Imperfect comb construction reveals the architectural abilities of honeybees

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Honeybees are renowned for their perfectly hexagonal honeycomb, hailed as the pinnacle of biological architecture for its ability to maximize storage area while minimizing building material. However, in natural nests, workers must regularly transition between different cell sizes, merge inconsistent combs, and optimize construction in constrained geometries. These spatial obstacles pose challenges to workers building perfect hexagons, but it is unknown to what extent workers act as architects versus simple automatons during these imperfect building scenarios. Using automated image analysis to extract the irregularities in natural comb building, we show that some building configurations are more difficult for the bees than others, and that workers overcome these challenges using a combination of building techniques, such as: intermediate-sized cells, regular motifs of irregular shapes, and gradual modifications of cell tilt. Remarkably, by anticipating these building challenges, workers achieve high-quality merges using limited local sensing, on par with analytical models that require global optimization. Unlike automatons building perfectly replicated hexagons, these building irregularities showcase the active role that workers take in shaping their nest and the true architectural abilities of honeybees.

Honeycomb is hailed as the pinnacle of biological architecture for its ability to maximize storage area while minimizing building material (1–4). In the 4th century, Pappus of Alexandria marveled at the “geometrical foresight” of the bees, while Darwin dubbed it “the most wonderful of all known instincts” (5, 6). To build a single cell, multiple bees must deposit, mold, and shape the precious wax; given that an unsupervised collective builds the structure, it is unknown whether each bee possess architectural abilities, or if they act as simple automatons (7–12).

Since the 1600s, hexagons were known to be a mathematically optimal solution, with examples such as stacked cylindrical columns, surface-minimizing soap bubbles, or rare geological features (13–16). This abiotic perspective led to work suggesting that perfect hexagons were mere byproducts of the wax properties ([17, 18]; but see ref. 19]), despite other organisms, like wasps, which build hexagons for the efficacy of honeybees as building agents, showed that perfect lattices, but this paper showcases them as similarly skilled architectures when fitting perfect lattices is impossible. Using automated image analysis, we quantified building challenges that bees face during natural construction and the methods they use to solve them. We found that workers preemptively change their building behavior in constrained geometries to make space for larger hexagonal cells, that irregular cell shapes come in regular combinations, and that bees change both tilt, size, and number of walls to meet different building challenges. Model comparisons, serving as upper and lower bounds for the efficacy of honeybees as building agents, showed that workers perform remarkably well given their lack of global sensing and central planning.
Fig. 1. An example of naturally built honeycomb, with arrows showing the five locations where workers had simultaneously initiated building; full sample sizes listed below (A). Bees build two types of comb: small-celled worker comb (B, i) and large-celled drone comb (B, ii). Histograms of cell areas (B, iii) and wall lengths (B, iv) show that the two cell types form distinct distributions (worker cells, red; drone cells, blue); the shaded areas in (B, iii) encompass 99.9% CI (shown also in C–F, ii). Worker comb: n = 11 colonies, 25 comb images, 43 instances, and 4,414 cells; drone comb: n = 11 colonies, 25 images, 39 instances, and 2,586 cells. Bees face two key challenges during comb building: transitioning from worker to drone-sized cells in a continuous piece of comb (C) and merging independent pieces of comb (D–F) (boxes in A; insets in C–F, i). Histogram of cell areas across the transition/merge (gray in C–F, i) as compared to perfect worker and drone cells (red and blue, respectively; random subsets from B, iii). Bees adjust cell areas (C–F, ii) and wall lengths (C–F, iii) as they build across the transition/merge (vertical dotted line in C–F, i–iii). Worker-to-drone cell transition: n = 7 colonies, 8 comb images, 17 instances, and 1,619 cells. Worker-to-drone cell merge: n = 9 colonies, 13 comb images, 24 instances, and 2,819 cells; drone-to-drone cell merge: n = 5 colonies, 13 comb images, 15 instances, and 2,309 cells; worker-to-worker cell merge: n = 8 colonies, 13 comb images, 23 instances, and 5,273 cells. Instances are defined as independent areas of interest (e.g., one comb image may have two instances of worker-to-worker cell merging). The black line and shading in C–F, ii and iii show mean ± SD and the data point colors denote number of walls in C–F, ii.
CI, worker cells: 22.5 to 29.6 mm in Fig. 1. Worker cells were smaller than drone cells; shaded boxes and black bars show the expected incidence when each cell type is picked independently according to its frequency of occurrence. The gray horizontal line shows expected incidence for a uniform distribution (full combinatorial sets shown in black dashes). The error bars show the full range of expected incidences following 10,000 random samples, with replacement.

