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Spatial Distribution and Age-specific Thermal Reaction of Worker Honeybees

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Abstract

Marked worker honeybees, *Apis mellifera*, on combs were photographed in 2 observation hives, and their spatial distributions were checked in detail. Younger workers were distributed more on the warmer central area, older workers, with stronger negative geotaxis, on the cooler peripheral areas of the comb. It is suggested that the distribution creates the cluster core-shell structure, which is based on thermogenic ability and relates to nest pattern based on cell utilization and the division of labor among workers.

Key words: spacing honeybees, age-specific reaction, negative geotaxis, thermoregulation, core-shell structure, division of labor

Introduction

Thermoregulation in honeybee colonies can be regarded as one of the major innovations in their biology (Seeley, 1985), and is supported by the behavioral mechanisms which compensate for a change in environmental temperature (Lindauer, 1961). The worker behavior associated with cluster expansion or contraction, however, has never been observed directly. Many worker behaviors have been explained by a hypothetical division of labor among workers or "age polyethism". Does thermoregulatory behavior have any connection with the famous age polyethism in honeybees first confirmed by Rösch (1925) ? Only Heinrich (1987) referred to age polyethism in thermoregulation, but he no more than pointed out how little we understand the individual bee's contribution to hive thermoregulation and the dependence of younger workers on hive microclimate.

In normal apiary hives, Free (1960) found younger workers on brood combs, while older workers were on storage combs. He considered that younger workers congregated to the center of the hive in order to nurse brood and older workers to the periphery in order to forage. Seeley (1982) examined the spatial distribution of the workers engaged in 13 tasks within his large observation hive. He discussed the adaptive origin of age polyethism, but did not consider the relation with thermoregulation.

The spatial distribution of adult drones on hives and combs has been investigated in apiary and observation hives, and is assumed to be linked with age-specific thermal reaction (Ohtani and Fukuda, 1977): Younger drones before flight stayed near the hive center and the comb center, while older ones were at peripheral areas. As drones are taskless (Mindt, 1962; Ohtani, 1974), they have no connection with age polyethism. It is possible that workers possess basically the same thermal reaction as drones, because younger workers preferred higher temperature to older ones in experimental conditions (Heran, 1952;

Brückner, 1976), and innermost bees were significantly younger than outermost bees in a one-frame observation hive (Harrison, 1987).

If the spatial distribution of workers could be observed directly in an observation hive, we would obtain further information on the relationship between thermoregulation and age polyethism. Although continuous videotape tracking of marked workers facilitates sutdy of behavioral dynamics, such technology is not always required for analysis of spatial distribution. We can get much informations from the fixed-moment data of still photographs shot with a camera. Therefore, data are extracted unpublished file photos and analyzed in this study of age-specific thermal reaction and spatial distribution of worker honeybees.

Material and Method

Honeybees were of the unpure Italian race (*Apis mellifera ligustica* Spinola) usually kept in Japanese apiaries. Several colonies were kept in a campus apiary of Hokkaido University for supplying brood combs for an incubator, from which newly emerged workers were picked up daily. They were marked individually on their mesosomal dorsa with numbered paper disks by sticking with a binding agent (Bond G17, Konishi Inc.), and kept in 2 observation hives during a period of different years. When worker numbers reached about 300 or 1500, the observational faces of hives were photographed at 05.00 h on October 4 in 1975 and on May 13 in 1979, and the workers positions on the comb were subsequently traced and analized.

Hive temperature was recorded as in Lavie (1954) by a temperature recorder (6 thermistor sensors, Chino Works, Ltd.) with the condition of no worker cover in 1979.

A more detail procedure will be described for each result.

Results and Discussions

1. Spacing on a one-sided comb (1975)

Thirty newly-emerged workers were labelled and released everyday in an observation hive with a one-sided comb whose cells were all empty. On subsequent days additional newly-emerged workers were introduced, thus forming an age-diverse population of marked worker honeybees. The releasing point was the hive floor anywhere. The procedure started on September 12, and a virgin queen was introduced 3 d(= days) later. When the oldest workers became 23 d old, 289 bees on the comb surface were photographed and drawn schematically in Fig. 1.

