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Comb construction in mixed-species colonies of honeybees, *Apis cerana* and *Apis mellifera*

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SUMMARY

Comb building in mixed-species colonies of *Apis cerana* and *Apis mellifera* was studied. Two types of cell-size foundation were made from the waxes of these species and inserted into mixed colonies headed either by an *A. cerana* or an *A. mellifera* queen. The colonies did not discriminate between the waxes but the *A. cerana* cell-size foundation was modified during comb building by the workers of both species. In pure *A. cerana* colonies workers did not accept any foundation but secreted wax and built on foundation in mixed colonies. Comb building is performed by small groups of workers through a mechanism of self-organisation. The two species cooperate in comb building and construct nearly normal combs but they contain many irregular cells. In pure *A. mellifera* colonies, the *A. cerana* cell size was modified and the queens were reluctant to lay eggs on such combs. In pure *A. cerana* colonies, the *A. mellifera* cell size was built without any modification but these cells were used either for drone brood rearing or for food storing. The principal elements of comb-building behaviour are common to both species, which indicates that they evolved prior to and were conserved after speciation.

Key words: *Apis cerana*, *Apis mellifera*, comb construction, mixed species.

INTRODUCTION

Nest-construction behaviour in social insects is a very complex and highly cooperative phenomenon (Wilson 1971; Belic et al., 1986). In honeybee colonies, the nests result from numerous kinds of operations performed by many individual bees (Hepburn, 1986). Although the task of comb construction, like many tasks performed in a honeybee colony, requires concerted actions by many nestmates, individuals are in fact very poorly informed and lack a central controller. Coordination relies on subtle mechanisms combining individual decision rules with specialised signals and informative local cues (Pratt, 2004). It has been suggested that the comb building of honeybees can be interpreted as a model of self-organisation (Belic et al., 1986; Bonabeau et al., 1997; Hepburn, 1998).

Indeed, regulation of behaviour through self-organisation (Bonabeau et al., 1997; Boomsma and Franks, 2006; Detrain and Deneubourg, 2006), specifically in honeybee societies, can be used to interpret behaviours including comb construction (Belic et al., 1986; Hepburn, 1998), as well as the arrangement of food storing and brood rearing on the combs (Camazine et al., 1990; Camazine, 1991) and the regulation of food collection behaviour (Jenkins et al., 1992).

Several studies on comb building in *Apis mellifera* have shown that some very simple building rules (Darchen, 1954; Hepburn and Whiffler, 1991), coupled to the physico-chemical properties of beeswax as a building material (Pirk et al., 2004; Buchwald et al., 2006), can parsimoniously explain several aspects of comb-building behaviour. However, the two sister-species, *Apis cerana* and *A. mellifera*, not only differ in the chemical components of their waxes (Aichholz and Lorbeer, 1999) but also have different worker cell sizes [5.2–5.4 mm in diameter for *A. mellifera* (Winston, 1987); 4.2–4.8 mm in diameter for *A. cerana* (Ruttner, 1988)]. In order to induce the colonies to build combs more quickly and with more regularity, artificial beeswax foundation embossed with the average cell sizes are commonly used for a particular species.

In other studies of mixed species, it has been shown that *A. cerana* and *A. mellifera* can decipher their differing dance languages (Su et al., 2008; Tan et al., 2008) but differ in their thermoregulatory fanning behaviour (Yang et al., 2010b). So, mixed colonies offer us a valuable opportunity for the integrative investigation of the relationships of the two species and provide us with a new perspective to study the theories of self-organisation in honeybees as well as in investigations of the evolution of behaviour. Division of labour in mixed-species colonies remains an intriguing issue not previously considered. In this paper we report studies on the comb-construction behaviour of mixed colonies of *A. cerana* and *A. mellifera* to answer several questions: (1) will the mixed colonies accept the waxes of both species? (2) Will pure colonies of *A. cerana* accept *A. mellifera* wax and *vice versa*? (3) Given that the bees are presented with beeswax foundation of different cell base sizes, are these accepted as such or are they modified? (4) Do *A. cerana* workers and *A. mellifera* workers cooperate heterospecifically in comb building or do they form separate, conspecific festoons? (5) Under the various conditions above, what cell sizes would result in the newly constructed combs? (6) Once constructed, how are these cells used in the economy of the nest?

