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# Inheritance of Behavioral Traditions underlying Nest Architecture in Stingless Bees

Viviana Di Pietro[1,7*], Cristiano Menezes[2], Maria Giulia de Britto Frediani[2], David José Pereira[2], Maxime Fajgenblat[3,4], Helena Mendes Ferreira[1], Tom Wenseleers[1,6**], Ricardo Caliari Oliveira[1,5,6***]

[1]Laboratory of Socioecology and Social Evolution, Department of Biology, KU Leuven, Naamsestraat 59, 3000 Leuven, Belgium

[2]Embrapa Environment, Laboratory of Entomology and Phytopathology, SP-340 road, 13918-110, Jaguariúna, Brazil

[3]Laboratory of Freshwater Ecology, Evolution and Conservation, KU Leuven, Leuven, Belgium

[4]Data Science Institute, I-BioStat, Hasselt University, Diepenbeek, Belgium

[5]Departament de Biologia Animal, de Biologia Vegetal i d'Ecologia - Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain

[6]These authors contributed equally

[7]Lead contact

[*,**,***]Correspondence: viviana.dipietro@kuleuven.be (V.D.P.), tom.wenseleers@kuleuven.be (T.W.) , ricardo.oliveira@uab.cat (R.C.O)

ORCID: 0000-0003-1116-9795 (V.D.P.); 0000-0002-8473-6298 (C.M.); 0000-0002-2233-1527 (M.F.); 0000-0003-2090-4528 (H.M.F); 0000-0002-1434-861X (T.W.); 0000-0002-8996-1291 (R.C.O.)

## Summary

The transmission of complex behavior and culture in humans has long been attributed to advanced forms of social learning[1,2], which play a crucial role in our technological advancement[3]. While similar phenomena of behavioral traditions and cultural inheritance have been observed in animals[1,2,4-6], including in primates[7], whales[8], birds[9], and even insects[10], the underlying mechanisms enabling the persistence of such animal traditions, particularly in insects, are less well understood. This study introduces pioneering evidence of enduring architectural traditions in the stingless bee *Scaptotrigona depilis*, which are maintained without any evidence for social learning. We demonstrate that *S. depilis* exhibits two distinct nest architectures, comprising either helicoidal or flat, stacked horizontal combs, which are transmitted across generations through stigmergy[11-17] – an environmental feedback mechanism whereby the presence of the existing comb structures guides subsequent construction behaviors, thereby leading to a form of environmental inheritance[18-20]. Cross-fostering experiments further show that genetic factors or prior experience do not drive the observed variation in nest architecture. Moreover, the experimental introduction of corkscrew dislocations within the combs prompted helicoidal building, confirming the use of stigmergic

building rules. At a theoretical level, we establish that the long-term equilibrium of building in the helicoidal pattern fits with the expectations of a two-state Markov chain model. Overall, our findings provide compelling evidence for the persistence of behavioral traditions in an insect based on a simple mechanism of environmental inheritance and stigmergic interactions, without requiring any sophisticated learning mechanism, thereby expanding our understanding of how traditions can be maintained in non-human species.

**Keywords**: animal traditions; environmental inheritance; stigmergy; nest architecture; stingless bees

## Results and discussion

The progress and prosperity of human civilizations have been significantly influenced by our ability to learn from others and accumulate knowledge, leading to technological progress and innovation that is stably transmitted through cultural mechanisms[21]. While long perceived to be exclusive to humans, behavioral traditions and social information transmission across generations have also been observed in a variety of non-human species[1,2,4-6,22]. In particular, primates[7], cetaceans[8], birds[9], and even insects[10] have all demonstrated the capacity to maintain and transmit complex behaviors or structures across generations. High cognitive skills and advanced forms of social learning have long been thought to be required for the stable maintenance of behavioral traditions and culture[7-9,23,24]. However, the persistence of distinct traditions also in species with simpler cognitive capacities, like insects[10,25], challenges the conventional understanding of cultural transmission, suggesting that alternative, less complex mechanisms might sometimes be at play[10,25-28].

Recent insights into the architectural diversity of stingless bee colonies hint at the existence of behavioral traditions that might not rely on direct social learning or high cognitive abilities[25]. In this case, it is likely that variation in nest architecture could be propagated based on the comb structure itself, whereby the current building style guides the building by later generations of bees. Such a process could lead to environmental and behavioral inheritance[18-20,27,28], even in the absence of any direct form of social learning. To test this theory, we here present the first study of behavioral innovation and social transmission of alternative nest architecture building patterns in a neotropical stingless bee, *Scaptotrigona depilis*. This species is one of 10 known stingless bee species that exhibit significant intraspecific variation in nest architecture[29], building its brood combs in either a helicoidal shape built continuously without

the support of a central pillar, or as horizontally stacked parallel layers, supported by a central pillar[12,14] (Figure 1A,B). Given that stingless bees generate perennial colonies and reproduce through swarming or managed splitting, we hypothesized that the observed architectural variation might be maintained over periods of time extending beyond the lifespan of individual worker cohorts.

To investigate the inheritance and prevalence of the two alternative nest architectural building patterns, we surveyed 413 *S. depilis* colonies in Jaguariúna, São Paulo State, Brazil, and monitored the incidence and changes in nest architecture over one-month periods in 2022 and 2023 (see STAR Methods). We hypothesized that the prevailing comb configuration would guide the construction of successive worker cohorts[25], with the frequencies of helicoidal and parallel architectures reflecting the stable equilibrium of a two-state continuous time Markov chain model. Specifically, if $a$ and $b$ represent the monthly transition rates to helicoidal and parallel comb structures, each occurring at frequencies $H$ and $P$, we predicted the dynamics to adhere to the following system of ordinary differential equations:

$$\frac{dH}{dt} = aP - bH$$

$$\frac{dP}{dt} = bH - aP$$

At equilibrium ($dH/dt = 0$ and $dP/dt = 0$), and given that $H + P = 1$, the expected equilibrium proportion building in the helicoidal configuration is $H^* = a/(a + b)$, while for the parallel configuration it is $P^* = b/(a + b)$. Our empirical data show that parallel-building colonies ($n$ = 398) transitioned to a helicoidal architecture at a rate of $a$ = 4.5% per month (binomial 95% CrI 2.8-6.9% per month), while helicoidal-building colonies ($n$ = 15) switched to parallel at a rate $b$ = 87% per month (binomial 95% CrI 64-97% per month). These rates did not differ significantly between the study years, supporting the Markov chain model's assumption of constant transition rates (binomial GLM, posthoc tests, $z$ ratio = -1.53, $p$ = 0.13 and $z$ ratio = 0.32, $p$ = 0.75 for contrasts in switching rates $a$ and $b$ between years).