Older workers generally stayed remote from an electric bulb (40W) which was used as the heat source, and concentrated particularly near the lower right corner which received the cold air current (about 11℃) from the hive entrance. The correlation between workers' age and the distance from the bulb is significant for the popultation as a whole (degrees of freedom = 287), given as $r=0.4253$ ($p<0.001$). The mean distance from the bulb was plotted for each same-age group of workers (Fig. 2). We found significant differences between mean distances of adjoining groups younger than 8 d ($r=0.9736$, $p<0.001$, d.f.= 6), but a gradual increase among age groups until 15 d old was not significant ($r=0.6410$, $p>0.1$, d.f.= 5). Correlation coefficient of all plots is 0.7402 ($p<0.005$, d.f.=15).

S1 (Summary 1): *On a one-sided comb*

Fig. 1. Distribution of workers on a one-sided comb. Schematized from a photograph taken at 05.00 h on October 4 in 1975. Numerals on mesosomata denote the age (d), and older workers are more darkly shaded. Q within double circles indicates a virgin queen. An electric bulb (40W) as a heat source is set 10 cm distant from the comb surface.

with a thermal gradient, younger workers were distributed on warmer cell surface, while older workers were far toward the cooler area near the hive entrance.

A glance at Fig. 1 shows that older workers seem to exhibit a stronger negative geotaxis. This tendency is more precisely presented in Fig. 3, in which the upward directing ratio ($\pm 60°$) is highest in the oldest workers (17-22 d old), whereas younger workers (1-4 d old) were most weakly geonegative. A significant difference in the upward directing ratio was obtained only between the youngest and oldest workers ($p<0.05$, binominal test).

The upward directing ratio seemed to occur more highly in the left half than in the right half of 3 out of 4 graphs in Fig. 3. A significant difference between ratios in the left and right halves, however, was detected only in the youngest workers by the binominal test (22 vs. 10). This suggests that the youngest workers are more sensitive and attracted by the heat source.

S2: *On the one-sided comb, negative geotaxis tended to increase with age, and*

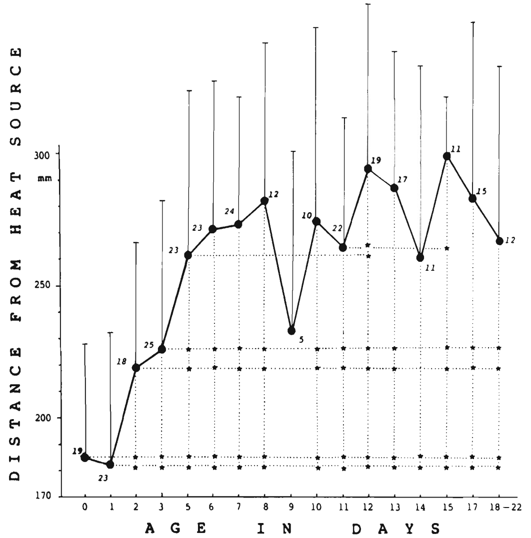

Fig. 2. Relationship between mean distances (mm) from the electric bulb (ordinate) and workers' age (abscissa) in Fig. 1. Numerals in the graph indicate the worker numbers in the same age groups. Small black stars on the cross points of dot lines indicate the significant differences in mean distances between 2 age groups (Mann-Whitney U-test). Vertical lines denote the halves of standard deviations in mean distances.

the youngest workers directed their heads to the heat source.

2. Distribution in a heated observation hive (1979)

The above results were obtained under conditions of a small colony (only 289 workers), a virgin queen and a one-sided comb without brood on October. Moreover, no hive temperatures were measured. Therefore, observations under more favorable conditions to bees were planned, with measuring of hive temperature included.

