Rowe,[1] Maritza R. Spott,[1] and Benjamin Koger[2,3,8,9]

[1]Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA
[2]Department of Collective Behaviour, Max Planck Institute of Animal Behavior, 78464 Konstanz, Germany
[3]Centre for the Advanced Study of Collective Behaviour, University of Konstanz, 78464 Konstanz, Germany
[4]Wissenschaftskolleg zu Berlin, 14193 Berlin, Germany
[5]Department of Biological Sciences, New Jersey Institute of Technology, Newark, NJ 07102, USA
[6]Meliponini and Apini Research Laboratory, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[7]Department of Mathematics and Statistics, Auburn University, Auburn, AL 36849, USA
[8]School of Computing, University of Wyoming, Laramie, WY 82071, USA
[9]Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, USA
[10]X (formerly Twitter): @SmithBeeLab
[11]Lead contact
*Correspondence: msmith@ab.mpg.de
https://doi.org/10.1016/j.cub.2024.10.022

SUMMARY

Symmetry is pervasive across the tree of life,[1–5] and organisms (including humans) build symmetrical structures for reproduction, locomotion, or aesthetics.[6–9] Symmetry, however, does not necessarily span across levels of biological organization (e.g., symmetrical body plans often have asymmetric organs).[10] If and how symmetry exists in structures built by social insect collectives, where there is no blueprint or central organizer, remains an open question.[11] Here, we show that honey bees actively organize nest contents symmetrically on either side of their double-sided comb; 79% ± 7% of cell contents match their backside counterpart, creating a mirror image inside the nest. Experimentally restricting colonies to opposite sides of comb, we find that independent colonies will symmetrically mimic each other's nest organization. We then examine the mechanism by which independent colonies can indirectly coordinate nest symmetry, showing that 100% of colonies (n = 6) perfectly co-localize their brood nest with a randomly positioned heat source, indicating that heat drives nest site initiation and early brood production. Nest symmetry also has adaptive benefits: two-sided nests grow more quickly, rear more brood, and have a more stable thermal environment than one-sided nests do. Finally, examining the evolutionary origins, we show that symmetry persists in three-dimensional (3D) nests of *Apis mellifera* and across multiple *Apis* species, coinciding with the onset of double-sided combs, which made it possible to symmetrically stockpile nest contents. This work shows that, similar to molecular mechanisms that create symmetry in multicellular organisms, there are behavioral processes that create functional symmetry in the collective organization of animal architecture.

RESULTS AND DISCUSSION

Superorganisms, and the nests they build, provide a unique opportunity to test fundamental biological principles at a different level of biological organization.[12] A superorganism is formed when tens to millions of multicellular individuals combine to form a functional unit, such as social insect colonies.[13] Nests are a key feature of superorganisms, and this extended phenotype is critical for the colony's survival, growth, and reproduction.[14,15] In the Western honey bee, *Apis mellifera*, the nest comprises multiple parallel combs, although the ancestral form was a single comb.[16,17] Each comb is built as a double-sided sheet of hexagonal cells, and the contents of each cell are the result of the combined decisions of the colony members.[18] The nest is analogous to the superorganism's body, with individuals moving freely throughout the physical structure.[19] Honey bee nests show remarkable similarities with multicellular organisms: workers actively thermoregulate,[20] perform gas exchange,[21] and spatially partition their nests (e.g., honey versus brood; somatic versus reproductive comb).[22,23] Given that the nest is similar to an organism's body, how does symmetry persist, if at all, in the extended phenotype?

Axes of nest symmetry

We begin at the proximate levels of analysis,[24] mechanism and development, to assess potential axes of symmetry in natural-comb observation hives. Six colonies of *A. mellifera* were installed into observation hives with sufficient capacity to house a full-sized colony and its nest (cavity volume > 40 L; see STAR Methods). The workers built and stockpiled their nest as they

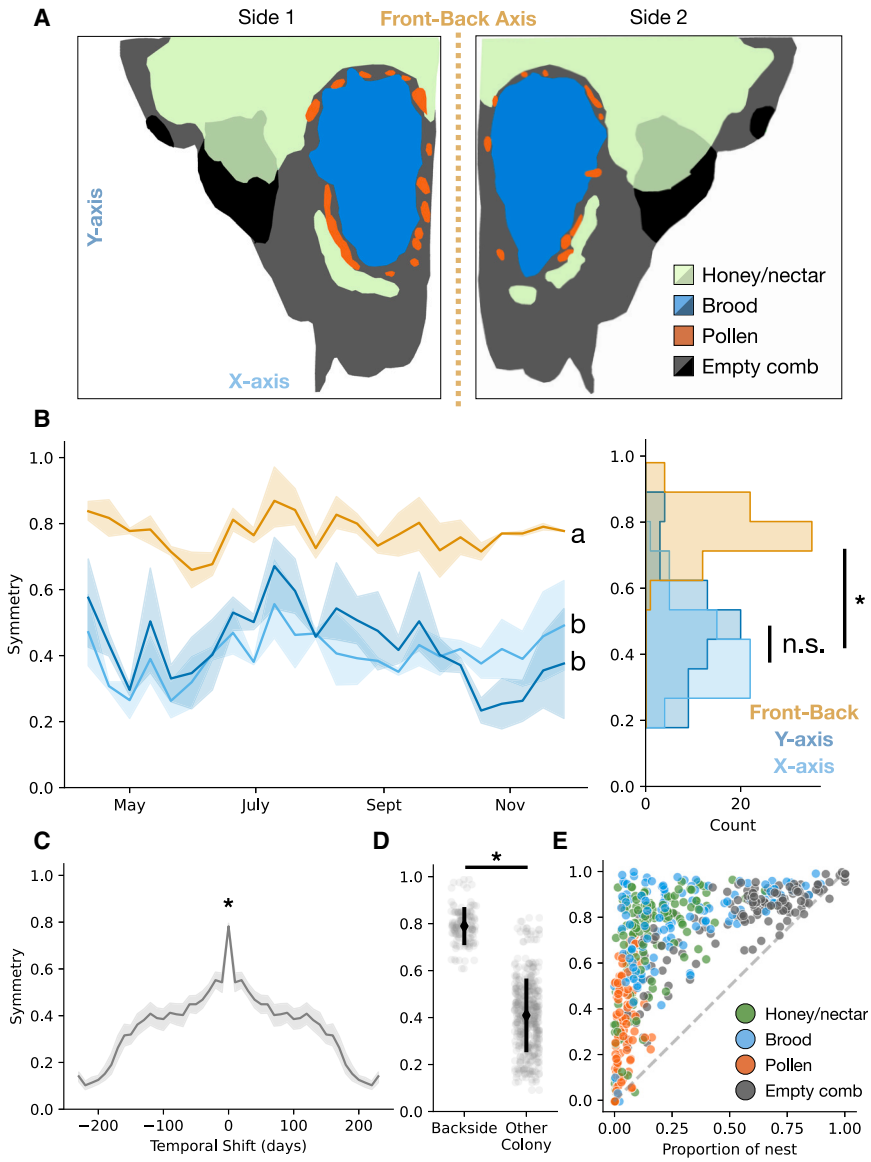

Figure 1. Honey bee nest contents are symmetrically organized on either side of the comb

(A) Honey bee nest maps, showing nest contents for both sides of the comb (representative example, see also Figures S3 and S4). Color legend is for worker comb, with drone comb in darker tone.

(B) Plot of nest symmetry for each axis over time (left) and as a histogram (right).

(C) Front-back symmetry for a given colony on the same day (temporal shift = 0 days) versus nest maps from the past (negative values) and future (positive values). Note that the focal map is compared with maps of its backside, shifted temporally.

(D) Comparison of front-back symmetry for a focal map and its backside versus when paired with other colonies.

(E) Front-back symmetry for each of four nest contents, plotted against their relative proportion of the nest. Dotted line shows the 1:1 ratio (letters and asterisks denote significant differences; LMM with pairwise comparisons; line plot shading shows 95 CI; error bars show mean ± SD).

wished, and we mapped the nest contents over time (148 total nest maps, 242 days).

Nests built within the large observation hives were skewed toward the entrance, as previously shown,[25] but a nest does not have a defined anterior-posterior or dorsal-ventral axis. Combs, however, are consistently planar and double sided, allowing us to define three natural axes of potential symmetry: horizontal (x axis), vertical (y axis), and front-back (i.e., an arbitrarily chosen focal side of comb and its backside). Symmetry was calculated as the proportion of all comb contents that exactly match the contents on the other side of the given axis (same date, same colony; 0 = no match, 1 = perfect match; Figures 1A and 1B). For all tests of symmetry, unless otherwise specified, we employed a linear mixed model (LMM) with the colony as a random effect to control for correlation across responses (see STAR Methods).