Fig. 2. An example of honeycomb with cell centers marked and colored by number of walls (hexagons marked with small orange dots). Note that the analysis does not include cells within 20 mm of the outer perimeter due to image distortion and edge effects (A). Proportion of irregular-shaped cells, across comb transitions and merging (black bars denote 95% CI; B). Frequency of irregular-shape pairs (C), triplet clusters (D), and triplet lines (E). In C through E, the black dashes show the expected incidence when each cell type is picked independently according to its frequency of occurrence. The error bars show the full range of expected incidences following 10,000 random samples, with replacement. The gray horizontal line shows expected incidence for a uniform distribution (full combinatorial sets shown in SI Appendix, Fig. S3).

mellifera ligustica (23) (worker cells, n = 4,414: cell area 25.7 ± 0.9 mm², wall length 3.2 ± 0.1 mm, wall-to-wall length 5.4 ± 0.2 mm, and cell angles 120.0 ± 2.5°; drone cells, n = 2,586: cell area 37.5 ± 1.5 mm², wall length 3.8 ± 0.2 mm, wall-to-wall length 6.6 ± 0.2 mm, and cell angles 120.0 ± 3.2°; mean ± SD, Fig. 1A and B and SI Appendix, Fig. S1). For all metrics other than cell angles, worker cells were smaller than drone cells; shaded boxes in Fig. 1B, iii encompass 99.9% of cell areas, with no overlap (99.9 CI, worker cells: 22.5 to 29.6 mm²; drone cells: 32.0 to 44.8 mm²). The same red- and blue-shaded boxes are transposed and plotted in Fig. 1C–F, ii to show the distribution of these perfect cell areas.

To transition between the two different cell sizes within a single plane of comb, workers built intermediate-sized cells as they approached the transition by gradually increasing wall lengths (Fig. 1, i–iii). The idea of bees using intermediate-sized cells to perform worker-to-drone cell transitions was first proposed 200+ years ago (7); here we provide a quantitative description of this process and show that (1) workers do not have two fixed cell sizes, and (2) workers preemptively modify cells to accommodate the larger cells. Therefore, while drone cells are the first visible reproductive investment, these intermediate-sized cells are their architectural precursors.

Nest construction is a distributed process, and combs initiated in separate locations (arrows in Fig. 1A) must be merged to optimize space and structural stability. Merging can occur between any combination of cell types: worker cell with drone cell, drone cell with drone cell, or worker cell with worker cell (Fig. 1A and C–F, i).

When merging worker and drone cells, the bees built intermediate-sized cells along the merge line by gradually increasing the wall lengths (Fig. 1D, i–iii). This building behavior was similar to the worker-to-drone cell transition but with more variation at the merge line (Fig. 1C, ii and iii versus D, ii and iii).

During a drone-to-drone cell merge, workers increased the distribution of cell areas (Fig. 1E, i) but preferentially shifted toward smaller cells (Fig. 1E, i and ii). The mean wall length decreases at the merge and variation increases (Fig. 1E, iii). This drop indicates that when merging two pieces of drone comb, the cells are already at the upper limit of cell area, and the workers prefer to build smaller cells, which can still be used for rearing workers despite being surrounded by drone cells. Therefore, even when building reproductive cells, workers still use nonreproductive cells as an architectural contingency plan.