The calculated equilibrium frequency of the helicoidal architecture $H^*$ of 5.0% (parametric bootstrapping 95% CI: 2.9-7.6%, $n$ = 15) aligns with the observed average frequency over both years (binomial GLM, 4.3% with 95% CI 2.7-6.7%, $n$ = 413, $z$ ratio = -0.652, $p$ = 0.51) and was also not significantly impacted by season or year (Jan.-Febr. 2022 vs. Aug.-Sept. 2023),

hive entrance orientation, or position within the bee yard (Figure S1 & Table S1). It has been suggested that a helicoidal nest architecture could be a thermoregulatory adaptation helping to maintain the brood area at an optimum temperature[30,31]. Nevertheless, our results indirectly dismiss this hypothesis, since we would then have expected the helicoidal building type to be spatially clustered or to be more common in the warm season (Jan.-Feb.), which was not the case (Table S1). The inclination of the hives also did not impact the frequency of both types. When 10 colonies were tilted, none of them switched to building helicoidal combs within 2 weeks, and on a per-month basis the rate of switching to the helicoidal type was not significantly elevated compared to 2022 parallel-building control colonies ($n$ = 299) (Firth's penalized logistic regression, $z$ ratio = 0.66, $p$ = 0.51). The lack of any significant environmental effects argues against variation in nest architecture being driven by changes in the external environment[30,31]. Our results confirm the long-term persistence of both architectural phenotypes, but with the helicoidal form continuing for a shorter average duration ($T_H = 1/b$ = 1.2 months, 1.0-1.5 95% CI) than the parallel form ($T_P = 1/a$ = 23.4 months, 14.7-39.8 95% CI). Yet, the average duration of building in the helicoidal pattern still surpassed the typical comb-building period of a single worker cohort, which transitions to other tasks after approximately two to three weeks[29]. Consequently, our results imply that information on how combs are constructed is transmitted across different worker cohorts and hence that some form of environmental and behavioral inheritance is going on[18-20,27,28].

To investigate the specific mechanism by which this transmission occurs, we carried out a full factorial cross-fostering experiment in which we provided experienced workers sourced from colonies building in either the helicoidal or parallel pattern with a comb lattice structure from a genetically unrelated colony that was matching or was opposite to their own (see STAR Methods, Figure 1, $n$ = 6 replicates per treatment combination). According to our stigmergy-based environmental inheritance hypothesis, the expectation was that presenting an altered comb structure in itself should cause the experienced workers to switch to a new building style, merely by being guided by the comb structure itself. In that case, social information on how combs are built would be socially transmitted across worker cohorts, but it would be based on environmental inheritance driven by stigmergy, whereby the change in the structure of the environment – the change in the comb lattice structure – would act as a stimulus for continued construction in a manner consistent with the existing architecture[11-17]. This form of social transmission emphasizes the role of the artifacts themselves – in this case, the comb structure – as repositories and conduits of social information, thereby bypassing the need for direct

observation of behavior or instruction. By contrast, if the experienced workers were predisposed towards building in a particular style, based on their genetic makeup or prior experience, the expectation was that they should continue to do so even when provided with a comb template of the alternative type.

Our cross-fostering experiment results confirm the environmental inheritance hypothesis. When exposed to alternative architectural styles, experienced workers did not adhere to their original construction patterns, thereby implying that nest architectural variation was not controlled by genes of large effect or by prior experience. Instead, they promptly adjusted their behavior to align with the provided comb structure, as evidenced by the rapid change in building patterns observed within the first week – a change that remained constant for several weeks (Figure 2). These results are consistent with the hypothesis that stigmergy underlies the observed environmental inheritance and the persistence of distinct nest architectural behavioral traditions. Nevertheless, over time, most colonies did eventually revert to building in the parallel configuration, consistent with the dominance of this type in the wild. Given that the observed changes in nest architecture were not affected by the original building style of the experienced workers, our results clearly argue against genetic factors of large effects (e.g. supergenes[32,33]) or direct social learning playing a significant role. Instead, our results support the concept that behavioral traditions can be maintained through environmental cues and stigmergic responses, aligning with the broader definition of traditions as patterns perpetuated over time by mechanisms that do not require direct social learning[27,28].

To test if stigmergic building rules underpin the observed variation in nest architecture, we carried out one final experiment in which we incised the top comb of parallel-building colonies to mimic "corkscrew dislocations", which are known theoretical precursors to helicoidal comb building[14]. In line with theoretical predictions[14], we found that this manipulation of the comb substrate caused a marked increase in the transition to a helicoidal architecture compared to control colonies (Figure 3). This result corroborates that the physical structure of the comb itself can direct subsequent construction, in line with the concept of stigmergy[11,13,16].