MATERIALS AND METHODS

Organisation of the mixed colonies

Mixed colonies of both *Apis cerana* L. and *Apis mellifera* L. workers were established. Three colonies were headed by *A. cerana* queens;

and, reciprocally, three colonies were headed by *A. mellifera* queens. Sealed brood about to emerge as young adults of each species was placed in the colonies of the other species (Tan et al., 2006). Wax-building behaviour was investigated when the newly emerged workers of the two species were about 10–18 days old, the peak age of wax secretion (Rösch, 1927; Hepburn et al., 1984; Seeley, 1995). Each of three pure *A. cerana* and *A. mellifera* colonies with the same age cohort of workers were selected as control groups. The colonies were equalised for size, numbers of combs and adult bees, nectar and pollen stores and brood. This was achieved simply by adding or removing combs.

Wax foundation

The experiments on the utilisation of the newly built combs in the pure *A. cerana* and *A. mellifera* colonies were done at an apiary of Yunnan Agricultural University, Kunming, China, to refine the final experimental protocol. In these experiments, beeswax was extracted from the combs of both *A. cerana* and *A. mellifera* and then used to make small sheets of beeswax foundation of two worker cell sizes: *A. cerana*, about 4.75 mm diameter and *A. mellifera*, 5.35 mm diameter using a silicon rubber mould (Hepburn et al., 2009). The foundations were about 25 mm long × 80 mm wide × 2 mm thick for the *A. mellifera* queen-headed mixed colonies, and about 12.5 mm long × 80 mm wide × 2 mm thick for the pure-species colonies and the *A. cerana* queen-headed mixed colonies, because they were more concentrated in the middle areas, the foundation widths were cut by half (honeycomb foundation used in commercial beekeeping is made from beeswax by pressing a blank sheet of wax between metal moulds on which hexagons are embossed so as to create a facsimile of natural comb).

We inserted both *A. cerana* cell-size (4.75 mm diameter) foundation and *A. mellifera* cell-size (5.35 mm diameter) foundation into pure *A. cerana* colonies and pure *A. mellifera* colonies. The experiments on cell size and wax discrimination, and comb-building cooperation were conducted with colonies of *A. cerana cerana* and *A. mellifera ligustica* at an apiary at the Ratchaburi Campus of King Mongkut's University of Technology Thonburi, Thailand. The same four types of beeswax foundation sheets (two cell sizes and two wax types) were fixed on the top bar of a frame, their relative positions determined by random number assignment. They were then inserted into the centre of the hives.

Observations

We used a video camera (Sony handycam DCR-DVD803E PAL) and DVD recorder (Sony DVD+RW, DPW60DSS2) to record the comb-building behaviour of the test and control colonies for 10 s intervals three times a day, the videos were taken every day for all of the replicates (Table 1). On replay of the video clips, we were able to obtain detailed information on: (1) how many workers of each species were engaged in which type of comb building; (2) how many starting sites were used to extend the building of new combs; (3) whether they formed a mixed-species building chain and cooperated with each other in comb building; (4) how many workers of each species were in each festoon; and (5) when building was complete. When the foundation sheets had been extended beyond their original lengths by the addition of several centimetres of new wax, the combs were removed from the hive and represented one sample for that colony. These combs were replaced by a new top bar with the same four kinds of foundation. The cell sizes and cell patterns were measured.

Statistical analyses

Chi-square tests were used to test for differences in the numbers of modified cells and patterns of cell orientation between the four types of colonies of *A. mellifera* and *A. cerana* queen-headed mixed colonies, and pure *A. cerana* and *A. mellifera* colonies. To test for differences in the mean numbers of workers engaged in comb building and the mean cell size of the newly built combs between the four types of colonies, we used analysis of variance (ANOVA) and Tukey *post-hoc* tests. Homogeneity of the variances between types of colonies was checked using Levene's test. Paired samples *t*-tests were used to compare the mean number and proportions of *A. cerana* workers with *A. mellifera* workers in the *A. mellifera* and *A. cerana* queen-headed mixed colonies (Johnson and Wichern, 2002). The means and standard deviations of each variable were calculated. All tests were performed using Statistica (version 8, www.statsoft.com; StatSoft).

RESULTS

Cell-size and wax discrimination

Pure *A. cerana* colonies ignored all sheets of beeswax foundation and began building new combs either from the top bar or from the lower edges of the foundation sheets (Fig. 1A). By contrast, the pure *A. mellifera* colonies accepted all sheets of both *A. cerana* and *A. mellifera* foundation and built cells on both cell sizes (Fig. 1B). In the two types of mixed colonies, all the four types of foundations were accepted (Fig. 1C,D); workers of both species were seen building cells on the foundations (Fig. 2, Table 1). None of these colonies showed any preference for a particular type of foundation with respect to wax type or cell size (repeated-measures ANOVA: $P>0.05$, Table 1).