The front-back axis, which divides comb into a front and backside, consistently had the highest nest symmetry (symmetry score along front-back axis: 0.79 ± 0.07; x axis: 0.41 ± 0.12; y axis: 0.47 ± 0.16; LMM with pairwise comparisons; Figure 1B). This creates a mirror image, where, on average, 79% of the hexagonal cells on one side of the comb exactly match the cell contents on its backside; significantly higher than random (symmetry versus random match [SVRM], see STAR Methods, $p < 0.0001$). Nests exhibited this front-back symmetry throughout the year (Figure 1B), even when colonies reproduced via colony fission (May–June), and were equally symmetrical in first-year colonies initiating their nests as in second-year established colonies (front-back symmetry in first-year colonies: 0.77 ± 0.07; second-year colonies: 0.82 ± 0.06; LMM with pairwise comparisons; Figures S3 and S4). Note that, because each cell has a solid wax base, individual bees cannot be in direct contact with both sides of the comb simultaneously. To access cells on the opposite side, bees must pass around the comb edge or through peripheral galleries along the cavity walls.[16]

Front-back symmetry is not a result of stereotypical developmental patterns; it is both temporal and colony specific. If nest maps from either side of the same colony are temporally shifted by only 10 days, symmetry drops from 0.79 ± 0.07 to 0.53 ± 0.20 (all non-paired dates: 0.42 ± 0.20; LMM with pairwise comparisons; Figure 1C). Similarly, symmetry drops when nest maps are compared between different colonies on the same date (0.41 ± 0.15; LMM with pairwise comparisons; Figure 1D). Combined, these data show that (1) nests exhibit front-back symmetry, (2) workers are continuously maintaining front-back symmetry throughout the colony's life,

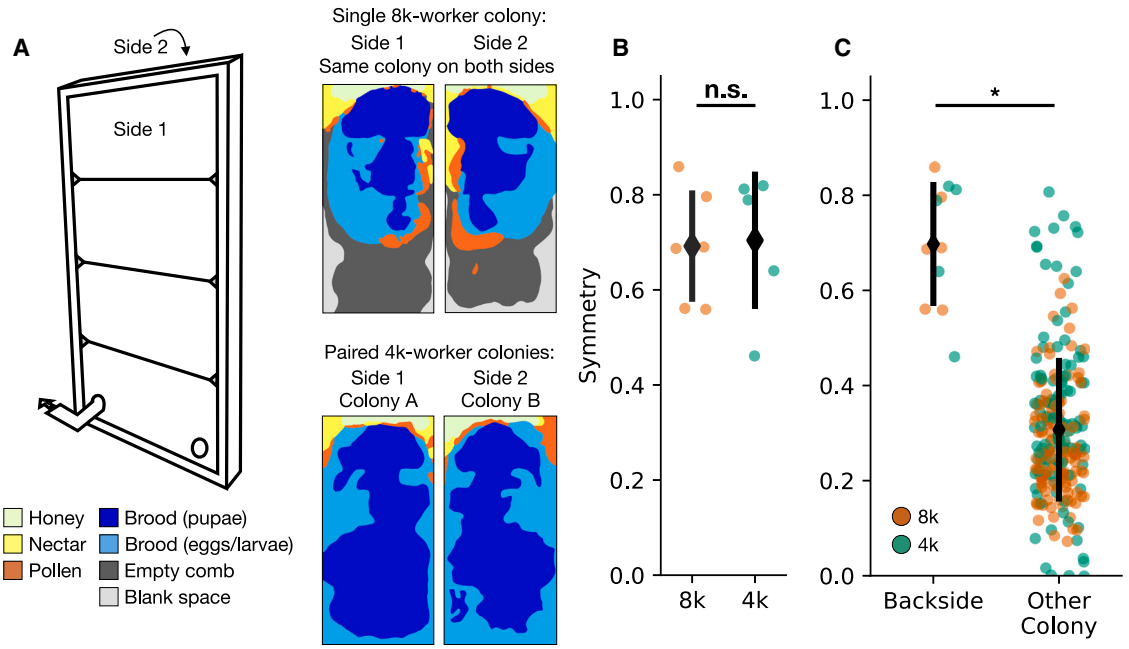

Figure 2. Colonies do not require direct contact with both sides of their nest to create nest symmetry

(A) Illustration of the one-sided observation hive and examples of nest maps (representative examples, see also Figure S5). Independent 4,000-worker colonies are installed on either side, without access to each other. The 8,000-worker colonies have access to both sides, via small passageways cut along the edges of the nest. Entrance tunnel shown at lower left corner; access to sucrose solution feeder at lower right (same provided on opposite side, not shown in illustration).

(B) Nest symmetry for colonies with access to both sides of the nest (8,000) versus independent colonies installed on either side of the one-sided observation hive (4,000). Symmetry scores are not significantly different.

(C) Nest symmetry is significantly higher for a focal map and its backside than when paired with maps of other colonies (asterisks denote significant differences; n.s., not significant; LMM with pairwise comparison; error bars show mean ± SD).

See also Figure S1.

and (3) front-back symmetry is both date and colony specific (Figures 1A–1D).

To determine whether all nest contents were equally symmetrical along the front-back axis, we calculated the proportion of each nest's contents that matched on either side of the nest (honey/nectar, brood, pollen, and empty comb). The highest front-back symmetry scores were for brood and empty comb, followed by honey/nectar, with pollen showing the lowest front-back symmetry (brood: 0.78 ± 0.20; empty comb: 0.79 ± 0.17; honey/nectar: 0.66 ± 0.26; pollen: 0.33 ± 0.20). Pollen, however, is a small proportion of the overall nest (brood: 0.27 ± 0.25; honey: 0.14 ± 0.14; empty comb: 0.55 ± 0.27; pollen: 0.03 ± 0.04), which makes it susceptible to minor errors during nest mapping. Taking into account their respective proportions, all nest contents are significantly more symmetrical than random (SVRM, $p < 0.0001$, Figure 1E). Therefore, workers are actively creating a nest that is symmetrical on the front and back sides of the comb, which creates a mirror image. Note that this applies both to where they do put resources (e.g., queen laying eggs and workers storing nectar) and where they do not (e.g., comb cells that are temporarily empty). Hereafter, we refer to symmetry along the front-back axis as simply "nest symmetry" to denote the symmetrical arrangement of nest contents.

Nest symmetry across independent colonies

We next conducted an experimental study to determine how colonies create a symmetrical nest on either side of the double-sided comb. Using custom-built observation hives, we restricted a colony to a single side, where they could initiate, build, and stockpile their nest as they wished (see STAR Methods). Workers, however, cannot chew through the surface upon which their nest is built and therefore create a one-sided nest (Figure 2A). In one experimental setup, we established separate 4,000-worker colonies on each side of the same one-sided observation hive (n = 5 observation hives; 10 colonies; 10 nest maps). These independent colonies each build a one-sided nest on their side of the shared comb-building surface, but they have no direct contact with one another (Figure S1; see STAR Methods). In the other experimental setup, we established 8,000-worker colonies in identical observation hives, but these colonies have access to both sides of the nest via small holes at the periphery (n = 6 observation hives; 6 colonies; 12 nest maps). If workers need direct contact with both sides of the comb to generate nest symmetry, then symmetry is predicted to be lost in the paired-4,000-worker colonies (but not in the 8,000-worker colonies) because each colony is restricted to its own side.

Independent colonies built nests that were as symmetrical on either side of the observation hive as colonies that had access to both sides of the observation hive (symmetry in paired-4,000-worker colonies: 0.70 ± 0.15; symmetry in 8,000-worker colonies: 0.69 ± 0.12; LMM with pairwise comparisons, Figure 2B). Across both treatment groups, symmetry was significantly higher for nest maps that were paired with their backside

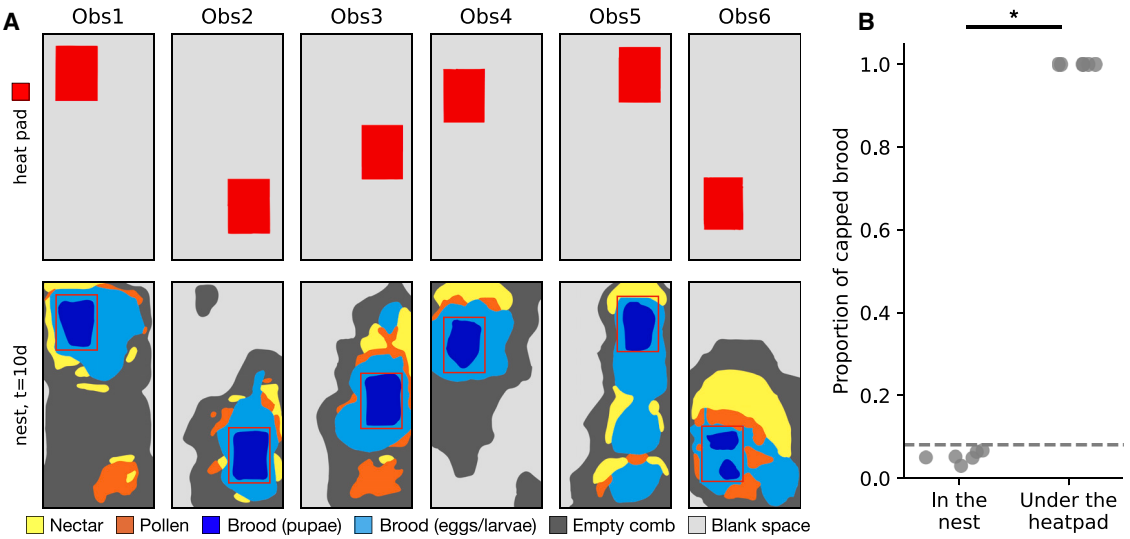

Figure 3. Temperature determines where a colony will position its nest and rear brood

(A) Heat pads (red rectangles, top row) were randomly placed on one side of empty observation hives. Colonies were then installed on the other side and mapped after 10 days (bottom row: colors denote comb contents; heat pad outlined in red).