Workers do regulate the proportion of drone cells in the colony (32), and unlike when merging drone cells, we did not find that the workers used reproductive cells to accommodate the worker-to-worker cell merge. Here too, the distribution of cell areas increases at the merge line, and wall lengths become more variable (Fig. 1F, i–iii). However, unlike the drone-to-drone cell merge, during a worker-to-worker cell merge, the variation is equally distributed, with workers choosing to deviate both above and below their typical worker-sized cells (Fig. 1F, ii and iii versus E, ii and iii).

All the adjustments that workers made during comb transitions and merging occurred over an area 1 to 2 cells wide within 10 to 15 mm of either side of the transition/merge (Fig. 1C–F, i and ii). Given that a worker’s tarsi-tarsi span can easily cover 2 to 3 cells (hind leg: 8 mm; wall-to-wall cell length: 5.4 to 6.6 mm; SI Appendix, Fig. S1), workers likely use physical contact with the growing comb to determine which building techniques to employ. Comparing transitions (Fig. 1C) and merging (Fig. 1D–F) shows the difficulty of unifying independent pieces of comb and also an additional building technique. Given the distribution of
“perfect” cell areas (Fig. 1B), only 7 of 1,619 cells (0.4%) fell outside of these bounds during the worker-to-drone cell transition as compared to 63 of 2,819 cells (2.2%) during a worker-to-drone cell merge (generalized linear mixed-effects models [GLMM]; \( P = 0.001 \)). We found similar rates of these deviant cell sizes during the other merging types (drone-to-drone cell: 64 of 2,209 cells, 2.8%; worker-to-worker cell: 109 of 5,273 cells, 2.1%; GLMM + pairwise comparisons: merging drone-to-drone versus worker-to-worker versus worker-to-drone, \( P > 0.05 \), not significant; worker-to-drone cell transitions versus each of the three merge types, \( P < 0.005 \)). Therefore, the irregular-sized cells are a result of having to merge combs, not of merging combs with different cell sizes. Although some of these cells are still viable for brood rearing or honey storage, there is an upper limit of cell size for rearing drones (33), and miniscule cells are often filled with wax (SI Appendix, Fig. S2). Presumably, the selective pressure to build a nest quickly and simultaneously (34) outweighs the cost of the unusable cells that occur during comb merging.

In addition to building irregular-sized cells, the bees also built irregular shapes. Across all instances of comb transition and merging, 4.4% of the cells were nonhexagonal (\( n = 21 \) combs; total cells: 12,020; nonhexagonal cells: 534; color denotes number of walls in Fig. 1 C–F, ii and Fig. 2A). As expected, the bees built a mix of 4-, 5-, 7-, 8-, and 9-sided cells (30), but the bees preferentially built 5- and 7-sided cells, which together accounted for 92.7% of the nonhexagonal cells (4-sided cells: 31, 5.8%; 5-sided cells: 233, 43.6%; 7-sided cells: 262, 49.1%; 8-sided cells: 7, 1.3%; 9-sided cells: 1, 0.2%; Fig. 2B).

In our dataset, we observed 5- and 7-sided cells that were often paired, and 4-sided cells wedged between two 7-sided cells (Fig. 2A). Even after taking into account the incidence of irregular shapes, we still found a higher frequency of 5–7 and 4–7 pairs and an under-representation of 7–7, 5–5, and 4–5 pairs (\( P < 0.0001 \); Fig. 2C). We also found an over-representation of certain triplet combinations, such as 4–7-7 triplet clusters and linear sequences of 5–7–5 and 7–5–7 sided cells (\( P < 0.0001 \); Fig. 2D and E) as well as under-representations, such as 7–7–7 and 5–5–5 triplet clusters and linear sequences of 5–7–7 and 5–7–7–sided cells (\( P < 0.0001 \); Fig. 2D and E). This shows that bees not only have a preference for certain types of irregular shapes but also combinations of those irregular shapes. Curiously, this same motif, pentagons paired with heptagons, is found in graphene grain boundaries, where two sheets of hexagonal carbon lattice are merged (35–38). In graphene, these defects increase the material’s strength (35, 38), but whether these same benefits exist for honeycomb is unknown. This structural parallel marks a striking example of geometric convergence between molecular forces and collective nest building.