Table 1. Mean numbers (±s.d.) of worker bees engaged in comb building on the four types of foundation

Foundations		Host colonies					
		A. cerana queen-headed mixed colonies (*N*=3, *n*=14 replicates)		*A. mellifera* queen-headed mixed colonies (*N*=3, *n*=10 replicates)		Pure *A. cerana* colonies (*N*=3, *n*=12 replicates)	Pure *A. mellifera* colonies (*N*=3, *n*=12 replicates)
Waxes	Cell size	*A. cerana* workers	*A. mellifera* workers	*A. cerana* workers	*A. mellifera* workers	*A. cerana* workers	*A. mellifera* workers
Apis cerana	*A. cerana*	3.5±2.2	18.0±5.7	3.3±2.1	18.2±9.0	–	16.8±9.8
	A. mellifera	5.1±2.4	16.6±6.1	2.5±2.3	17.0±7.5	–	21.2±9.7
Apis mellifera	*A. cerana*	4.1±2.4	17.0±3.3	1.4±1.2	18.1±8.2	–	19.3±10.4
	A. mellifera	3.4±3.3	16.5±4.9	1.9±2.0	19.2±4.5	–	15.8±10.6
	P-value	0.221	0.743	0.110	0.863	–	0.216

N is the number of pure colonies and *n* is the number of repetitions.

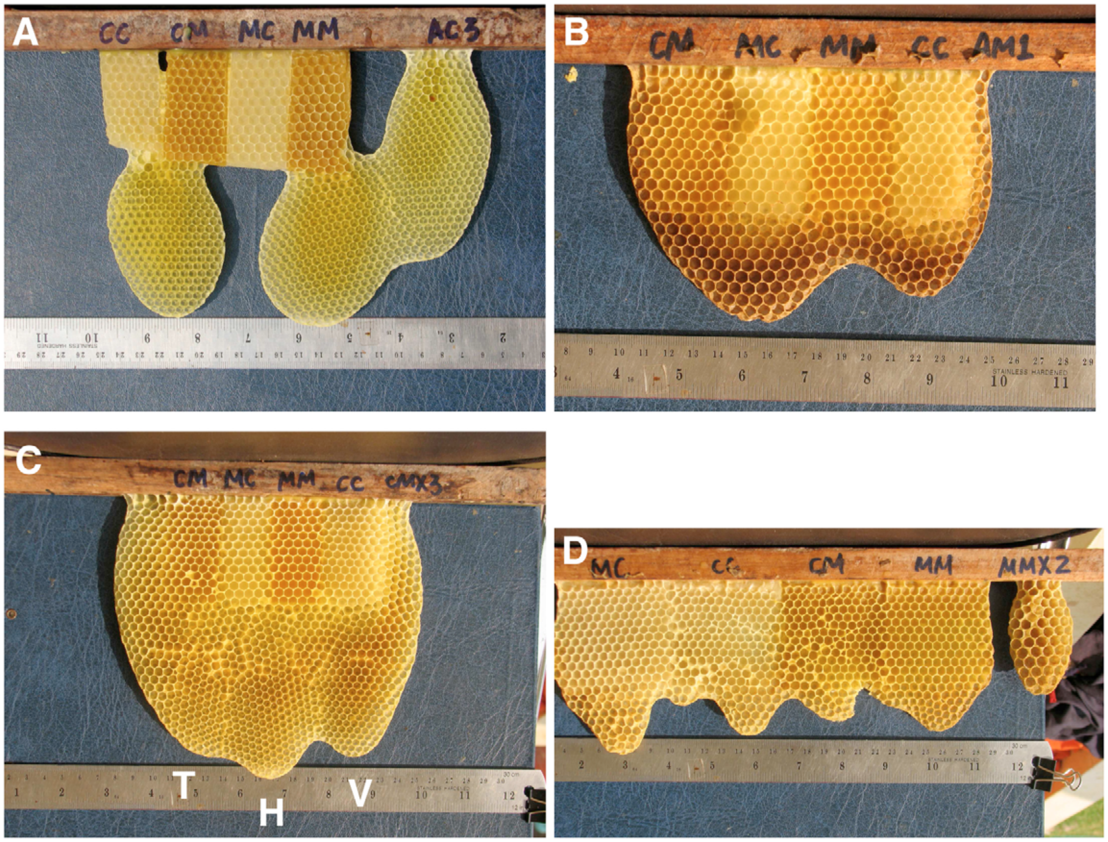

Fig. 1. Combs built in the four types of colonies. (A) Pure *Apis cerana*; (B) pure *Apis mellifera;* (C) *Apis cerana* queen-headed; and (D) *Apis mellifera* queen-headed colony (photographed in a horizontal perspective due to different widths). Abbreviations on the top bars are: CC=*A. cerana* cell-size foundation made from *A. cerana* wax; CM=*A. cerana* cell-size foundation made from *A. mellifera* wax; MM=*A. mellifera* cell-size foundation made from *A. mellifera* wax; MC=*A. mellifera* cell-size foundation made from *A. cerana* wax; cell direction patterns of newly built combs, V=vertical, H=horizontal, T=tilted. AC3=an example comb built by a pure *A. cerana* colony (colony 3). AM1=an example comb built by a pure *A. mellifera* colony (colony 1). CMX3=an example comb built by a mixed colony *A. cerana* queen headed (colony 3). MMX2=an example comb built by a mixed colony *A. mellifera* queen headed (colony 2).