Despite our clear conclusions on the mode of inheritance of alternative nest architectures in this species, several important questions remain. It is unclear, for example, what factors cause the actual transitions between building styles, and why helicoidal building tends to persist for a much shorter time than parallel building. In all likelihood, transitions to parallel building are initiated by the building of a central wax support pillar[12] (Figure 1A), while transitions to

helicoidal building are caused by occasional mistakes in the vertical alignment of newly built cells[14]. If correct, this suggests that the parallel configuration is the default building pattern and that switching to the helicoidal pattern could be regarded as an error-correction mechanism to absorb occasional building mistakes and avoid costly interruptions in cell building. Similar error-correction mechanisms during comb construction have previously been described in other social insects[34-37]. Honeybees, for example, initiate comb building at multiple points within a frame, and to be able to fuse those comb sections they occasionally switch to building pentagonal or heptagonal cells instead of the standard hexagonal ones[36,37]. This adaptability reflects a sophisticated combination of stigmergy and behavioral flexibility, where bees respond dynamically to the challenges presented by their construction environment[36,37]. In any case, our findings indicate no clear superiority of either architectural style, given that neither significantly impacted cell construction rates (Figure S4). This absence of a distinct advantage may explain the coexistence of both architectural styles in *S. depilis*, despite the exclusive adoption of the helicoidal style in some other stingless bee species[12,14,29].

Another important outstanding question is if the persistence of distinct nest architectures in our system could also involve more complex forms of social learning, which some authors take as a defining characteristic of behavioral or cultural transmission[6,38,39]. Indeed, Chittka & Rossi[25] suggested that stingless bees might to some extent be able to copy how to build their comb merely by observing and being exposed to the existing comb structure, which would be akin to an indirect form of social learning known as product learning[40]. At present, we cannot exclude that the stigmergy we describe also involves such a learning process and is not just based on innate responses, but conclusive evidence to support this theory is currently lacking. Personally, we believe it is more likely that the stigmergic building rules are largely innate, and that the same set of building rules produce different nest architectures depending on the initial comb substrate, or that particular innate building rules are conditionally expressed in function of the existing architecture.

Testing whether or not the inheritance of alternative nest architecture is purely innate remains a task for future work and would require extensive and complex experiments. Establishing the involvement of social learning would entail showing that prior exposure to a specific comb structure affects the construction approach in subsequent generations, following swarming events. Suggestive evidence for this has been collected in the honeybee, where colonies maintain their original comb building orientation after swarming[41]. Nevertheless, as this study

did not use a cross-fostering setup, it could not preclude that the building style persisted merely as a result of varying genetic predispositions[41]. In addition, definite conclusions for our system might be precluded by the fact that colonies presumably have a propensity to revert back to parallel building when placed in an empty hive without any comb substrate.

In conclusion, our findings provide a compelling case for the role of stigmergy and environmental feedback in the transmission of nest-building traditions in stingless bees. This mechanism facilitates the persistence of distinct architectural styles within colonies, thereby producing behavioral traditions that persist across the lifespan of individual bees, even in the absence of any evidence for learning or advanced cognitive processing. Hence, our study strengthens the case that animal traditions and the non-genetic transmission of behavior can be based on cognitively simple processes[27,28], such as via stigmergy-mediated environmental feedback. Future research should aim to dissect the precise nature of these stigmergic interactions further and explore whether beyond innate responses, there might also be a layer of learning that influences these behaviours[25]. These investigations could shed light on the continuum between innate building rules and the potential for learning in shaping the cultural and behavioral landscapes of non-human societies. Our study not only advances our understanding of stingless bee behavior but also contributes to the broader discussion on the mechanisms of behavioral transmission and tradition maintenance in animal societies.

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**Author contributions**

R.C.O. and T.W. conceived and supervised the study. V.D.P, C.M. and R.C.O.: experimental design. V.D.P, C.M., M.G.B.F. and D.J.P.: behavioral experiments. T.W.: theoretical modelling. V.D.P., M.F., R.C.O., T.W. and H.M.F.: data analysis. V.D.P and H.M.F wrote the

first draft of the manuscript. All authors contributed to revising and proofreading and approved of the final version before submission.

**Declaration of interests**

The authors declare no competing interests.

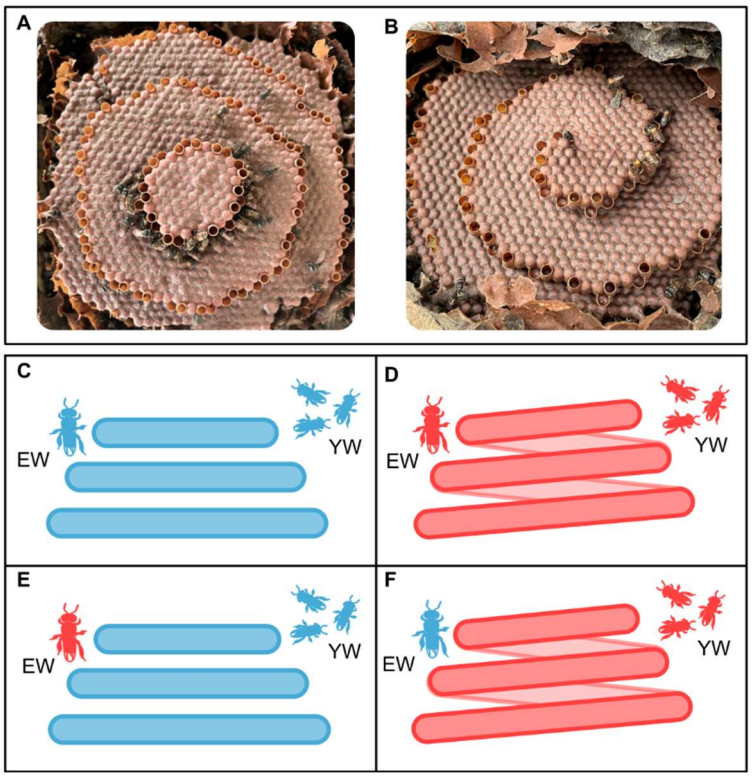


**Figure 1. Cross-fostering experiment to study how intraspecific variation in nest architecture in the stingless bee *Scaptotrigona depilis* is transmitted.** Strikingly, workers in this species construct their brood combs either in a parallel arrangement (**A**) with a central support pillar from which the first cell of the comb is built (*) or in a helicoidal pattern (**B**), without any central pillar (+). To study the mechanism by which this variation in nest architecture is transmitted, we carried out a full factorial cross-fostering experiment (panels **C-F**), where we provided experienced workers (EW), deriving from colonies with either parallel (blue) or helicoidal building patterns (red), with a comb lattice structure that was either of the same type or the alternative type. The combs used as a template in the experiment contained late-stage genetically unrelated brood that was ready to emerge, to allow for interactions and potential social information exchange between experienced workers and naïve young workers (EW and YW).