A comb with about 200 adult workers and a mated queen was set in a one-frame observation hive provided with a heater and thermostat on the bottom. About 400 newly emerged workers were labelled and released everyday into the observation hive for the first 6 days; thereafter about 50 workers were daily labelled and released (cf. Table 1, N). The releasing point was usually the left upper corner of the wooden comb frame, to avoid the heater at the hive bottom. The queen laid eggs every day. As no-marked bees had disappeared from the hive when the oldest marked bees became 27 d old, 1352

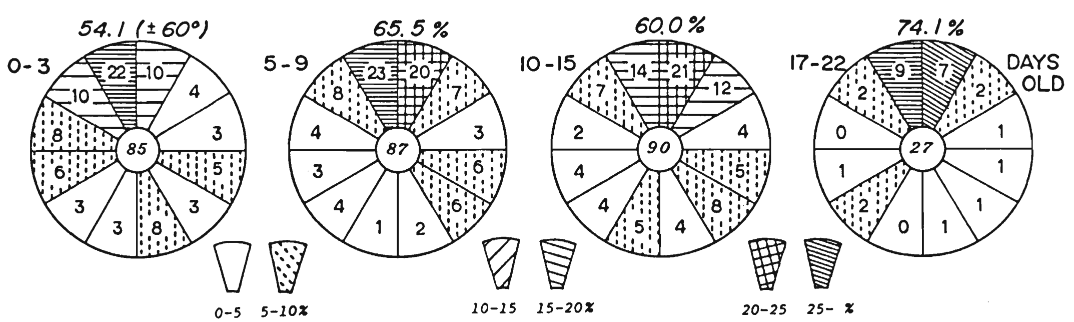

Fig. 3. Directions of body axis in workers of 4 age groups (shown at the upper left side of each circle graph) in Fig. 1. Numerals in each graph give the total number of worker on the center and the number of workers in each 30° sector whose relative frequency is shown by an appropriate shading (divided into 6 groups; see the bottom). The percentage of workers which directed upward with a range of ±60° is shown at the top of each graph.

Table 1. Mean distances from the center worker cluster (A: white stars in Fig. 5) or from the center of brood area (B: black stars) in each age group.

	AGE GROUP	<1>				<2>								<3>				<4>					Correlation
	AGE IN DAYS	5	6	8	9	10	11	12	13	14	15	16	17	19	20	22	23	24	25	26	27	TOTAL	Coefficient With Age
	TOTAL MARKED BEES (N)	50	50	100	50	50	50	50	50	50	17	43	38	50	50	400	400	400	400	400	355	3053	
S SIDE	A (mm)	53.8	86.9	97.6	100.1	118.5	99.9	91.9	121.6	106.8	77.8	90.3	50.5	111.6	104.3	109.7	127.5	119.4	100.4	121.4	92.0		→ r=0.3920
	SD	42.3	25.3	42.6	41.8	44.0	36.2	29.7	43.2	58.5	21.7	42.5	37.5	44.6	27.8	49.8	50.7	44.1	49.8	53.6	49.0		cf. r=0.1274
			r=0.8848						r= -0.7341						r=0.6809				r= -0.5483				(d.f.=549)
						r= -0.0137												r= -0.1645					
	B (mm)	77.8	64.5	101.5	116.5	121.5	88.5	93.3	108.7	126.8	106.8	82.3	85.8	127.3	122.2	110.5	126.7	119.7	104.5	129.4	120.1		→ r=0.5818**
	SD	11.1	30.2	55.7	49.0	52.8	42.9	41.6	59.8	63.7	36.8	36.6	38.6	60.7	39.4	51.1	53.7	51.3	53.2	63.8	54.7		cf. r=0.1274
			r=0.8951						r= -0.3863						r= -0.3023				r=0.3269				(d.f.=549)
						r=0.2536												r= -0.1710					
	TOTAL (sn)	4	8	28	10	13	15	19	16	17	5	12	8	13	12	134	69	59	48	40	21	551 (43.0 %)	
	sn/(sn+nn)x100	16.7	29.6	28.9	20.8	30.2	31.9	40.4	39.0	36.2	38.5	35.3	40.0	40.6	36.4	47.9	53.5	55.1	49.0	58.8	46.7		→ r=0.9007****
N SIDE	A (mm)	83.6	79.5	92.7	114.0	110.9	108.0	118.9	106.8	108.6	95.1	116.3	112.2	92.2	107.8	115.9	121.0	118.4	134.9	127.6	116.5		→ r=0.6990****
	SD	41.4	40.5	39.5	48.2	52.6	47.0	51.7	50.0	50.3	46.8	45.7	39.3	39.9	43.9	49.4	48.4	52.3	48.4	46.2	57.0		cf. r=0.1966
			r=0.8779						r= -0.0771						r=0.9532 *				r= -0.1965				(d.f.=727)
						r=0.6543*												r= 0.7726*					
	B (mm)	86.8	89.9	94.4	112.0	129.2	116.6	128.6	97.6	113.0	112.6	111.8	113.1	102.5	110.0	113.4	118.6	117.9	125.6	117.3	113.3		→ r=0.4852*
	SD	42.2	45.3	43.6	48.5	50.8	56.3	57.2	44.6	61.4	53.8	49.4	44.4	48.2	48.4	52.2	49.0	51.0	48.3	47.2	55.4		cf. r=0.1120
			r=0.8904						r= -0.4886						r=0.9628 *				r= -0.5551				(d.f.=727)
						r= 0.5237												r= 0.6782					
	TOTAL (nn)	20	19	69	38	30	32	28	25	30	8	22	12	19	21	146	60	48	50	28	24	729	
	AVERAGE (only A)	68.7	83.2	95.2	107.1	114.7	104.0	105.4	114.2	107.7	86.5	103.3	81.4	101.9	106.1	112.8	124.3	118.9	117.7	124.5	104.3		→ r=0.6101***
	NO. OF BEES ON WOODEN FRAME (wn)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	1	6	6	3		
	TOTAL (sn+nn+wn)	24	27	97	48	43	47	47	41	47	13	34	20	32	34	280	132	108	104	74	48	1300	
	(sn+nn+wn)/N x 100	48.0	54.0	97.0	96.0	86.0	94.0	94.0	82.0	94.0	76.5	79.1	52.6	64.0	68.0	70.0	33.0	27.0	26.0	18.5	13.5	42.6 %	