Cell-size modification of foundation sheets

All of the *A. mellifera* cell-size sheets of foundation were built to their original size without any modification (Table 2) but the *A. cerana* cell-size foundation sheets were modified in all colonies except the pure *A. cerana* colonies. Some of these cells were squeezed to make space for enlarging neighbouring cells. The percentages of combs that had modified cells in the test and control groups are shown in Table 2. In *A. mellifera* queen-headed mixed colonies, all of the *A. cerana* foundation sheets were modified and nearly all in the pure *A. mellifera* colonies, which is significantly different from the *A. cerana* queen-headed mixed colonies and pure *A. cerana* colonies (Chi-square: χ^2_3=71.7, P<0.001).

Freely built combs

On completion of the trials of comb building on the artificial foundation sheets, the workers from the four types of colonies were observed to start building new combs at several sites (Table 3). Pure *A. mellifera* colonies and *A. mellifera* queen-headed mixed colonies had significantly more festoons at new comb-building sites than *A. cerana* and pure *A. cerana* queen-headed colonies (ANOVA: $F_{3,44}$=15.9, P<0.001; Table 3). In *A. cerana* queen-headed mixed colonies, workers of both species were seen working together in festoons, although significantly more *A. mellifera* workers were involved (42.1±6.2% *A. cerana* workers, 57.9±6.2% *A. mellifera* workers; paired *t*-test: t_{13}=4.9, P<0.001). Similarly, in *A. mellifera* queen-headed mixed colonies, significantly more *A. mellifera* workers than *A. cerana* workers were engaged in comb building in the festoons (32.5±4.8% *A. cerana* workers, 67.5±4.8%, *A. mellifera* workers; paired *t*-test: t_9=9.8, P<0.001; Table 3). In total, significantly more workers were engaged in comb building in the mixed colonies than in the pure *A. cerana* and pure *A. mellifera* colonies (ANOVA: $F_{3,44}$=11.3, P<0.001; Table 3).

As for irregular cells on the new combs, pure *A. cerana* and pure *A. mellifera* colonies built significantly fewer irregular cells (0.8% and 2.7%, respectively), than did the mixed colonies (9.1% and

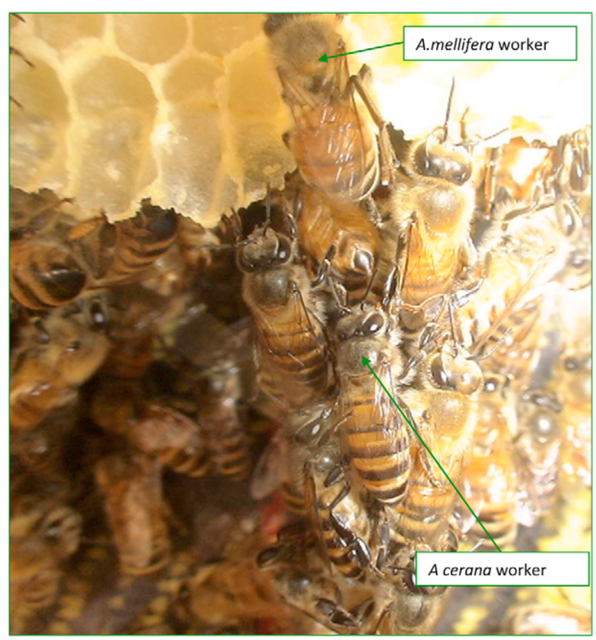

Fig. 2. Comb building by a mixed chain of *Apis cerana* and *Apis mellifera* workers.

Fig. 3. Utilisation of combs built on two types of cell-size foundation in pure *Apis cerana* colonies: the *Apis mellifera* size cells (left) were used for food storing while the *A. cerana* size cells (right) were used for brood rearing.

10.8%, respectively), most of which were located at the seams of combs which had been started at different sites (ANOVA: $F_{3,44}$=30.0, P=0.003; Table 3). The *A. cerana* queen-headed mixed colonies showed significantly greater variation in the patterns of cell orientation on the newly built combs than *A. mellifera* queen-headed colonies, pure *A. cerana* and *A. mellifera* colonies; different festoons on one comb may build patterns different from others (Chi-squared: χ^2_6=27.9, P<0.001; Fig. 1C; Table 3). *Apis mellifera* queen-headed colonies built new combs mainly in vertical and horizontal patterns (Fig. 1D); in pure *A. cerana* and *A. mellifera* colonies, the patterns of cell orientation were more homogeneous and mainly vertical (Figs 1A,B; Table 3).