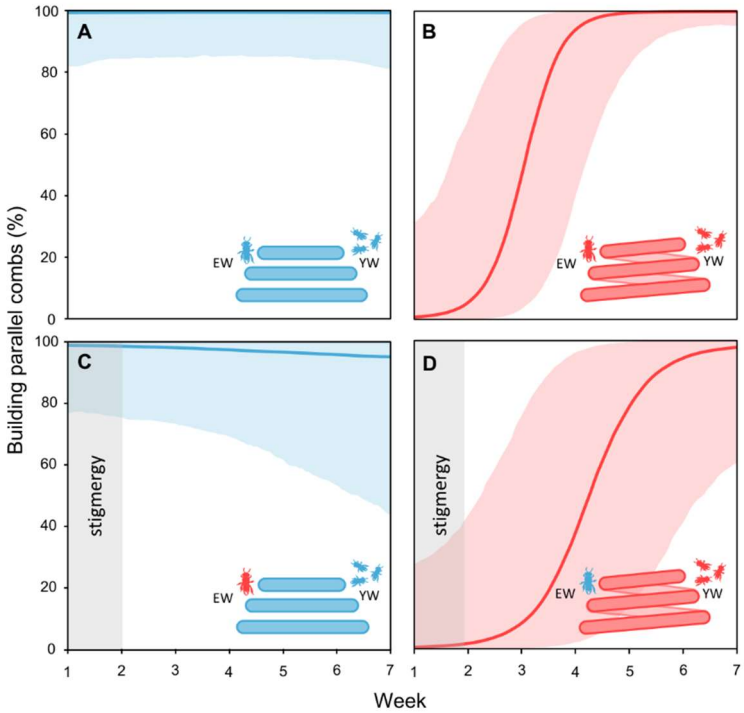


**Figure 2. Stigmergy, rather than genetics or prior experience, drives the inheritance of alternative nest architectures**. Estimated changes in nest architecture over 7 weeks in our cross-fostering experiment ($n$ = 6 replicates/treatment). When experienced workers (EW), with a history of building in either parallel or helicoidal styles, encountered the opposing comb structure, they immediately switched to building in the presented style (panels **C** and **D**). This swift change was likely driven by stigmergy, where the existing comb structure guided subsequent building. These results also clearly show that nest architecture was not determined by prior experience or genetic makeup. As the weeks progressed, most colonies eventually switched towards the parallel style (panels **A-D**), mirroring its dominance in the wild and the observed preferential switching to this building style. The presence of EWs from parallel-building colonies did not accelerate this shift (panels **B** and **D**), thereby implying that direct social learning did not significantly influence building patterns. The shaded areas denote 95% credible intervals from a Bayesian Gaussian process logistic regression model (see STAR Methods). For detailed results see Figures S2 & S3.

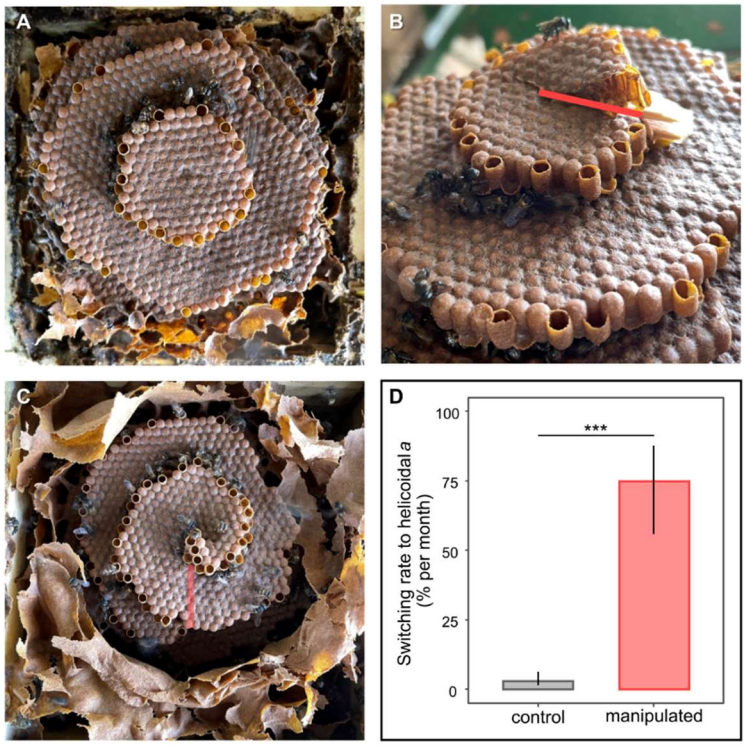


**Figure 3. Stigmergy underlies the transition to helicoidal comb building.** To investigate the role of stigmergy in the social transmission of helicoidal comb construction, we modified the top comb of colonies that were previously building in a parallel pattern by incising and tilting one side to simulate "screw dislocations", a theoretically identified initiator of helicoidal comb building[14] (**A**,**B**). With only the comb's lattice structure changed and all workers inexperienced in helicoidal building, a subsequent shift to this construction style would implicate stigmergy. Results confirmed this hypothesis: 43% of the manipulated colonies (9/21) transitioned to helicoidal building within one week (**c**, red line indicates initial incision, obscured by newly built helicoidal comb), which amounted to a monthly transition rate to helicoidal building of 75.0% [55.8-87.7] 95% CI ($n$ = 21) compared to a mere 3.1% [1.5-6.3] 95% CI in the parallel-building 2022 controls ($n$ = 299) (binomial GLM analysis, odds ratio to switch over one month: 95.1 [30.3-299.2] 95% CI, $z$ ratio = 7.79, $p$ < 1E-14).