(B) Pupae (dark blue in A) comprised 5% ± 1% of the total nest area, but 100% was co-localized with the heat pad (dotted line denotes percent of nest area which heat pad covers; asterisks denote significant differences; chi-squared test).

counterpart than when paired with nest maps taken from other colonies (symmetry with backside nest map: 0.70 ± 0.15; other nest map: 0.31 ± 0.14; LMM with pairwise comparisons; Figure 2C). Of 22 total maps (10 from 4,000-worker colonies, 12 from 8,000-worker colonies), 19 (86.4%) had the highest symmetry with their backside counterpart, a significantly higher pairing compared with a null hypothesis where all 22 maps were unrelated to each other (expected pairing with their backside under the null: 1 of 22; observed pairing with backside: 19 of 22; chi-squared test, χ^2 (1, 22) = 339, $p < 0.0001$). Therefore, each nest was specifically symmetrical with the nest that was built on the other side of the observation hive, regardless of whether workers had direct contact with both sides of their nest (8,000-worker colonies) or did not have direct contact with both sides (paired-4,000-worker colonies). Therefore, colonies are indirectly coordinating nest symmetry, even when each side of the nest is inaccessible, and built/organized by an independent colony.

Temperature drives nest organization

We next investigated how independent colonies could generate nest symmetry across an impermeable barrier. Presumably, workers on one side of the nest respond to cues produced by the colony on the other side and vice versa. Chemical and visual cues are unlikely, due to the 2-mm-thick plastic barrier and that colonies were kept in the dark to mimic natural conditions. Mechanical and thermal cues, however, could be transmitted through the substrate.[26–28] Given that colonies tightly regulate their brood temperature between 33°C and 36°C,[29] we tested whether mimicking this temperature profile on one side of the nest would influence a colony's nest organization on the other side. Using the same one-sided observation hive design (Figure 2A), we randomly placed a 15 × 20 cm^2 heat pad set to 36°C (Figure 3A). We then installed a 4,000-worker colony on the other side of the observation hive. After 10 days, we mapped each nest to determine if the heat pad influenced where colonies initiated their nests and reared their brood (observers were blind to the location of the heat pad; $n = 6$, see STAR Methods).

Across all six colonies, 100% of the pupae were spatially aligned with the position of the heat pad (SVRM, $p < 0.0001$, Figures 3A and 3B). Eggs develop into pupae after 9 days; therefore, the first cells that workers built and the queen laid eggs into were located atop the heat pad. Note that the heat pad comprised only 8.9% of the total available cavity area. Evidently, temperature dictates where brood will be positioned, which creates a symmetrical brood pattern on either side of the comb. Although temperature has been proposed as a potential mechanism for self-organized spatial patterns,[18,30] our results provide the first empirical support for thermal cues driving collective nest organization. Furthermore, previous modeling work did not consider the double-sided aspect of the comb, nor that self-organization had the potential to create a symmetrical nest.

Presumably, aligning the brood on either side of the comb helps with thermoregulation and provides a mechanism for workers to indirectly coordinate nest symmetry. Akin to the Spemann-Mangold organizer, a small group of embryonic cells that sends signals to neighboring cells to establish the adult body plan in vertebrates,[31] temperature defines the position of the brood nest. From this nest-level organizer, other comb contents can be arranged sequentially (pollen closest to the brood, then honey/nectar).[16] Note, however, that all nest contents are symmetrical in natural nests, not just the brood (Figures 1A and 1E), and that honey stores are symmetrical even when colonies have no brood (Figure S4). Therefore, there are likely additional mechanisms (e.g., nest entrance) that workers use to symmetrically organize their food resources.

Functional benefits of nest symmetry

We now shift from the proximate levels of analysis to the ultimate: adaptive function and evolutionary context.[24] First, we experimentally tested whether a 2-sided nest, which could be organized symmetrically, provides colonies with a functional benefit over a 1-sided nest, which cannot be organized symmetrically. Each 4,000-worker colony was given the same amount of space to build and stockpile their nest, but in one setup, colonies had access to one side of an 84 × 21 cm^2 nest, and in the other, they had access to both sides of a 42 × 21 cm^2 nest (i.e., the same total nest area, but one nest is one sided, and the other is two sided; colonies installed at the same time of year, in the same location, and with the same access to resources; see STAR Methods). After 10 days, we mapped their nest contents to compare the two treatment groups (one-sided colonies, $n = 8$; two-sided colonies, $n = 4$).

After only 10 days, colonies living in two-sided nests contained 58% more brood (eggs, larvae, and pupae) than colonies living in one-sided nests (brood: one sided: 1,179 ± 220 cm^2; two sided: 1,866 ± 291 cm^2; z-test with Bonferroni correction, $\alpha = 0.01$, $p < 0.0001$, Figure S2). Two-sided colonies also expanded into more of the available cavity space than one-sided colonies (occupied nest space: one sided: 78 ± 11%; two sided: 97 ± 3%) and therefore had significantly less "blank space" (one sided: 743 ± 386 cm^2; two sided: 115 ± 93 cm^2; z-test with Bonferroni correction, $\alpha = 0.01$, $p < 0.005$; Figure S2). The other nest contents (honey/nectar, pollen, and empty comb) showed no difference between one- and two-sided nests (honey/nectar: one sided 558 ± 243 cm^2; two sided 508 ± 88 cm^2; pollen: one sided: 128 ± 72 cm^2, two sided: 163 ± 95 cm^2; empty comb: one sided 836 ± 212 cm^2, two sided 791 ± 233 cm^2; z-test with Bonferroni correction, $\alpha = 0.01$, $p > 0.05$; Figure S2). Therefore, colonies building and living in a two-sided nest were able to rear more brood and expand their nest more quickly than equivalent colonies living in a one-sided nest.

Given that colonies in two-sided nests were more successful at expanding their brood area than colonies living in one-sided nests, we next examined the temperature profiles of their brood nests using embedded thermocouples. Although the mean brood nest temperature was remarkably similar between the two groups (one sided: 34.3°C ± 1.3°C; two sided: 34.5°C ± 0.8°C; mean ± SD), one-sided brood nests had a larger temperature variance (ratio of two-sided variance over one-sided variance: 0.452, F-test for homogeneity of variance, $p < 0.0001$, Figure S2). Colonies strive to keep their developing brood at a stable 33°C–36°C,[20] and deviations outside these boundaries impact adult performance.[29,32] For brood reared in 2-sided nests, 93.3% of the temperature readings fell within these limits versus 79.0% in one-sided nests (GLM: $p < 0.05$, Figure S2). Therefore, a two-sided nest provides additional thermal stability, keeping brood within their preferred range, which is critical for brood development.

To standardize across treatment groups (individual colony living in a one-sided versus two-sided nest), each colony had only one entrance tunnel, which created an element of asymmetry in our two-sided nests (entrance side versus non-entrance side). The two-sided nests still showed nest symmetry overall (0.67 ± 0.07, SVRM, $p < 0.0001$, Figure S6), but pollen was nest-side specific, with 99.4% ± 1.2% of pollen deposited on the entrance side (Figure S6). Pollen foragers must deposit pollen into cells themselves.[33] When foragers are routed to one side of the nest, pollen stores lose their nest symmetry (pollen symmetry: 0.10 ± 0.20; SVRM, $p > 0.05$). Therefore, forager traffic flow is a likely factor in creating pollen symmetry, as observed in natural-comb nests (Figures 1A and 1E). Note that foragers could still access the other side of the two-sided nest but only via small passageways at the edge (as in Figure 2A). Furthermore, despite these limited passageways, the brood nest was still symmetrical in the two-sided nests (brood: 0.87 ± 0.09; SVRM, $p < 0.0001$). Therefore, the queen must have distributed her egg-laying efforts symmetrically across both sides of the nest (as well as workers providing brood care).

Nest symmetry in 3D *A. mellifera* nests

We next explored the evolutionary context for nest symmetry, in both the three-dimensional (3D) nests of *A. mellifera* and the single-comb nests of other *Apis* species. In the previous experiments, we worked with colonies living in single-comb observation hives, but *A. mellifera* is a cavity-nesting bee that builds a 3D nest with multiple parallel combs (Figure 4A).[17,34] To determine whether front-back symmetry exists in each comb of a 3D nest, we installed six colonies into bee boxes and allowed each colony to build a natural-comb nest (i.e., multiple parallel combs, without wax or plastic comb foundation provided). After 22 days, we photographed both sides of each comb and used a neural network to automatically classify cell contents to determine if workers stockpiled combs in a 3D nest symmetrically (Figure 4B, STAR Methods).

In total, these six colonies built 48 combs (6–9 combs per colony) which generated 96 maps (two maps per comb; Figure S7). In these 3D nests, each comb is significantly more symmetrical than random (SVRM, $p < 0.0001$). Each comb exhibits front-back symmetry, creating a mirror image of itself (Figures 4B and S8), but combs are not interchangeable within the same colony, or across colonies (symmetry with backside: 0.71 ± 0.09; symmetry with comb from same colony but not backside: 0.47 ± 0.15; symmetry with comb from other colony: 0.45 ± 0.13; LMM with pairwise comparisons; Figure 4C). Therefore, *A. mellifera* workers create symmetry within each comb of their 3D nest structure. This also confirms that the symmetry we observed across entire nests in our observation hives (Figures 1 and 2) is not an artifact of the experimental setup but rather reveals the symmetry within a 3D nest.