The different mixed colonies built significantly different sized cells (ANOVA: $F_{3,44}$=34.8, P<0.001; Table 3). The largest cells were built by *A. mellifera* queen-headed mixed colonies. The cells built in the pure *A. mellifera* colonies and *A. mellifera*-queen-headed mixed colonies were like those *A. mellifera* drone cells (European type, 6.0–6.3 mm) whereas in the *A. cerana* queen-headed mixed colonies, the cells had a diameter of 5.41±0.27 mm, which is like normal *A. mellifera* worker size cells. The pure *A. cerana* colonies

Table 2. Percentages of *A. cerana* cell-size foundations with modified signs

Colony type	*Apis cerana* cell-size foundations: Number	*Apis cerana* cell-size foundations: Percentage with modified signs (%)	*Apis mellifera* cell-size foundations: Number	*Apis mellifera* cell-size foundations: Percentage with modified signs (%)
Pure *A. cerana* (*N*=3, *n*=12 replicates)	24	0	24	0
Pure *A. mellifera* (*N*=3, *n*=12 replicates)	24	83.3	24	0
A. cerana queen-headed mixed (*N*=3, *n*=14 replicates)	28	10.7	28	0
A. mellifera queen-headed mixed (*N*=3, *n*=10 replicates)	20	100	20	0

N is the number of pure colonies and *n* is the number of repetitions.

Table 3. Means (±s.d.) of characteristics of freely built combs

	Apis cerana queen-headed mixed colonies (*N*=3, *n*=14 replicates)	*Apis mellifera* queen-headed mixed colonies (*N*=3, *n*=10 replicates)	Pure *A. cerana* colonies (*N*=3, *n*=12 replicates)	Pure *A. mellifera* colonies (*N*=3, *n*=12 replicates)
Number of festoons	2.3^{b}±0.5	4.2^{a}±1.4	1.9^{b}±0.9	3.9^{a}±1.1
Number of *A. cerana* workers on the festoons	61.4±13.4	36.8±10.7	108.0±29.1	–
Number of *A. mellifera* workers on the festoons	84.6±16.1	75.6±16.3	–	90.3±25.0
Total number of two species workers on the festoons	146.1^{a}±22.0	112.4^{b}±24.5	108.0^{b} ±29.1	90.3^{b}±25.0
Percentage of irregular cells (%)	9.1^{a}±3.6	10.8^{a}±4.7	0.8^{b}±0.5	2.7^{b}±1.7
Patterns of the newly built combs: V=vertical H=horizontal T=tilted R=rosette	V+H: 29% V+H+T: 22% V+T: 21% V: 14%; T: 7% V+H+R: 7%	V+H: 60% V: 40%	V: 75% V+H: 17% T: 8%	V: 83% V+H: 17%
Cell size of the newly built combs (mm)	5.41^{b}±0.27	5.93^{a}±0.61	4.38^{c}±0.06	$5.74^{a,b}$±0.61

Means within one row followed by the same letter are not significantly different (Tukey multiple comparisons: P>0.05). *N* is the number of pure colonies and *n* is the number of repetitions.