## STAR Methods

## RESOURCE AVAILABILITY

### *Lead contact*

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Viviana Di Pietro (viviana.dipietro@kuleuven.be).

### *Materials availability*

This study did not generate new unique reagents.

### *Data and code availability*

All datasets used for analysis in this study have been deposited at Mendeley Data (10.17632/bhjp3m2sbp.1) and will be publicly available as of the date of publication. Accession numbers are listed in the key resources table. For review the data can be downloaded through this link provided by Mendeley Data: https://data.mendeley.com/preview/bhjp3m2sbp?a=cd4b4d79-ba16-4248-8754-1af2f5eda915

All original code has been deposited at Mendeley Data and is publicly available as of the date of publication. DOI is listed in the key resources table.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

## EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Stingless bees, a group of more than 500 highly eusocial bees in the tribe of the Meliponini, display an astounding diversity in terms of diet, behaviour and nest architecture, not only between but also within species[29,42,43]. Unlike honeybees, which construct vertical parallel wax combs and use them both for food storage and brood rearing[44], the nest building of stingless bees is more complex and diverse[42]. They use a range of building materials, including soil particles, resin, plant fibres, and even excrement[29,42,45,46] with food pots and brood combs being spatially separated[42]. Nest architecture can vary both across and within species, with brood cells being arranged either in horizontal combs, vertical combs, clustered cells or some intermediates between these forms[29]. Our focus in this study is on *Scaptotrigona depilis*, a Neotropical stingless bees and one of 10 species that are known to show large bimodal intraspecific variation in nest architecture[29]. The species constructs nests with combs consisting either of flat, stacked parallel combs, supported by a central pillar, or builds combs arranged in a helicoidal configuration, in which case there is no central support pillar. Both forms of comb can coexist within the same colony simultaneosly[47], suggesting that minor changes in building behaviour can cause a switch between both configurations[14,48] and the switch from one configuration to the other can occur at any stage during building. The precise trigger for such transitions remains unknown. The colonies used for this study were kept at Embrapa Meio

Ambiente in Jaguariúna, São Paulo, Brazil. These colonies were housed in identical wooden boxes (19 x 19 x 16 cm) and the bees were allowed to forage freely in their natural environment.

## METHOD DETAILS

*Observational data collection*

To determine the frequency of helicoidal and parallel comb building in the stingless bee *Scaptotrigona depilis* and determine the rate of switching between both types within the same colony, we recorded the nest architecture of 413 colonies at one month intervals, in January-February 2022 ($n = 241$), and August-September 2023 ($n = 172$). Hive entrance orientation and position in the bee yard (placed individually on a stand or placed on the upper or lower shelf under a shelter) were noted to evaluate environmental influences on nest architecture.

*Cross-fostering experiment*

Our factorial cross-fostering experiment involved four groups with six colonies each: two control groups with congruent comb structures and worker backgrounds, and two treatment groups with mismatched comb structures and worker backgrounds (Figure 1). Standardized hives (19 x 19 x 16 cm) were populated with three to four layers of brood combs from which bees where ready to emerge, the original mother queen and approximately 600 genetically unrelated bees of varying ages, from the youngest to foragers, of a given nest architectural background. After a one-week acclimation period during which colonies were kept closed and where they were provided with wax, pollen and honey, we tracked nest architecture weekly for seven weeks, in a blinded manner, between February and March 2022.

*Stigmergy experiment and hive inclination effect*

To investigate the role of stigmergy in the social transmission of helicoidal comb building, we experimentally manipulated small newly built brood comb layers (diameter ca. 5 cm) of 21 parallel-building colonies by making an incision and tilting up one side to mimic "screw dislocations", which theoretically have been posited to initiate helicoidal comb building[14] (Figure 3). After seven days, we recorded the nest architecture to check if helicoidal comb building had stably persisted.

In a similar fashion, we examined whether inclination had any impact on nest configuration by selecting 10 colonies that were building in parallel and tilting them at an angle of 20 degrees. Following a two-week interval, we examined these colonies to determine if any of them had transitioned to helicoidal architecture.

342 *Colony performance analysis*

343 To test if nest architecture affected colony performance, six free-foraging observation hives
344 (three per nest architecture) of similar colony size were set up and videotaped to allow us to
345 measure and compare cell building rates. The brood combs were exposed by removing the
346 involucrum, and colony temperature was maintained with halogen lights. The number of newly
347 built cells over 5 subsequent days were measured from the video footage using ImageJ's "Cell
348 Counter" plugin[49]. Colony size was controlled for by using that same plugin to count the
349 average number of workers visible in 20 video frames collected at 30-second intervals for a
350 period of 10 minutes.

351

352 **QUANTIFICATION AND STATISTICAL ANALYSIS**

353 *Observational data collection*

354 Bayesian binomial credible intervals for the frequency of each nest architecture and the
355 monthly transition rates were estimated under a Jeffrey's prior using the *propCI* function from
356 the *prevalence* package[50] in R. We employed binomial generalized linear models (GLM) with
357 a logit link function to test for annual and environmental variations in architecture switching
358 rates, using the *glm* function in R and carried out two-sided Sidak posthoc comparisons with
359 the *emmeans* package[51] (Figure S1). To derive confidence intervals for the equilibrium
360 frequency of helicoidal types and the expected duration of architectural forms, we applied
361 parametric bootstrapping with one million replicates. All statistical analyses were performed
362 in R v. 4.2.2[52].