The difficulty of merging comb also depends on the cell’s relative position and tilt. Presumably, it is easier for the bees to merge two pieces of comb when the respective cell tilts are aligned because then the walls and vertices will not collide (see model with full parameter sweep for cell tilt and cell offset in SI Appendix, Fig. S5). To test this hypothesis, we first calculated the cell tilt for each hexagonal cell (Fig. 3A), binned each instance into quartiles based on the difference in cell tilt, and then plotted the proportion of nonhexagonal cells across the transition/merge (Fig. 3B). For comb transitions, all instances fell into the first quartile, showing that bees do not change the cell tilt as they transition from building worker- to drone-sized cells (Fig. 3B). When merging combs, however, the two pieces of comb are built independently, and so the difference in cell tilt can be greater. Across all types of cell transitions versus each of the three merge types, Fig. 3B. We also found a higher frequency of 5–7 and 4–7 pairs and an under-representation of 7–7, 5–5, and 4–5 pairs (\( P < 0.0001 \); Fig. 2C). We also found an over-representation of certain triplet combinations, such as 4–7-7 triplet clusters and linear sequences of 5–7–5 and 7–5–7 sided cells (\( P < 0.0001 \); Fig. 2D and E) as well as under-representations, such as 7–7–7 and 5–5–5 triplet clusters and linear sequences of 5–7–7 and 5–7–7–sided cells (\( P < 0.0001 \); Fig. 2D and E). This shows that bees not only have a preference for certain types of irregular shapes but also combinations of those irregular shapes. Curiously, this same motif, pentagons paired with heptagons, is found in graphene grain boundaries, where two sheets of hexagonal carbon lattice are merged (35–38). In graphene, these defects increase the material’s strength (35, 38), but whether these same benefits exist for honeycomb is unknown. This structural parallel marks a striking example of geometric convergence between molecular forces and collective nest building.

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merging, as the difference in cell tilt increases, so too does the proportion of nonhexagonal cells (Fig. 3B). Indeed, when the difference in cell tilt is the largest (fourth quartile), over 60% of the cells at the merge line are nonhexagonal, highlighting the importance of cell tilt during a merge.

Given that cells with similar tilts are easier to merge (i.e., require fewer nonhexagonal cells), we investigated whether bees adjust cell tilt to facilitate comb merging. In Fig. 3C, we plotted the cell tilt for each individual cell in four examples of worker-to-worker cell merging (total instances of worker-to-worker cell merging: 23). Remarkably, the data indicate that bees leverage the fact that combs with similar cell tilt are easier to merge—and they gradually change the cell tilt across the merge line to match the two independent pieces of comb (e.g., first quartile, Fig. 3C). Even when the difference in cell tilt was large, the bees built hexagonal cells at the merge line with intermediate cell tilt, thereby splitting the
difference between the two pieces of comb (e.g., third quartile, Fig. 3C). The bees effectively ‘roll’ the hexagonal cells into the gap when merging combs. If the tilt difference is small, these rolling cells can maintain their hexagonal shape, but when the tilt difference is large, the bees use nonhexagonal shapes to merge the combs.

Finally, to determine to what extent we could capture different aspects of the bee’s building behavior, we built two merge strategy models of differing complexity. The first model is purposefully static to show the limitations of a rigid merging strategy. In this ‘naive model’ only hexagonal cells are built, but they are allowed to overlap at the merge line by a small area, epsilon (Fig. 4 A and B). As epsilon increases, wasted space decreases, but to perform as well as the bees, cells would need to overlap between 8 and 16 mm\(^2\); 30 to 60% of a worker cell (Fig. 4D).