Prior work on pattern formation in honey bee nests has focused on a single side of comb (i.e., a 2D plane).[11,18,30,35] Although this approach is useful for examining the role of blueprints, templates, and how simple behaviors can combine to create stereotypical self-organized patterns, it does not address the overall arrangement of multiple combs within a 3D nest. Indeed, existing models are at the comb-level, which often predict combs to be interchangeable because each comb is organized using the same mechanism. Our results, however, show that combs are not interchangeable across colonies or even within the same colony (Figure 4C). Therefore, workers are influenced by the wider 3D configuration of their nest,[36] beyond a single comb, and adapt their nest contents accordingly.

Nest symmetry across *Apis* species

All species of *Apis* build double-sided combs of hexagonal cells.[34,37] The ancestral state, however, was not a multi-comb

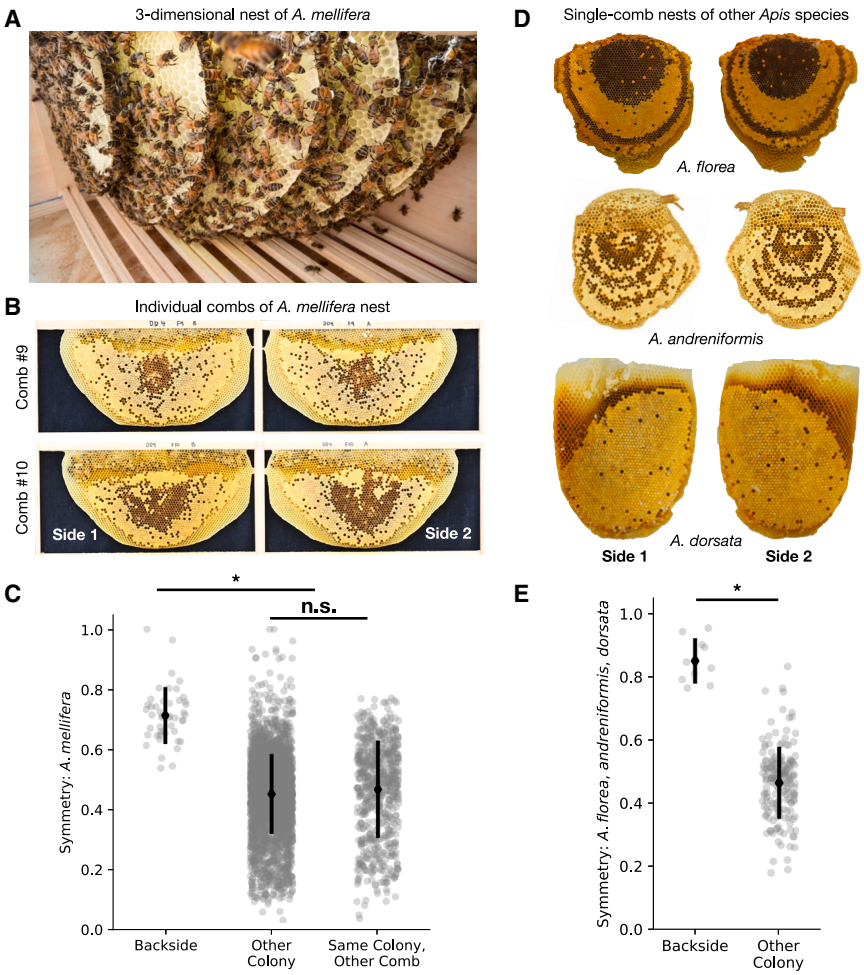

Figure 4. The evolutionary context of nest symmetry

(A) The 3D nest of *A. mellifera* contains multiple parallel combs built within a cavity.

(B) Photographs of two example combs in a 3D nest.

(C) In *A. mellifera* nests, each comb is symmetrical with its backside, but combs are not symmetrical across colonies or with other combs from the same colony.

(D and E) The single-comb nests of *A. florea* ($n = 5$), *A. andreniformis* ($n = 4$), and *A. dorsata* ($n = 1$) also exhibit front-back symmetry, which is specific to each nest and its backside counterpart (asterisks denote significant differences; n.s., not significant; LMM with pairwise comparison; error bars show mean ± SD).

See also nest maps in Figures S7 and S8.

nest built within a cavity but an entire nest that was composed of a single-comb exposed to the elements, similar to the present-day dwarf and giant honey bees (Figure 4D).[17,34] To determine whether front-back symmetry is unique to *A. mellifera* or shared across *Apis*, we collected wild nests of *A. florea* ($n = 5$), *A. andreniformis* ($n = 4$), and *A. dorsata* ($n = 1$) from Thailand, photographed both sides of the comb, and mapped their nest contents (Figure 4D and S8; see STAR Methods).

Across all three species ($n = 10$ nests total; 20 nest maps), nest contents are significantly more symmetrical than random (SVRM, $p < 0.001$), creating a mirror image (Figure S8). Nest maps are not interchangeable across colonies; symmetry was highest when nests were paired with their backside counterpart (symmetry with backside: 0.85 ± 0.07; with other nest map: 0.47 ± 0.11; LMM with pairwise comparisons; Figures 4D and S8). We also sequentially calculated nest symmetry for each nest map against all others; of 20 total maps, 18 (90%) had the highest symmetry with their backside counterpart (expected pairing with their backside under the null: 1 of 20; observed pairing with the backside: 18 of 20; chi-squared test, χ^2 (1, 12) = 132, $p < 0.0001$). Therefore, nest symmetry is not unique to *A. mellifera* but is shared across *Apis*.

Combined, these results show that nest symmetry likely evolved at the onset of double-sided comb, which arose with *Apis*, 50–60 mya.[38] Double-sided comb is typically attributed to its wax savings,[1,39] but workers in a hypothetical proto nest presumably also gained thermodynamic benefits from stacking brood together on both sides. Similar to facultatively social carpenter bees that save energy by reusing and renovating nests, these early benefits can drive both the formation of social groups and nest structure.[40,41] Once *A. mellifera* moved their nests from exposed locations into cavities, they had to modify their nest structure to fit within the spatial constraints of a fixed cavity. Although this forced them to abandon their ancestral single comb in favor of multiple parallel combs, they maintained front-back symmetry within each comb for its adaptive benefits (Figure S2).

Symmetry in the extended phenotype

How life organizes itself is a fundamental developmental and evolutionary question. Uniting investigations across all four levels of analysis,[24] we show the following: social insects create and maintain symmetrical nests (Figures 1, 2, and 4), thermal cues drive brood organization (Figure 3), there is a functional benefit to living in a symmetrical nest (Figure S2), and the evolutionary origins of symmetrical nests (Figure 4). There is no a priori expectation for symmetry in the organization of honey bee nests because the contents of each cell are determined by the collective behavioral actions of the colony. Indeed, even when independent colonies stockpile opposite sides of a nest, a mirror image is still created (Figure 2).

Although symmetry is pervasive in external body plans, the benefits of symmetry are typically attributed to locomotion[2] or conserved developmental pathways.[42] Even molecular compounds tend to be symmetrical, although this may be due to simplicity in replication, not inherent benefits of a symmetrical form.[43] Our work shows that symmetry extends beyond the body plan, into the extended phenotype of the superorganism and that architectural symmetry provides the colony with clear

adaptive benefits (brood thermoregulation and colony growth). Examples of front-back symmetry are rare and, to our knowledge, limited to human-built objects, such as pre-Columbian textiles that are woven with symmetrical patterns[44] or distortion-eliminating lenses, such as the Cooke Triplet.[45] In human architecture, top-down planning can also create front-back symmetry, for example, shared plumbing lines can define the location of the kitchen and bath across apartments on either side of the building. Unlike honey bees, independent households do not further mirror the organization of each other's residences (e.g., the position of the bed, dining table, and bookcase).

An organism's appearance is the result of evolutionary pressures, and those same pressures apply to the structures organisms build, such as nests. Symmetry is found across the tree of life, and the benefits of a symmetrical form extend to the physical structures organisms build and organize (their extended phenotype[14]). We show a novel example of biological symmetry, which coincides with the onset of double-sided comb, a unique feature of *Apis* architecture.[17,34] Similar to the molecular pathways that generate and maintain symmetry in unicellular and multicellular organisms,[46,47] our work provides a basis for behavioral processes that give rise to architectural symmetry in the superorganism.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Michael L. Smith (msmith@ab.mpg.de, mls0154@auburn.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All nest maps analyzed in this study are available in the supplemental information.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

We thank the Ag Lands crew for completing lab renovations in time for the field season; C.C. Carthel, O.S. Smith, C.D. Dickinson, and K.R. McInerney for help with data collection and processing; and C.J. Ballen, B.A. Klein, R.C. Range, and T.D. Seeley for discussions on symmetry. This work was supported by the National Science Foundation (grant no. 2216835), the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2117 – 422037984, a CASCB Project Proposal, the Alabama Agricultural Experiment Station, and the USDA Hatch Act program.