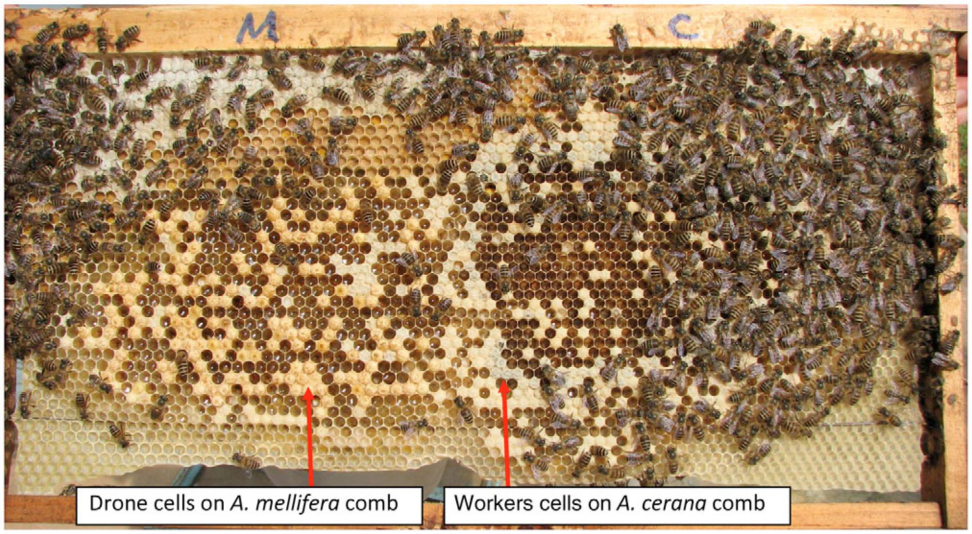

Fig. 4. Utilisation of combs built on two types of cell-size foundation in pure *Apis cerana* colonies: the *Apis mellifera* size cells (left) were used for drone brood rearing (with typical capping apertures) while the *A. cerana* size cells (right) were used for rearing worker brood.

built cells of 4.38±0.06 mm in size, which is the normal *A. cerana* worker cell size.

Utilisation of the newly built combs in the pure *A. cerana* and *A. mellifera* colonies

In these experiments, we inserted both *A. cerana* cell-size (4.75 mm diameter) foundation and *A. mellifera* cell-size (5.35 mm diameter) foundation into pure *A. cerana* colonies and pure *A. mellifera* colonies with the following results. Pure *A. cerana* colonies accepted both foundation types and built cells without altering the original cell base. Pure *A. mellifera* colonies accepted both foundation wax types but changed the *A. cerana* cell size to their normally larger cells with the inclusion of many irregular cells.

Once the control combs had been constructed, *A. cerana* colonies differed from *A. mellifera* colonies in the subsequent use of these cells. The pure *A. cerana* colonies used the *A. mellifera* size cells either for food storing (Fig. 3) or drone brood rearing while the *A. cerana* size cells were normally used for rearing worker brood (Fig. 4). In pure *A. mellifera* colonies, the queens laid eggs into both *A. mellifera* size cells and *A. cerana* size cells but they all showed a preference for *A. mellifera* size cells and laid eggs into the former cells first and more regularly (Fig. 5).

DISCUSSION

General comb building

It is common knowledge that the cavity-dwelling honeybees build multiple, parallel combs and that this parallelism is recognised as a building rule (Darchen, 1954; Hepburn, 1986; Hepburn and Muller, 1988). Comb-constructing bees work in a dark cavity or hive where there is no central source of information. When construction begins, the workers cling together in elongated chains or festoons, forming a dense cluster that facilitates an equable temperature for wax secretion and manipulation (Hepburn, 1986). Numerous comb-building workers, with active wax glands, engage in the task of comb construction. But, instead of building a single comb together, several festoons begin at independent sites and begin building several cells (hence combs) simultaneously and only later connect them with some irregular transitional cells (Hepburn, 1986; Hepburn and Whiffler, 1991). In this case, the parallelism rule can only be achieved indirectly at the finishing stage of comb building, with many irregular cells and seam connections between several branches started from separate sites (Hepburn and Whiffler, 1991). In this context it can be noted that *A. cerana* and *A. mellifera* workers cooperate heterospecifically in the same festoons in comb building.

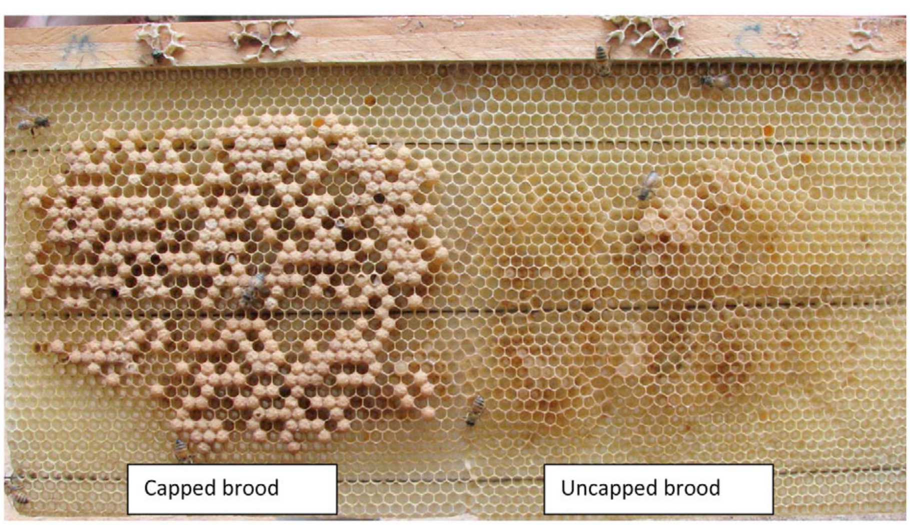

Fig. 5. Utilisation of combs built on two types of cell-size foundation in pure *Apis mellifera* colonies: the brood cells on the *A. mellifera* cells are capped already but the larvae on the *Apis cerana* side still need about three more days until capping, suggesting that the queens first laid eggs on the left side and only laid eggs in the *A. cerana* size cells somewhat later.