In the second model (the ‘global model’), hexagons are replicated until they reach the merge line without overlap. The leftover space is then used to compute a number of additional cells, after which all cell positions are optimized using virtual springs with rest lengths defined by the original comb. Finally, the new comb is computed as a Voronoi partition of the cell centers (Fig. 4 A-C, 1 and 6). Note that the model only optimizes the location of the cell centers, not the cell shapes. Nevertheless, the global model shows many similarities to the bees: nonhexagonal cells are built and at similar rates (Fig. 4E); merging instances that are difficult for the bees (i.e., required more irregular shapes) are also difficult for the model (Fig. 4F); when merging worker and drone cells, intermediate-sized hexagons are built, along with paired 5- and 7-sided cells (Fig. 4 C, 2 and G). Unlike the bees, the global model builds irregular cells that are within the bounds of worker-and-drone-sized cells (red and blue shading in Fig. 4G), so little space is wasted (Fig. 4D). This is likely the result of being able to adjust the cell centers along the entire merge line simultaneously, unlike the bees, whose wax cells are fixed once built.

The two models serve as an upper and lower bound for the efficacy of honeybees as building agents. Workers outperform the naive model by using irregular cell shapes, sizes, and tilts but underperform relative to the global model, likely because workers must optimize cells within constrained geometries. When faced with building tasks more complex than simple repetitive hexagons, both the global model and the bees arrive at similar strategies, including intermediate-sized cells and motifs of irregular shapes.

This rich repertoire of building behaviors suggests that there are cognitive processes behind the comb-building behavior of honeybees, not just hardwired instincts (12). While it is possible that self-organizing processes still play a role, future models must take into account the variety of cell shapes and sizes, not just the famously repetitive hexagons. By focusing on the challenges that are relevant to the honeybee’s natural history, at a per-cell basis, we show how architectural problems are overcome and the active role that workers take in shaping their nest.

Materials and Methods

Natural Comb Building. All experiments were performed using colonies of the Western honeybee, A. m. ligustica, headed by naturally mated queens sourced from Ithaca, NY, United States. On 6 July 2017, we provided empty wooden frames to seven honeybee colonies. The wooden frames (48 × 23 cm) provided space for the workers to build comb. None of the frames contained any embossed wax comb foundation or wire supports, so the workers were free to choose where, how much, and what type of comb to build. To promote comb building, we simulated a nectar flow by providing each colony with a 2-L 50:50 (vol:vol) sucrose solution feeder. Colonies were left undisturbed, except on July 13, 16, and 19, when we noted where the bees had begun to build on each frame. If a frame was filled with comb, we removed that frame from the colony. All frames were removed from the colonies by 25 July 2017. The frames were then cleared of any stored nectar and brood by separate colonies by placing the combs atop the inner cover of the colony. Once cleaned, frames were brought indoors for safekeeping until they were photographed.

These seven colonies built 18 frames of comb. To further supplement the dataset, we added five additional frames of comb that were built by workers in colonies under similar conditions (natural comb built upon empty frames without wax foundation or supporting wires). The total dataset, therefore, includes 23 frames of comb, which originated from 12 colonies.

For each comb, however, to establish the variation in worker- and drone-sized cells when workers have no barriers to building comb (i.e., no need to attach comb to a substrate or fit differently sized cells together).

Transitions are defined as transitions where workers transition from building worker-sized combs to drone-sized combs (see also ref. 2). For these areas of interest, we selected 30 mm on either side of the transition, which encompasses five to six worker-sized cells or four to five drone-sized cells. We picked transition areas manually to ensure that the 30-mm area on either side of the transition would not overlap with the edges of the comb or other areas of interest. "Transition" cells were used to determine how cells varied as the workers shifted from building worker-sized cells to building drone-sized cells (i.e., when workers fit different sized hexagons into a single comb).