AUTHOR CONTRIBUTIONS

Conceived the project, M.L.S.; designed research, M.L.S.; performed research, M.L.S., P.R.M., C.S.B., E.R.M., P.P., E.B.R., and M.R.S.; contributed analytic tools or materials, B.C., B.K., and R.M.; analyzed data, M.L.S., P.R.M., B.K., and R.M.; wrote the paper, M.L.S.; provided feedback on paper, P.R.M., C.S.B., B.C., B.K., E.R.M., P.P., E.B.R., M.R.S., and R.M.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
  - Natural-comb observation hives
  - Apparatus: one-sided observation hives
  - Assaying whether independent colonies create symmetrical nests
  - Heat-pad experiment
  - Nest symmetry in three-dimensional A. mellifera nests
  - Automated classification of comb contents in three-dimensional A. mellifera nests
  - Nest symmetry in the single-comb nests of A. florea, A. andreniformis, and A. dorsata
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Statistical analyses

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2024.10.022.

Received: June 5, 2024
Revised: August 16, 2024
Accepted: October 8, 2024
Published: November 7, 2024

REFERENCES

1. Thompson, D.W. (1942). On Growth and Form The comple (Cambridge University Press).
2. Abrams, M.J., Basinger, T., Yuan, W., Guo, C.L., and Goentoro, L. (2015). Self-repairing symmetry in jellyfish through mechanically driven reorganization. Proc. Natl. Acad. Sci. USA *112*, E3365–E3373. https://doi.org/10.1073/pnas.1502497112.
3. Hudson, A. (2000). Development of Symmetry in Plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. *51*, 349–370.
4. Hileman, L.C. (2014). Trends in flower symmetry evolution revealed through phylogenetic and developmental genetic advances. Philos. Trans. R. Soc. Lond. B Biol. Sci. *369*, 20130348. https://doi.org/10.1098/rstb.2013.0348.
5. Marshall, C.R. (2006). Explaining the Cambrian "explosion" of animals. Annu. Rev. Earth Planet. Sci. *34*, 355–384. https://doi.org/10.1146/annurev.earth.33.031504.103001.
6. Hansell, M. (2005). Animal Architecture (Oxford University Press).
7. Laidre, M.E. (2021). Animal architecture. Curr. Biol. *31*, R1458–R1464. https://doi.org/10.1016/j.cub.2021.09.082.
8. Borgia, G. (1985). Bower quality, number of decorations and mating success of male satin bowerbirds (Ptilonorhynchus violaceus): an experimental analysis. Anim. Behav. *33*, 266–271. https://doi.org/10.1016/S0003-3472(85)80140-8.
9. Gao, Y., and Yuille, A.L. (2017). Exploiting symmetry and/or manhattan properties for 3D object structure estimation from single and multiple images. Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition, 7408–7417. https://doi.org/10.1109/CVPR.2017.711.
10. Palmer, A.R. (2004). Symmetry breaking and the evolution of development. Science *306*, 828–833. https://doi.org/10.1126/science.1103707.
11. Camazine, S., Deneubourg, J.-L., Franks, N.R., Sneyd, J., Theraulaz, G., and Bonabeau, E. (2001). Self-Organization in Biological Systems (Princeton University Press).

12. Kennedy, P., Baron, G., Qiu, B., Freitak, D., Helanterä, H., Hunt, E.R., Manfredini, F., O'Shea-Wheller, T., Patalano, S., Pull, C.D., et al. (2017). Deconstructing superorganisms and societies to address big questions in biology. Trends Ecol. Evol. *32*, 861–872. https://doi.org/10.1016/j.tree.2017.08.004.
13. Hölldobler, B., and Wilson, E.O. (2009). The Superorganism: The Beauty, Elegance, and Strangeness of Insect Societies (WW Norton & Company).
14. Dawkins, R. (1982). The Extended Phenotype (Oxford University Press).
15. Turner, J.S. (2000). The Extended Organism: The Physiology of Animal-Built Structures (Harvard University Press).
16. Seeley, T.D., and Morse, R.A. (1976). The nest of the honey bee (Apis mellifera L.). Insectes Soc. *23*, 495–512. https://doi.org/10.1007/BF02223477.
17. Smith, M.L. (2021). Nest Structure: Honey Bees. Encycl. Soc. Insects, 626–632. https://doi.org/10.1007/978-3-319-90306-4_85-1.
18. Camazine, S. (1991). Self-organizing pattern formation on the combs of honey bee colonies. Behav. Ecol. Sociobiol. *28*, 61–76. https://doi.org/10.1007/BF00172140.
19. Seeley, T.D. (1989). The Honey Bee Colony as a Superorganism. Am. Sci. *77*, 546–553.
20. Jones, J.C., Myerscough, M.R., Graham, S., and Oldroyd, B.P. (2004). Honey Bee Nest Thermoregulation: Diversity Promotes Stability. Science *305*, 402–404. https://doi.org/10.1126/science.1096340.
21. Peters, J.M., Peleg, O., and Mahadevan, L. (2019). Collective ventilation in honeybee nests. J. R. Soc. Interface. *16*, 20180561. https://doi.org/10.1098/rsif.2018.0561.
22. Seeley, T.D. (1982). Adaptive significance of the age polyethism schedule in honeybee colonies. Behav. Ecol. Sociobiol. *11*, 287–293. https://doi.org/10.1007/BF00299306.
23. Smith, M.L. (2018). Queenless honey bees build infrastructure for direct reproduction until their new queen proves her worth. Evolution *72*, 2810–2817. https://doi.org/10.1111/evo.13628.
24. Tinbergen, N. (1963). On aims and methods of Ethology. Zeigschrift fur Tierpsychologie *20*, 410–433.
25. Marting, P.R., Koger, B., and Smith, M.L. (2023). Manipulating nest architecture reveals three-dimensional building strategies and colony resilience in honeybees. Proc. Biol. Sci. *290*, 20222565. https://doi.org/10.1098/rspb.2022.2565.
26. Humphrey, J.A.C., and Dykes, E.S. (2008). Thermal energy conduction in a honey bee comb due to cell-heating bees. J. Theor. Biol. *250*, 194–208. https://doi.org/10.1016/j.jtbi.2007.09.026.
27. Nieh, J.C., and Tautz, J. (2000). Behaviour-locked signal analysis reveals weak 200–300 Hz comb vibrations during the honeybee waggle dance. J. Exp. Biol. *203*, 1573–1579.
28. Kleinhenz, M., Bujok, B., Fuchs, S., and Tautz, J. (2003). Hot bees in empty broodnest cells: Heating from within. J. Exp. Biol. *206*, 4217–4231. https://doi.org/10.1242/jeb.00680.
29. Jones, J.C., Helliwell, P., Beekman, M., Maleszka, R., and Oldroyd, B.P. (2005). The effects of rearing temperature on developmental stability and learning and memory in the honey bee, Apis mellifera. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. *191*, 1121–1129. https://doi.org/10.1007/s00359-005-0035-z.
30. Montovan, K.J., Karst, N., Jones, L.E., and Seeley, T.D. (2013). Local behavioral rules sustain the cell allocation pattern in the combs of honey bee colonies (Apis mellifera). J. Theor. Biol. *336*, 75–86. https://doi.org/10.1016/j.jtbi.2013.07.010.
31. Spemann, H., and Mangold, H. (1924). über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. Arch. für Mikroskopische Anat. und Entwicklungsmechanik *100*, 599–638. https://doi.org/10.1007/BF02108133.
32. Tautz, J., Maier, S., Groh, C., Rössler, W., and Brockmann, A. (2003). Behavioral performance in adult honey bees is influenced by the temperature experienced during their pupal development. Proc. Natl. Acad. Sci. USA *100*, 7343–7347. https://doi.org/10.1073/pnas.1232346100.
33. Seeley, T.D. (1995). The Wisdom of the Hive: the Social Physiology of Honey Bee Colonies (Harvard University Press).
34. Oldroyd, B.P., and Wongsiri, S. (2006). Asian Honey Bees: Biology, Conservation, and Human Interactions (Harvard University Press).
35. Johnson, B.R. (2009). Pattern formation on the combs of honeybees: Increasing fitness by coupling self-organization with templates. Proc. Biol. Sci. *276*, 255–261. https://doi.org/10.1098/rspb.2008.0793.
36. Tschinkel, W.R. (2017). Do Florida harvester ant colonies (Pogonomyrmex badius) have a nest architecture "plan?". Ecology *98*, 1176–1178. https://doi.org/10.1002/ecy.1739.
37. Smith, M.L., Loope, K.J., Chuttong, B., Dobelmann, J., Makinson, J.C., Saga, T., Petersen, K.H., and Napp, N. (2023). Honey bees and social wasps reach convergent architectural solutions to nest- building problems. PLoS Biol. *21*, e3002211. https://doi.org/10.1371/journal.pbio.3002211.
38. Peters, R.S., Krogmann, L., Mayer, C., Donath, A., Gunkel, S., Meusemann, K., Kozlov, A., Podsiadlowski, L., Petersen, M., Lanfear, R., et al. (2017). Evolutionary History of the Hymenoptera. Curr. Biol. *27*, 1013–1018. https://doi.org/10.1016/j.cub.2017.01.027.
39. Hales, T.C. (2001). The honeycomb conjecture. Discrete Comput. Geom. *25*, 1–22. https://doi.org/10.1007/s004540010071.
40. Ostwald, M.M., Lyman, B.R., Shaffer, Z., and Fewell, J.H. (2020). Temporal and spatial dynamics of carpenter bee sociality revealed by CT imaging. Insectes Soc. *67*, 203–212. https://doi.org/10.1007/s00040-020-00761-w.
41. Ostwald, M.M., Fox, T.P., Harrison, J.F., and Fewell, J.H. (2021). Social consequences of energetically costly nest construction in a facultatively social bee. Proc. Biol. Sci. *288*, 20210033.
42. Friedman, M. (2008). The evolutionary origin of flatfish asymmetry. Nature *454*, 209–212. https://doi.org/10.1038/nature07108.
43. Johnston, I.G., Dingle, K., Greenbury, S.F., Camargo, C.Q., Doye, J.P.K., Ahnert, S.E., and Louis, A.A. (2022). Symmetry and simplicity spontaneously emerge from the algorithmic nature of evolution. Proc. Natl. Acad. Sci. USA *119*, e2113883119. https://doi.org/10.1073/pnas.2113883119.
44. Phillips, A. (2020). Colored strip patterns and front-back symmetry in warp-faced pre-Columbian textiles. Leonardo *53*, 304–308. https://doi.org/10.1162/leon_a_01691.
45. Kidger, M.J. (2001). Fundamental Optical Design (SPIE Press).
46. Turing, A. (1953). The chemical basis of morphogenesis. Philos. Trans. R. Soc. Lond. B *237*, 37–72.
47. Heger, P., Zheng, W., Rottmann, A., Panfilio, K.A., and Wiehe, T. (2020). The genetic factors of bilaterian evolution. eLife *9*, 1–45. https://doi.org/10.7554/eLife.45530.
48. Smith, M.L., Ostwald, M.M., and Seeley, T.D. (2016). Honey bee sociometry: tracking honey bee colonies and their nest contents from colony founding until death. Insectes Soc. *63*, 553–563. https://doi.org/10.1007/s00040-016-0499-6.
49. Otis, G.W. (1982). Weights of worker honeybees in swarms. J. Apic. Res. *21*, 88–92. https://doi.org/10.1080/00218839.1982.11100520.
50. Martin, H., and Lindauer, M. (1966). Sinnesphysiologische Leistungen beim Wabenbau der Honigbiene. Z Vergl Physiol. *53*, 372–404. https://doi.org/10.1007/BF00298103.
51. Smith, M.L., Napp, N., and Petersen, K.H. (2021). Imperfect comb construction reveals the architectural abilities of honeybees. Proc. Natl. Acad. Sci. USA *118*, 1–8. https://doi.org/10.1073/pnas.2103605118.
52. Nouvian, M., Reinhard, J., and Giurfa, M. (2016). The defensive response of the honeybee Apis mellifera. J. Exp. Biol. *219*, 3505–3517. https://doi.org/10.1242/jeb.143016.
53. Chen, L.C., Zhu, Y., Papandreou, G., Schroff, F., and Adam, H. (2018). Encoder-Decoder with Atrous Separable Convolution for Semantic Image Segmentation. Proc. Eur. Conf. Comput. Vis. 801–818.
54. TorchVision maintainers and Contributors (2016). TorchVision: PyTorch's Computer Vision library at GitHub.