Comb building in mixed-species colonies

It is somewhat strange that in the pure *A. cerana* colonies, none of the four types of foundations were accepted, although two of the four foundations were embossed with normal *A. cerana* cell size. In sharp contrast, in the pure *A. mellifera* colonies, the workers were seen building cells on both types of wax and both cell sizes. These results indicate that *A. mellifera* workers are more tolerant of wax and cell-size factors. This contrast is revisited in both types of mixed colonies where more *A. mellifera* workers than *A. cerana* workers were seen building comb, irrespective of the host queen. However, interestingly, *A. cerana* workers did engage in comb building on foundations of both waxes and the two cell sizes in the both types of the mixed colonies (Table 1). This certainly suggests that *A. mellifera* comb-building workers can stimulate *A. cerana* workers to start comb building. And, a comb-building stimulus appears reciprocal because in pure *A. mellifera* colonies, while 83.3% of the *A. cerana* cell-size foundation sheets were modified and expanded to *A. mellifera* cell-size, only 10.7% were modified in mixed colonies headed by *A. cerana* queens. In the *A. cerana* queen-headed mixed colonies, more *A. mellifera* workers were engaged in comb building in festoons, so it is not surprising that the cell sizes were similar to normal *A. mellifera* workers.

It is interesting to note that in this type (*A. cerana* queen-headed) of mixed colony, the festoons were formed predominately by *A. mellifera* workers with fewer *A. cerana* workers joining them. However, the combs built in the mixed colonies did have more irregular cells than were observed in any of the pure *A. cerana* or *A. mellifera* colonies. This seems to indicate that the *A. cerana* workers also play a role in determining final cell-size. Although they did cooperate with each other in festoons, the two species cannot really perform the comb-building task harmoniously. The fact that the combs in the pure *A. mellifera* colonies and *A. mellifera* queen-headed colonies were built into normal *A. mellifera* drone size cells may be related to the season in which we conducted the experiment.

In conclusion, the *A. cerana* workers, as a colony did not accept any type of beeswax foundation but as individuals can be stimulated by *A. mellifera* workers to engage in comb building. So, our results are consistent with the idea that honeybee comb-building behaviour is an example of self-organisation. We also confirm that in the mixed colonies, these two closely related honeybee species did in fact cooperate in comb building, even though irregular cells arise through their joint efforts. We can also infer that, although the comb-building workers are poorly informed and lack a central controller (Pratt, 2004), comb building is really a task that can only be finished by some smaller groups in which individuals closely cooperate to achieve progress. This might explain, in part, why *A. mellifera* workers do not dominate the comb-building effort.

The results presented here based on mixed-species colonies reinforce the conclusion that this experimental method is extremely useful for testing underlying mechanisms that evoke or suppress certain behaviours. Such an experimental context has been successfully used to elucidate disruption of social networks as in ovarial activation (Tan et al., 2009) as well as stimulation of social networks as in the dance language (Tan et al., 2008) and retinue behaviour towards queens (Yang et al., 2010a). The results from the comb-building experiments provide additional evidence for the value of mixed-species colonies as experimental probes.

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REFERENCES

Aichholz, R. and Lorbeer, E. (1999). Investigation of combwax of honeybees with high-temperature gas chromatography and high-temperature gas chromatography-chemical ionization mass spectrometry. I. High-temperature gas chromatography. *J. Chromatogr. A* **855**, 601-615.

Belic, M. R., Skarka, V., Deneubourg, J. L. and Lax, M. (1986). Mathematical model of honeycomb construction. *J. Math. Biol.* **24**, 437-449.

Bonabeau, E., Theraulaz, G., Deneubourg, J. L., Aron, S. and Camazine, S. (1997). Self-organization in social insects. *TREE* **12**, 188-193.

Boomsma, J. J. and Franks, N. R. (2006). Social insects: from selfish genes to self organisation and beyond. *TREE* **21**, 303-308.

Buchwald, R., Breed, M. D., Greenberg, A. R. and Otis, G. W. (2006). Interspecific variation in beeswax as a biological construction material. *J. Exp. Biol.* **209**, 3984-3989.

Camazine, S. (1991). Self-organizing pattern formation on the combs of honey bee colonies. *J. Behav. Ecol. Sociobiol.* **28**, 61-76.

Camazine, S., Sneyd, J., Jenkins, M. J. and Murray, J. D. (1990). A mathematical model of self-organized pattern formation on the combs of honeybee colonies. *J. Theor. Biol.* **147**, 553-551.