363 *Cross-fostering experiment*

364 We employed a Bayesian Gaussian process logistic regression model[53], fit using the *brms*
365 package[54], to analyses the change in nest architecture over time. This approach allowed us to
366 model time series data flexibly, accounting for potential autocorrelations and inter-colony
367 variability by including a random intercept for each colony. We assumed an exponentiated
368 quadratic covariance function to describe the covariance between two time points as a function
369 of the number of weeks that separates them. We selected weakly informative zero-centered
370 normal priors for intercepts and scales (with a *SD* of 3), and a moderately informative inverse
371 gamma prior (with a shape and scale of 5) for length scales. We ran four MCMC chains of
372 2,000 iterations each, of which the first 1,000 iterations were discarded as warmup.
373 Convergence diagnostics included inspection of traceplots and calculation of the Potential
374 Scale Reduction Factor (R-hat), which for all parameters was smaller than 1.01[55]. To visualize

375 the effects of time and treatment on the outcome type, we plotted the conditional temporal
376 trajectory of each treatment along with 95% equal-tailed credible intervals (Figure 2). We also
377 performed a prior sensitivity analysis to ascertain that our findings were robust against
378 alternative prior specifications (Figure S2). In addition, we visualized the posterior
379 distributions of the estimated fraction of parallel comb architecture for each treatment group at
380 each week using the *ggdist* package[56] (Figure S3). Posterior estimates were compared pairwise
381 among treatments and weeks.

382 *Stigmergy experiment and hive inclination effect*

383 A logit link binomial GLM was used to compare the rate of switching to helicoidal comb
384 building in manipulated colonies ($n$ = 21) vs. in unmanipulated parallel-building control
385 colonies observed over the same time period (February 2022, $n$ = 299). Differences in the
386 period over which switching was observed, i.e. one week versus one month, were adjusted by
387 including log(period) as a model offset. To facilitate comparison, switching rates were
388 expressed on a fixed per month basis.

389 A binomial GLM with logit link function was employed to compare the rate of transitioning to
390 helicoidal comb construction in tilted colonies ($n$ = 10) with that in unmanipulated control
391 colonies that naturally built parallel combs during the same observation period (February 2022,
392 $n$ = 299). Given that there was separation, this model was fitted using the *brglm2* package[57]
393 using a Jeffrey's prior. To account for differences in the observation period in the manipulated
394 vs. in the control colonies (two weeks versus one month), we made adjustments by including
395 log(period) as a model offset.

396 *Colony performance analysis*

397 The data were then analyzed using a Poisson GLMM with a log link function fitted using the
398 *lme4* package[58]. The model included time, nest architecture, and their interaction as fixed
399 effects, log(colony size) as a model offset and colony ID as a random intercept. Overdispersion
400 was addressed by incorporating an observational-level random effect. Two-sided Sidak post-
401 hoc pairwise comparisons and estimation of marginal means were performed with the *emmeans*
402 package[51].

403 **Supplemental Information figures and tables**

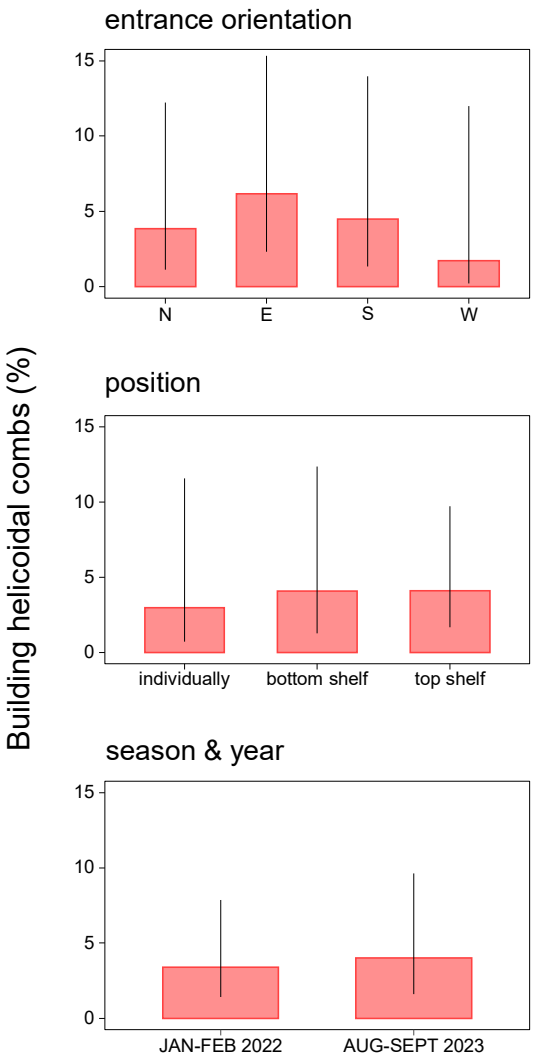


**Figure S1. The frequency of the helicoidal nest architecture was consistent across time and space and not significantly affected by environmental factors.** The frequency of the helicoidal nest architecture in *Scaptotrigona depilis* was not significantly affected by season & year (January-February 2022, $n = 241$ colonies, vs. August-September 2023, $n = 172$ colonies), hive entrance orientation (facing north, east, south or west) or position in the apiary (placed individually on a stand, or on the bottom or top shelf in the apiary) (main effects logistic regression, anova Type III likelihood-ratio tests, effect for year/season: $\chi^2_1 = 0.095$, $p = 0.76$, effect for hive entrance orientation: $\chi^2_3 = 2.65$, $p = 0.45$, effect for position in the apiary: $\chi^2_2 = 0.23$, $p = 0.89$). Plots show the expected marginal means and Sidak adjusted 95% confidence intervals, calculated using the *emmeans* package. As each colony was observed twice per year with a one month interval, we used prior observation weights of 0.5 in our logistic regression to avoid pseudoreplication.