55. Deng, J., Dong, W., Socher, R., Li, L.-J., Li, K., and Fei-Fei, L. (2009). ImageNet: A large-scale hierarchical image database. IEEE Conf. Comput. Vis. Pattern Recognit. 248–255. https://doi.org/10.1109/CVPR.2009.5206848.

56. Zhao, X., Vemulapalli, R., Mansfield, P.A., Gong, B., Green, B., Shapira, L., and Wu, Y. (2021). Contrastive Learning for Label Efficient Semantic Segmentation. Proc. IEEE/CVF Int. Conf. Comput. Vis. 10603–10613.

57. Lin, T.-Y., Goyal, P., Girshick, R., He, K., and Dollar, P. (2017). Focal Loss for Dense Object Detection. Proc. IEEE Int. Conf. Comput. Vis. 2980–2988.

58. Bates, D., Mächler, M., Bolker, B.M., and Walker, S.C. (2015). Fitting linear mixed-effects models using lme4. J. Stat. Softw. *67*, 1–48. https://doi.org/10.18637/jss.v067.i01.

59. Lenth, R.V. (2021). emmeans: Estimated marginal means, aka least-squares means.

60. Lewis, F., Butler, A., and Gilbert, L. (2011). A unified approach to model selection using the likelihood ratio test. Methods Ecol. Evol. *2*, 155–162. https://doi.org/10.1111/j.2041-210X.2010.00063.x.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Python version 3.9.4	Python Software Foundation	https://www.python.org/
R version 4.0.2	R Core Team	https://www.r-project.org/
Symmetry versus Random Match Procedure (SVRM)	This paper	STAR Methods
Other		
Nest maps	This paper	supplemental PDF

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

This study examines the nests built by colonies of honey bees (genus *Apis*).

A. mellifera: Nest maps of *A. mellifera* were obtained from colonies housed in large observation hives in Ithaca, NY (2013) and Auburn, AL (2021). Experimental manipulations (one-sided observation hives, heat pad trials, functional importance of 2-sided nests, three-dimensional symmetry) were performed on colonies located in Auburn, AL. For further details on colony size and source, please see method details below.

A. florea, *A. andreniformis*, and *A. dorsata*: Nest maps of these three species of *Apis* were obtained from photographs of wild nests collected in Thailand, from the Mae Rim district of Chiang Mai (*A. florea*), the Tatako district of Nakornsawan (*A. andreniformis*), and from the Sanpathong district of Chiang Mai (*A. dorsata*).

METHOD DETAILS

Natural-comb observation hives

To detect potential symmetry in undisturbed nests containing naturally-built combs, we installed colonies into large observation hives and tracked their nest contents as the colonies grew and developed. Each colony began as an artificial swarm, containing 11,200 ± 900 workers and a mated queen (worker population determined by weight, 7700 workers = 1 kg;[49]). We fed the swarms a 1:1 (v:v) sucrose solution *ad libitum* for 72–96 h before installation. The observation hives (8895 x 100 cm: Ithaca NY; 85 x 114 cm: Auburn AL) contained no comb or wax foundation, so that each colony was free to build their combs and stockpile their nest as they wished. A total of six colonies were tracked: four at the Liddell Field Station of Cornell University in Ithaca, NY (42°27'36.0'' N, 76°26'42.0'' W) installed between 6-10 July 2012 (further details in Smith et al.[48]), and two at the Smith Bee Lab of Auburn University in Auburn, AL (32°40'26.6'' N, 85°30'43.5'' W), installed on 4 April 2021.

To extract maps of the colony's nest contents, we traced the combs and their contents onto clear polyester sheets (nest contents, for both worker and drone comb: honey/nectar, pollen, brood – eggs/larvae/pupae, empty comb). The plastic sheets were then scanned using an architectural scanner, the maps were digitized using Adobe Illustrator, and down-sampled to 100 x 100 pixels (1 x 1 cm per pixel; ca. 2 x 2 worker cells[50,51]). In these maps, each comb content type has a designated pixel value. Colonies in Ithaca, NY were mapped once per month as mature colonies in their 2nd year: April – Sept 2013; colonies in Auburn, AL were mapped every 10 days as founding colonies in their 1st year: 4 April 2021 – 1 Dec 2021. In total, this generated 148 maps of natural-comb colonies living in observation hives.

These maps were used to explore potential axes of nest symmetry in the overall nest, and how symmetry changes over time. We first tested each axis (x, y, front-back) to see which, if any, were symmetrical (i.e., if the contents of the nest were symmetrical along a given axis). We define symmetry as the proportion of the nest whose nest contents exactly match the contents on the other side of the axis. For all tests of symmetry, unless otherwise specified, we employed a linear mixed-model (LMM) with the colony as a random effect to control for correlation across responses (given the image size of each nest, 100 x 100 pixels = 10^4 observations, we assume that the proportions representing symmetry can be modelled through a normal distribution). We used the LMM to test the significance of different effects and then, if significant, we report the marginal average effects and standard deviations of each (see statistical analyses).

For a given nest map, symmetry along the x- and y-axes is calculated by inverting the same map along that axis, and summing the number of pixels that overlap between the original map and the inverted map (symmetry = pixels that overlap / total pixels). To account for the nest shape, the x- and y-axes are drawn to bisect the nest vertically (x) and horizontally (y), such that the number of pixels on either side of the axis matches (i.e., the axis bisects the nest, not the cavity space). To calculate symmetry along the front-back axis, pixel overlap is calculated between the focal map and a map of the comb's backside (i.e., same colony, same date, opposite side of comb).