Darchen, R. (1954). Quelques regulations sociales dans les constructions chez les abeilles. *Insectes Soc.* **1**, 219-228.

Detrain, C. and Deneubourg, J. L. (2006). Self-organized structures in a superorganism: do ants 'behave' like molecules? *Physics Life. Rev.* **3**, 162-187.

Hepburn, H. R. (1986). *Honeybees and Wax: An Experimental Natural History.* Berlin: Springer-Verlag.

Hepburn, H. R. (1998). Reciprocal interactions between honeybees and combs in the integration of some colony function in *Apis mellifera* L. *Apidologie* **29**, 47-66.

Hepburn, H. R. and Muller, W. J. (1988). Wax secretion in honeybees. Thermal integrity of the festoon. *Naturwissenschaften* **75**, 628-629.

Hepburn, H. R. and Whiffler, L. A. (1991). Construction defects define pattern and method in comb building by honeybees. *Apidologie* **22**, 381-388.

Hepburn, H. R., Hugo, J., Mitchell, D., Nijland, M. J. M. and Scrimgeour, A. (1984). On the energetic costs of wax production by the African honeybee, *Apis mellifera adansonii. S. Afr. J. Sci.* **80**, 363-368.

Hepburn, H., Radloff, S. E., Duangphakdee, O. and Phiancharoen, M. (2009). Interspecific utilization of wax in comb building by honeybees. *Naturwissenschaften* **96**, 719-723.

Jenkins, M. J., Sneyd, J., Camazine, S. and Murray, J. D. (1992). On a simplified model for pattern formation in honey bee colonies. *J. Math. Biol.* **30**, 281-306.

Johnson, R. A. and Wichern, D. W. (2002). *Applied Multivariate Statistical Analysis*, 5th edn. Upper Saddle River: Prentice Hall.

Pirk, C. W. W., Hepburn, H. R., Radloff, S. E. and Tautz, J. (2004). Honeybee combs: construction through a liquid equilibrium process? *Naturwissenschaften* **91**, 350-353.

Pratt, S. C. (2004). Collective control of the timing and type of comb construction by honey bees (*Apis mellifera*). *Apidologie* **35**,193-205.

Rösch, G. A. (1927). Untersuchungen über die Arbeitsteilung und das Alter der Baubienen. Weiterer Bautrag zur Frage nach der Arbeitsteilung im Bienenstaat. *Zeit. Vergl. Physiol.* **12**, 1-71.

Ruttner, F. (1988). *Biogeography and Taxonomy of Honeybee.* New York: Springer-Verlag.

Seeley, T. D. (1995). *The Wisdom of Hive: the Social Physiology of Honey Bee Colonies.* London: Harvard University Press.

Su, S., Cai, F., Si, A., Zhang, S. and Tautz, J. (2008). East learns from west: asiatic honeybees can understand dance language of european honeybees. *PLoS ONE* **3**, e2365.

Tan, K., Hepburn, H. R., He, S. Y., Radloff, S. E., Neumann, P. and Fang, X. (2006). Gigantism in honeybees: *Apis cerana* queens reared in mixed-species colonies. *Naturwissenschaften* **93**, 315-320.

Tan, K., Yang, M. X., Radloff, S. E., Hepburn, H. R., Zhang, Z. Y., Luo, L. J. and Li, H. (2008). Dancing to different tunes: heterospecific deciphering of the honeybee waggle dance. *Naturwissenschaften* **95**, 1165-1168.

Tan, K., Yang, M. X., Radloff, S. E., Pirk, C. W. W., Crewe, R. M., Phiancharoen, M., Hepburn, H. R. and Oldroyd B. P. (2009). Worker reproduction in mixed-species colonies of honey bees. *J. Behav. Ecol.* **20**, 1106-1110.

Wilson, E. O. (1971). *The Insect Societies.* Cambridge: Belknap Press, Harvard University Press.

Winston, M. L. (1987). *The Biology of the Honey Bee.* London: Harvard University Press.

Yang, M. X., Tan, K., Radloff, S. E., Pirk C. W. W. and Hepburn, H. R. (2010a). Hetero-specific queen retinue behavior of worker bees in mixed-species colonies of *Apis cerana* and *Apis mellifera*. *Apidologie* **41**, 54-61.

Yang, M. X., Wang, Z.-W., Li, H., Zhang, Z.-Y., Tan, K., Radloff, S. E. and Hepburn, H. R. (2010b). Thermoregulation in mixed species colonies of honeybees (*Apis cerana* and *Apis mellifera*). *J. Insect. Physiol.* doi:10.1016/j.jinsphys.2009.12.011.