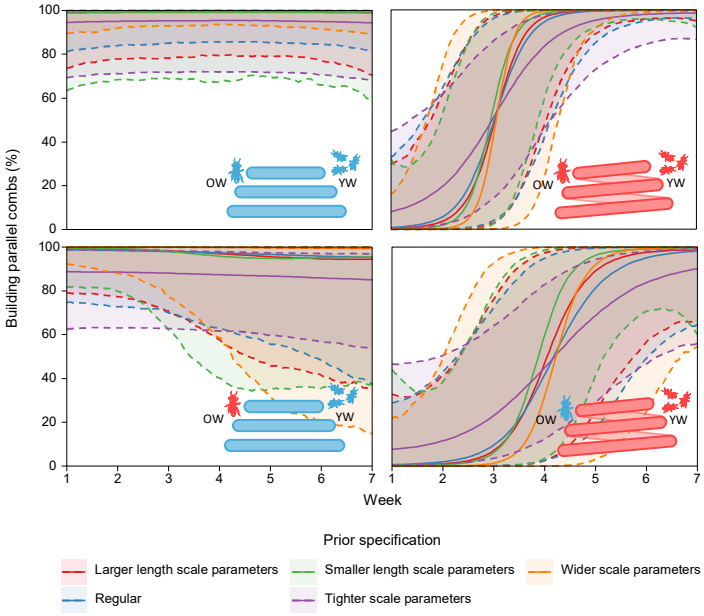


**Figure S2. Prior sensitivity analysis of nest architecture change over time in the cross-fostering experiment**. Plots show the estimated percentage of colonies building parallel combs through time for the four treatment groups ($n$ = 6 replicates per treatment combination, Figures 1 & 2) under five different prior specifications for a Gaussian process logistic regression fitted using *brms*, in which colony was included as a random intercept ($n$ = 6 colony replicates per treatment combination, data were collected weekly). Full lines indicate posterior mean trajectories, while dashed lines and the shaded zones show 95% credible intervals. The regular prior specification (a Normal (0, 3) prior on the model intercept, an Inv-Gamma (5, 5) prior on the Gaussian process' length scale, a Normal (0, 3) prior on the Gaussian process' marginal scale and a Normal (0, 3) prior on the random intercepts' scale), used for the main analysis (Figure 2), is shown in blue. Results of an alternative model in which the Gaussian process' length scale is replaced by an Inv-Gamma (18.5, 10) prior, favoring slower temporal changes, is shown in red. Results of a model in which the Gaussian process' length scale is replaced by an Inv-Gamma (18.5, 5) prior, favoring faster temporal changes, is shown in green. A model in which wider Normal (0, 9) priors on the model intercept, the Gaussian process' marginal scale and the random intercepts' scale are used, is shown in orange. Finally, a model in which tighter Normal (0, 1) priors on the model intercept, the Gaussian process' marginal scale and the random intercepts' scale are used, is shown in purple. The prior sensitivity analysis reveals that alternative prior specifications do not affect the general patterns and qualitative conclusions presented in the main analysis, and only lead to minor changes in the width of the credible intervals.