Apparatus: one-sided observation hives

Natural comb is double-sided, to get from one side of the comb to the other, workers pass around the edges, or via peripheral galleries.[16] To restrict colonies to a single side of comb, we built one-sided observation hives (Figure 2A). This modified version of an observation hive allows colonies to initiate, build, and stockpile a nest as they wish, but the plastic foundation prevents workers from walking or chewing through to the other side, thereby creating a one-sided nest. To form a single planar surface for the workers to build cells upon, the plastic foundation is coated with a thin layer of beeswax and slid into a wooden frame (84 x 41 cm) that holds four pieces of plastic foundation (21.2 x 42.5 x 0.2 cm; Betterbee, Greenwich, NY). The wooden frame is then stabilized between two pieces of glass, with 2 cm between the plastic foundation and the glass on either side; sufficient space for a colony to build one-sided comb upon the plastic foundation. In some experiments, we allowed the colony to access both sides of the one-sided observation hive, by clipping the corners of the plastic foundation (2 cm^2). This created peripheral galleries which allowed individuals to freely pass from one side of the observation hive to the other. To provide colonies with an entrance so that workers could forage outside, we drilled a 3.5 cm diameter hole into the lower corner of the glass; a second hole provided the colony with access to a sucrose-solution feeder (1:1, by volume) to standardize nectar availability across colonies. All colonies were kept in the dark throughout the experiment, to mimic natural nest conditions.

Assaying whether independent colonies create symmetrical nests

In this experiment, we tested whether workers needed access to both sides of the nest to generate symmetry, and whether independent colonies living on either side of the observation hive would build nests that were mirror-images of one another. Each one-sided observation hive was stocked with either: (a) one colony containing 8000 workers, a queen, and access to both sides of the nest, or (b) two colonies, each containing 4000 workers and a queen, but restricted to one side of the nest (i.e., the one-sided observation hive houses two colonies living on either side of the plastic foundation).

All colonies were started as swarms and fed a 1:1 (v:v) sucrose solution *ad libitum* for 72–96 h before installation. We performed two rounds of this experiment. In the first round, colonies were installed on 11 April 2022, and mapped after 18 days, on 29 April 2022 (three observation hives containing 8000-worker colonies; three observation hives containing pairs of 4000-worker colonies; nine colonies total). In the second round, colonies were installed on 10 May 2022, and mapped after 15 days, on 25 May 2022 (three observation hives containing 8000-worker colonies; two observation hives containing pairs of 4000-worker colonies; seven colonies total). When installing pairs of 4000-worker colonies (one colony on either side of the observation hive), the two colonies were installed within 10 minutes of each other.

Nest contents on both sides of each observation hive were mapped and processed, as detailed above (honey, nectar, pollen, brood – eggs/larvae, brood – pupae, empty comb, blank space). Blank space was defined as locations where the workers did not form hexagonal cells upon the plastic foundation; empty comb was defined as locations where workers did form hexagonal cells, but the cells were empty. To prevent unintentional bias, the same person did not map both sides of an observation hive; each person was blind to the nest contents on the other side. In total, these experiments generated 22 nest maps: 12 maps from 8000-worker colonies (six colonies, two sides per colony); 10 maps from 4000-worker colonies (ten colonies, one side per colony). Nest maps were down-sampled to 84 x 41 pixels (1 x 1 cm per pixel; 2 x 2 worker cells[50,51]).

To confirm that the 4000-worker colonies living on either side of the one-sided observation hives remained independent after installation, we performed a survival analysis on marked foragers (see also Figure S1). At each colony's entrance, we captured 40+ foragers and marked the tip of their abdomens with a colony-unique paint color (POSCA, Mitsubishi Pencil Co., Japan). We then split the group: half were reintroduced to their own colony, and half were reintroduced to the colony on the other side of the observation hive. Colonies are hostile to non-resident conspecifics,[52] so if the colonies are independent entities, then higher survival is predicted for marked foragers that are reintroduced to the same colony from which they were collected (and lower survival for marked foragers introduced to the colony on the other side of the observation hive). All foragers were reintroduced via the feeder hole, and so they were in direct contact with the colony (i.e., marked foragers were not placed at the colony entrance). The next night, we inspected each colony and counted the number of marked bees within. We then calculated the survival probability for marked foragers that were reintroduced to their own colony (residents), versus marked foragers that were reintroduced to the colony on the other side of the observation hive (non-residents).

Foragers reintroduced to their resident colony had significantly higher survival rates than foragers introduced to the other colony (resident survival: 95 ± 28%; non-resident: 3 ± 3%; t-test with unequal variance; $P < 0.0001$; Figure S1). Note that survival rates can exceed 100% when marked foragers manage to depart from the non-resident colony and return to their resident colony via the nest entrance. Therefore, despite their proximity, the two colonies on either side of the one-sided observation hive remained independent entities.

Heat-pad experiment

In this experiment, we tested whether temperature influences how colonies organize their nests. We randomly placed a heating pad (15 x 20 cm; Zoo Med Labs Inc., CA) on one side of each one-sided observation hive and maintained it at 36°C (digital thermostat controller, BN-LINK, CA). On the other side, we installed a colony (4000 workers, a single queen, swarms prepared as detailed above). Six colonies were installed into observation hives on 20 June 2022, and mapped after 10 days, on 29 June 2022. When mapping nests, the observer was blind to the location of the heating pad, which was only revealed after the nest contents had been transcribed.

Assaying the functional importance of living in a 2-sided nest

In this experiment, we tested whether living in a 1-sided versus 2-sided nest has an impact on a colony's ability to grow, stockpile, and thermoregulate their nest (See also Figures S2 and S6).

For the 1-sided colonies, we kept the one-sided observation hives as described above, installing a single colony into one side of the observation hive, and leaving the other side empty (glass still covered both sides of the observation hive). For the two-sided colonies, we modified the wooden frame to hold two pieces of plastic foundation (42 x 41 cm) instead of four (84 x 41 cm; as in Figure 2A). To allow the colony free access to both sides of the plastic foundation, we clipped 2 cm corners from the plastic foundation in the 2-sided colonies. Therefore, the colonies in the 1-sided and 2-sided nests had the same total area upon which they could build their nest. Colonies were standardized across treatment groups (4000 workers, a single queen, swarms prepared as detailed above). To standardize access to the outside environment, each colony only had a single entrance hole available (i.e., in the 2-sided nests, only one side had an entrance tunnel attached).

To determine if colonies living in 1-sided versus 2-sided nests had different temperature profiles, we installed thermocouples into the brood nests of each colony (one thermocouple for each one-sided nest, one thermocouple for each side of each two-sided nest). Given that we would not know where the brood nest would be located upon installing the colonies, we opened each observation hive after three days to position the thermocouple. HOBO data loggers recorded the temperature of the K-type thermocouples once per minute (Onset, MA).

We performed two rounds of this experiment. In the first round, colonies were installed on 13 July 2022, and mapped after 10 days, on 22 July 2022 (four one-sided colonies; two two-sided colonies; six colonies total). In the second round, colonies were installed on 23 Aug 2022, and mapped after 10 days, on 1 Sept 2022 (four one-sided colonies; two two-sided colonies; six colonies total). In total, these experiments generated 16 nest maps: eight maps from one-sided colonies (eight colonies, one side per colony); eight maps from two-sided colonies (four colonies, two sides per colony). One-sided colony maps were down-sampled to 84 x 41 pixels; two-sided colony maps were down-sampled to 42 x 41 pixels (1 x 1 cm per pixel; 2 x 2 worker cells[50,51]).

Nest symmetry in three-dimensional A. mellifera nests

Apis mellifera colonies build nests with multiple parallel combs in a cavity. To determine whether nest symmetry persists in the combs of a three-dimensional nest, we installed six *A. mellifera* colonies into bee boxes and allowed each colony to build a nest. Each bee box (Langstroth deep: 47 x 38 x 25 cm), contained 10 wooden bee frames (43 x 20 cm) for bees to build their comb upon. Frames were oriented perpendicular to the south-facing entrance (frame 1: furthest from entrance, frame 10: closest to nest entrance). Frames had no wire supports or plastic/wax foundation, so workers were free to build and stockpile their nest as they wished. Each colony was initiated as a swarm, with 1.4kg of bees (ca. 10,700 workers), and a naturally mated queen (Gardner Apiaries, Baxley, GA). Colonies were fed a 1:1 (by volume) sucrose solution for 3 days prior to installation on 4 April 2021 at Auburn University (32° 40' 26.62'' N, 85° 30' 43.55'' W).

These colonies were repeatedly photographed for a separate experiment, which examined nest growth, but not comb contents (for further details, see "control treatment" in[25]). Each side of each comb was photographed in the apiary using a portable photography rig (348 x 66 x 44 cm) and a Nikon Z50 camera (aperture = f7.1, shutter speed = 1/80s, focal length = 135mm). To determine whether workers were stockpiling their combs symmetrically in these naturally-built three-dimensional nests, we used the comb images taken 22 days after nest initiation (26 April 2021) to allow colonies to complete one full worker-brood cycle. In total, these six colonies generated 48 combs (6-9 combs per colony) which generated 96 nest maps (two maps per comb).