Merging is defined as locations where workers merge two pieces of comb into one. Merging occurs because workers build comb simultaneously on different parts of the substrate, eventually joining them into a single plane of comb (2-plane alignment). For these areas of interest, we selected 30 mm on either side of the merge line, as was done for transitions. Instances of merging were further classified by the type of combs that are being merged: worker-to-drone cells, drone-to-drone cells, and worker-to-worker cells. Merging cells were used to determine how workers join cells that may differ in size, position, and tilt.

Automated Image Analysis of Comb Cells. To automatically detect individual cells and their vertices from the images, we first found the center of each cell by applying Adaptive Gaussian thresholding followed by Laplacian of the Gaussian blob detection. Then, the adjacent cell centers were connected by a line, and the gradient magnitude was calculated for all pixels along this line. The location of maximum gradient difference defined the center of the cell wall. Perpendicular lines were then drawn from the center of each cell wall, and where these lines intersect defined the vertices of the cell. The vertices were then connected to form the cell. To confirm that the cell centers and vertices matched the individual cells in the comb image, each image was visually inspected with the results overlaid and corrected, if necessary.

Extracting Cell Metrics and Data Analysis. For each cell, we extracted the following metrics: wall lengths, wall-to-wall length, interior cell angles, cell areas, and cell tilt. Wall lengths were calculated as the distance between

Photography Setup. To obtain high-quality images of the comb in a two-dimensional planar arrangement, we used a Canon EOS Rebel T6i camera (24.2 Megapixel CMOS APS-C sensor) with a long focal-length macro lens (100 mm f/2.8), controlled lighting, and a black felt background. The camera was mounted on a tripod 5 m from the comb to avoid distortion at the edges of the image. At this distance and camera resolution, 1 mm = 13.18 pixels.

Identifying Areas of Interest. In each photograph, we identified areas of interest, which fell into three types: "perfect comb," "transitions," and "merging." A given comb could have multiple instances of these three types of comb building areas of interest. "Perfect comb" is defined as areas of repeated worker- or drone-sized cells, more than 30 mm from the edges of the wooden frame, and more than 20 mm away from the edges of the comb, transitions, or merging areas. "Perfect comb" cells were used to establish the variation in worker- and drone-sized cells when workers have no barriers to building comb (i.e., no need to attach comb to a substrate or fit differently sized cells together).

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each vertex and its subsequent vertex. Wall-to-wall lengths (short diagonal) were calculated as the distance between the 0 to 2nd, 1 to 3rd, and 2 to 4th vertex. Cell angles (interior angle) were calculated for each vertex relative to its two neighboring vertices. Cell areas were calculated as the sum of the triangles that were formed by each pair of neighboring vertices and the center of the cell. Cell tilt (i.e., the orientation of the cell relative to the x-y plane) was calculated using the Kabasch Algorithm. For each hexagonal cell, we calculated the optimal rotation for a perfect, similarly sized, reference hexagon to align with the observed data and used that rotation angle as the plane. Cell tilt (i.e., the orientation of the cell relative to the x-y plane) was calculated using the Kabasch Algorithm. For each hexagonal cell, we calculated the optimal rotation for a perfect, similarly sized, reference hexagon to align with the observed data and used that rotation angle as the plane. Data processing and visualizations were done using the Python programming language (Python Software Foundation, version 3.6).

Statistical analyses were performed in R version 4.0.2 (39), with the packages stats, lme4, and emmeans (39–41). To test whether irregular cells varied depending on the type of comb-building challenge, we used GLMM with colony ID, frame number, and comb side as nested random factors. A null model was compared to a model containing the type of comb-building challenge as an explanatory variable (i.e., transition and three merge types). The best-fit model was determined using likelihood ratio tests (42). Pairwise comparisons with a Tukey adjustment were then used to determine which, if any, classifications were significantly different from one another (worker-to-drone cell transition, worker-to-worker cell merge, drone-to-drone cell merge, and worker-to-worker cell merge).