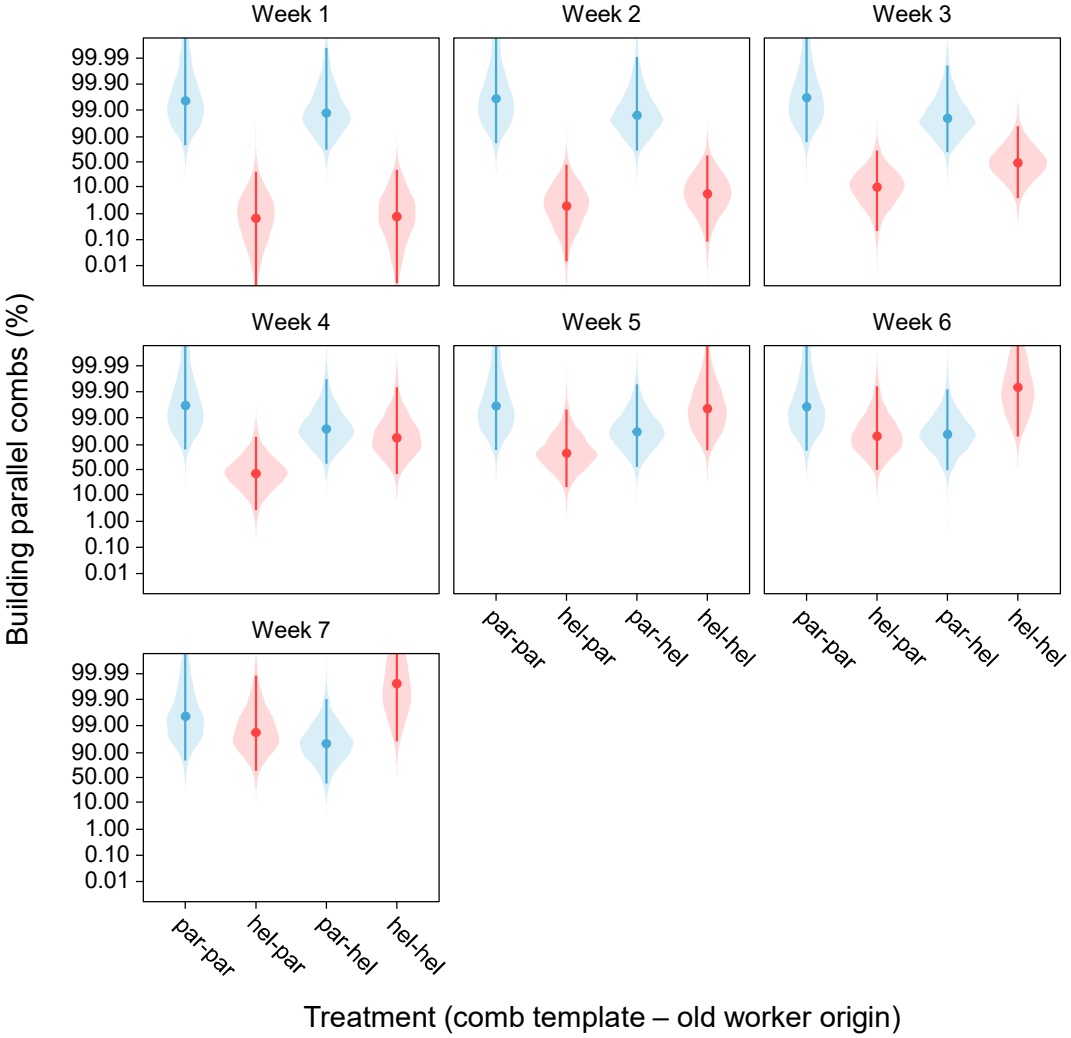


**Figure S3. Weekly estimates of nest architecture variation in transplant experiment.** The figure presents the model's predicted probabilities of colonies building in the parallel pattern across different treatments plus their uncertainty at 1 to 7 weeks after the start of the experiment (points and line ranges show median posteriors plus 95% credible intervals, $n$ = 6 replicates per treatment combination). Treatments show the combinations of the provided comb lattice template (parallel or helicoidal) and the origin of the experienced old workers (from colonies that previously built in the parallel or helicoidal arrangement), i.e. "par-par", "hel-par", "par-hel" or "hel-hel" (cf. Figures 1 & 2). The estimates are derived from a Bayesian Gaussian process logistic regression model, which incorporated random intercepts for individual bee colonies. The colors distinguish between treatments, with blue and red indicating the architecture of the provided comb lattice structure (parallel or helicoidal).

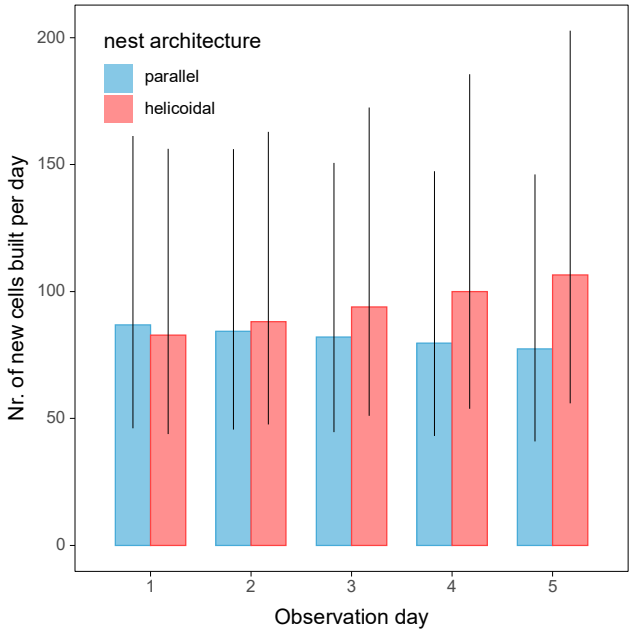


**Figure S4. Mean cell building rates in colonies with parallel and helicoidal nest architectures.** This figure illustrates the average number of new cells constructed per day over a period of 5 days in colonies kept in observation hives that were constructing their combs in either a parallel or a helicoidal arrangement ($n$ = 6 colony replicates for each). The plotted estimates show marginal means and 95% confidence intervals derived from a Poisson generalized linear mixed model (GLMM), calculated using the *emmeans* package (for details see Table S1). No significant differences were found at any observation day, which shows that nest architecture did not significantly affect colony performance.

**Table S1. Analysis of cell building rates in colonies with parallel and helicoidal nest architectures.** (A) Model coefficients of a Poisson generalized linear mixed model (GLMM) in which we compared the number of new cells built per day, measured at 24-hour intervals for a period of five days in function of nest architecture ($n$ = 6 colony replicates per type). The model accounts for fixed effects time (day of observation) and nest architecture plus their interaction, and included the natural logarithm of colony size as a model offset (average count of workers visible in 20 video frames collected at 30-second intervals for a period of 10 minutes). Colony ID was included as a random intercept and an observation-level random effect was included to take into account overdispersion. (B) Pairwise contrasts in daily cell building rates between the two nest architectures show no significant differences in building efficiency (ratio in the number of new cells built per day for colonies of average size) at any of the observation days. The table shows coefficients, standard errors (*SE*), lower and upper 95% confidence intervals (*LCL* and *UCL*), $z$ values and $p$ values (*NS* = not significant, i.e. $p > 0.05$).

| **(A) Model coefficients** | **term** | **coefficient** | ***SE*** | ***z* value** | ***p* value** | |
|---|---|---|---|---|---|---|
| fixed effect | (Intercept) | -0.15 | 0.19 | -0.80 | 0.42 | |
| fixed effect | scale(time) | 0.03 | 0.05 | 0.57 | 0.57 | *NS* |
| fixed effect | nest_architecture1 | -0.04 | 0.19 | -0.23 | 0.82 | *NS* |
| fixed effect | scale(time):nest_architecture1 | -0.08 | 0.05 | -1.54 | 0.12 | *NS* |
| random intercept: observation | *SD*(Intercept) | 0.19 | | | | |
| random intercept: colony ID | *SD*(Intercept) | 0.46 | | | | |
| **(B) Contrast (day)** | **ratio** | ***LCL*** | ***UCL*** | ***z* ratio** | ***p* value** | |
| helicoidal / parallel (1) | 0.95 | 0.43 | 2.09 | -0.12 | 0.90 | *NS* |
| helicoidal / parallel (2) | 1.04 | 0.49 | 2.24 | 0.11 | 0.91 | *NS* |
| helicoidal / parallel (3) | 1.14 | 0.54 | 2.43 | 0.35 | 0.73 | *NS* |
| helicoidal / parallel (4) | 1.26 | 0.58 | 2.69 | 0.58 | 0.56 | *NS* |
| helicoidal / parallel (5) | 1.38 | 0.62 | 3.04 | 0.79 | 0.43 | *NS* |

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