Automated classification of comb contents in three-dimensional A. mellifera nests

We trained a convolutional neural network to automatically classify image pixels as belonging to one of many possible nest content classes, including: honey, nectar, pollen, egg, larvae, pupae, and empty comb. To align with the experiments described above, we combined individual classes into the following four groups: honey/nectar, pollen, brood (egg/larvae/pupae), and empty comb. We used DeepLabV3+ with a resnext50_32x4d backbone, as implemented by torchvision, with initial weights learned from the ImageNet dataset.[53–55] The annotated training set consisted of 1204 comb image crops and the validation set consisted of 322 annotated comb image crops. Image crops ranged in size from 806 x 802 pixels to 1795 x 1387 pixels although the majority were 900 x 900 pixels. Every pixel in every annotated crop was labelled by a human labeller. Following Zhao et al., we first used a contrastive loss pretraining regime to set initial model weights.[56] After pretraining, we trained our semantic segmentation model with focal loss using a gamma value of 1.[57] For both the pretraining and training stage, we used the same image augmentation regime: random 736 x 736 image crops, random vertical and horizontal flipping, random brightness and contrast scaling, and random blurring. All images were normalized to the ImageNet mean and standard deviation. The model was trained with stochastic gradient descent with an initial learning rate of 0.05 and a momentum value of 0.5. The learning rate was decreased by a factor of 10 if 80 epochs passed since the last minimum recoded loss value on the validation set. The model with the lowest validation loss was used. After training, each whole uncropped comb image was passed through the model. The class with the highest predicted probability was used as the label for each pixel in each comb image.

After automated processing, each image was verified by a human to ensure accurate labelling. Beyond human visual verification, to estimate overall model performance, we calculated a range of metrics. We first calculated the overall model accuracy by dividing the number of correctly labelled pixels of the four content groups in the validation images by the total number of pixels of those groups in the validation images. This overall model accuracy was 90.3%. Additionally, to ensure model performance for each class was

acceptable, for each content group we also calculated recall (correctly predicted pixels of a class / actual number of pixels of that class), precision (correctly predicted pixels of a class / all predicted pixels of a class), and IOU (intersection over union of the predictions and ground truth for a class).

Performance of trained model on the human annotated validation set

Metric	Honey/nectar	Pollen	Brood (egg/larvae/pupae)	Empty comb
Recall	0.89	0.83	0.82	0.94
Precision	0.94	0.89	0.89	0.89
IOU	0.85	0.76	0.75	0.84

Nest symmetry in the single-comb nests of A. florea, A. andreniformis, and A. dorsata

Wild nests of *A. florea* (n=5) were collected from the Mae Rim district of Chiang Mai,

A. andreniformis (n=4) from the Tatako district of Nakornsawan, and *A. dorsata* (n=1) from the Sanpathong district of Chiang Mai, Thailand. Each nest was photographed from both sides, in the lab, using an Olympus E-M10 camera. Due to some nests being slightly damaged in transport, we could only reliably identify cells across all images that contained a wax capping (honey) or paper capping (brood – pupae), and so defined all other cells as empty (i.e., an "empty" cell might contain: egg, larvae, pollen, or nectar). Worker and drone cells could be differentiated by their cell size in *A. florea* and *A. andreniformis*, but these cell-types are indistinguishable in *A. dorsata*. Nest images were mapped, digitized, and down-sampled to 80 x 45 pixels (ca. 1-2 worker cells per pixel wall-length). To prevent unintentional bias, the same person did not map both sides of a nest, and each person was blind to the nest contents on the other side. In total, we collected 10 nests which generated 20 nest maps (two maps per colony).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses

Statistical analyses were performed in Python version 3.9.4 (Python Software Foundation) and R version 4.0.2 (R Core Team).

Symmetry versus Random Match Procedure (SVRM)

To test if nests were more symmetrical than a random matching of comb contents, let us denote p as the observed proportion of matching pixels between two nest images, and p_0 as the expected proportion of matching pixels if the two nest structures were independent of each other. More specifically, let X_1 and X_2 represent the respective ordered vectors of n pixel values for two images of the same dimensions (i.e., a vector of n pixels for the first image and of n pixels for the second image, ordered in the same way according to their positions in the respective images). To simplify calculations, nest maps were flipped along the y-axis to align their overlap, such that the nest entrance of the natural comb observation hives was always located in the lower right corner). Moreover, let Y represent a specific comb content of interest (e.g., honey, nectar, pollen), then the proportion of matching pixels is computed as:

$$p = \frac{1}{n}\sum_{i=1}^{n} 1_{\{X_1^i = Y \text{ and } X_2^i = Y\}},$$

where X^i represents the i^{th} pixel of X and $1_{\{Z\}}$ is the indicator function which is equal to one if z is true and zero otherwise. In other words, we compute the proportion of pixels that correspond to content Y in the same position for both images. In particular, if computing the proportion of total matching pixels (i.e., not focusing on a specific comb content), then the proportion of matching pixels is computed as:

$$p = \frac{1}{n}\sum_{i=1}^{n} 1_{\{X_1^i = X_2^i\}},$$

I.e., the proportion of pixels that match in content (hence not only a specific content Y) in the same position for both images. Moreover, we define:

$$\pi_j = \frac{1}{n}\sum_{i=1}^{n} 1_{\{X_j^i = Y\}},$$

as the (marginal) proportion of pixels corresponding to comb content Y for image j (j = 1,2). Alternatively, if we focus on the total proportion of matching pixels, then π_j represents the proportion of comb contents in image j that are present in both images (j = 1,2). As a consequence, for each comparison we can compute $p_0 = \pi_1 \times \pi_2$ based on the assumption of independence between images.

Using this notation, we formulate the following hypotheses:

$$H_0 : p = p_0$$
$$H_A : p > p_0$$

The logic of this test is that, if two nests are symmetrical, or close to symmetrical, the proportion of matching pixels p will be significantly higher than the proportion of matching pixels under the hypotheses of independence p_0 (i.e., random re-organization of the pixels in each nest). To test these hypotheses, whether focusing on a specific comb content Y or on the total content overlap, for each experimental condition we devised the following procedure based on the idea of the chi-squared test of independence:

1. For each pair of nest images (i.e., the vectors X_1 and X_2), we computed their respective proportions of comb content (i.e., π_1 and π_2).
2. We then computed the expected number of matching pixels for each type of comb content if the two nests were independent from each other (i.e., $p_0 = \pi_1 \times \pi_2$).
3. We then computed the proportion of matching contents p on the observed nest images.
4. We finally ran the z-test for difference of proportions using the above hypotheses to obtain a p-value.

Assuming we have a total of K images in an experimental condition of interest (e.g., natural comb observation hives), then by excluding the comparison of an image to itself (which would result in perfect symmetry), we have a total of comparisons given by:

$$m = \frac{K \times (K - 1)}{2}.$$

Therefore, the above four-step procedure is run m times. Once this procedure was run on all possible combinations of nest images within an experimental condition, we used a Bonferroni correction for m multiple comparisons (i.e., significance level given by $0.05/m$) to select the combinations whose observed matching pixels were significantly higher than the matching pixels under random re-organization. We will refer to the images whose p-values are below the Bonferroni-corrected significance level as "detected symmetries" whereas the others will be referred to as "undetected symmetries". If the hypothesis of symmetry is valid, we would expect the highest degree of symmetry to occur within the same date and colony. Therefore, we ran a final test to understand if the proportion of expected symmetrical nests (i.e., combinations from the same colony and the same date) among the "detected symmetries" (which we denote as p_s) was higher than the proportion of expected symmetrical nests among the "undetected symmetries" (which we denote as p_a). Hence, the set of hypotheses are:

- $H_0 : p_s = p_a$
- $H_A : p_s > p_a$

We again used a z-test for proportions with a directed alternative hypothesis (i.e., higher proportion among significant combinations) to deliver the final SVRM p-value reported in the manuscript.

We run the above procedure and obtain the final SVRM p-value for all experimental conditions and, for each of these, for all contents of interest: overall symmetry, honey/nectar, brood, pollen, empty comb.

Linear Mixed Effects Models (LMM)

Symmetry was calculated as the proportion of comb contents that exactly matched between two nest maps, or along a given axis (i.e., the number of pixels that have the same value; 1 pixel = 1 x 1 cm; pixel values denote comb content type). To differentiate between potential axes of symmetry (x-, y-, and front-back axis), first versus second year colonies, treatment groups (e.g., 8000-worker colonies versus paired 4000-worker colonies) and temporal/colony specificity, we used an LMM, with symmetry as the response variable and colony ID as the random factor.[58,59] Best-fit models (with or without explanatory variables) were determined using the likelihood ratio test.[60] Pairwise comparisons were then performed on the best-fit model, using the emmeans package and a 95% confidence interval.

Chi-Squared Test

We used a chi-squared test to determine whether nests were universally interchangeable (8k and 4k nest maps, Figures 2 and S5; comparing *A. florea*, *A. andreniformis*, and *A. dorsata* nest maps, Figures 4 and S8), and to compare pollen storage between the entrance side and non-entrance side of the 2-sided nests (Figure S6).

Z-Test with Bonferroni Correction

We used a z-test to compare nest contents between the 1-sided and 2-sided nests (Figure S2). To account for multiple comparisons (honey/nectar, pollen, total brood, empty comb, blank space), we applied a Bonferroni correction (i.e., $\alpha = 0.05/5 = 0.01$).

Fligner-Killeen Test and F-Test

To differentiate between the temperature profiles of 1-sided and 2-sided nests, we first used a Fligner-Killeen test of homogeneity of variances to determine if there was a difference in the variability of temperatures between 1-sided and 2-sided nests, with no assumption on the distribution on the temperatures. If significant, having confirmed the approximate normal distribution of the temperatures in each group, we used an F-test for homogeneity of variances with a directed alternative hypothesis (i.e., the variance of temperatures for two-sided nests is smaller than that of one-sided nests).