To test whether workers were building combinations of irregular cells (“motifs”), we first calculated the expected incidence of irregular pairs and triplets using the multinomial distribution, assuming independent sampling. We then tested whether the observed frequency was significantly different from the expected frequency using the prop.test and binom.test functions in the stats package (39), with a Bonferroni correction for repeated measures. To visualize the range of expected incidences, we randomly sampled irregular cells (with replacement) and recalculated expected incidences based on this random sample. Error bars shown in Fig. 2 C–E and SI Appendix, Fig. S5 show the full range of expected values based on 10,000 iterations.

Models of Comb Merging. To model merge regions, we approached the problem from the perspective of merging two, perfectly regular, hexagonal tilings. For each observed merge line, we fitted a perfect tiling to each side (outside of the transition region) by defining an origin and two non-orthogonal basis vectors for how cell centers should be placed in space. We fitted these three points (six parameters) to the observed data using nonlinear least squares for a patch of seven cells. After fitting the parameters, the two basis vectors were 30 degrees apart, and their length corresponded to the intercell spacing for the particular type of comb. Each merge line produced two such coordinate systems (Fig. 4A), which then allowed us to predict cell centers and vertices for regularly spaced cells. We used these observed coordinate systems to test two merge strategies of different complexity.

Naive Model. The first model was purely local and did not attempt to construct any nonideal cells. For each merge region, we filled the space with candidate cells until they intersected with the merge line (i.e., when the predicted cell would have vertices on either side of the merge line) (SI Appendix, Fig. S4). We built a set of these intersecting cells for each region and then iteratively added them in. They were allowed to be added only if they did not intersect any existing cells by more than epsilon. For each merge line, we performed this procedure in both set orders and then picked the one that resulted in more area covered by cells. When epsilon = 0, the result is a partial covering of space with perfect cells that do not intersect at all. As epsilon increases, more space is covered, but the cells have overlap.

The naive model can further be used as an indicator of the difficulty of merging particular combs given the rotation of the cells on either side, the horizontal and vertical displacement, and the orientation of the merge line. SI Appendix, Fig. S5 shows an example sweep over two of these parameters. With zero offset and perfect angular alignment, all space can be divided up into the preferred area distribution. With any angular difference, the potential for wasted space becomes significantly more difficult; however, the model indicates that the most difficult situation is when the combs are misaligned in a particular horizontal range, independent of the epsilon.

Global Optimization Model. The second model used global optimization to find a complete covering of space with possibly deformed cells. The approach has four steps (SI Appendix, Fig. S6):

1) The procedure starts with the output from the naive model above with (epsilon = 0) to find areas that could not be covered with perfectly regular cells. We then divided the leftover area by the area of a perfect cell to find the number, N, of additional cells that needed to be placed. Since the perfect cells on either side of the merge line could have different areas, we computed the number of added cells for both sides and then averaged and rounded the result.

2) We then iteratively added N cell centers by finding the points in the noncovered area that were maximally distant from all previously placed cell centers. We picked the furthest one and repeated this procedure N times. This gave us the right number of candidate cells and reasonable initial locations for optimization.

3) We optimized cell placement by placing virtual springs between adjacent cell centers where the rest lengths initially corresponded to the spacing in the perfectly regular comb. For springs that connected added points, the connection could be ambiguous, and we resolved these situations by triangulating the point sets with edges and choosing edge combinations that were closest to regular comb center-center distances. In merge areas, this would produce cell centers that were over (more than six) or under (less than six) connected. Springs that connected centers from different regions used the average length, as did cells that were placed in the second step and did not have an obvious rest length. We then adjusted the position of cell centers that were 1-3-1 1.5 cell lengths from the merge line by finding the minimum energy configuration of the system using sequential least squares optimization. Finally, we computed the new comb as the Voronoi partition produced by these adjusted cell centers.

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