* P<0.05; ** P<0.01; *** P<0.005; **** P<0.001

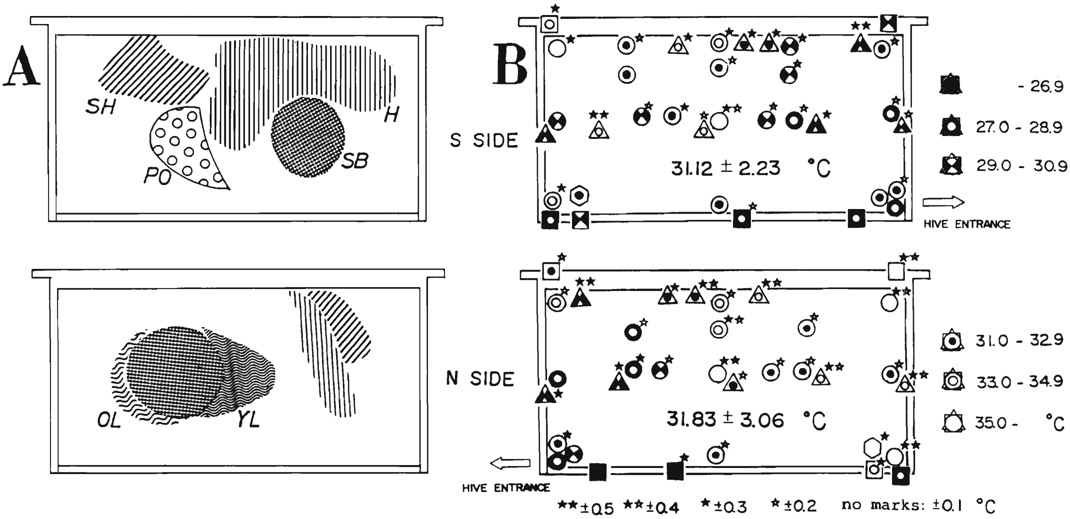

Fig. 4. Spatial arrangement of cell utility (A) and temperature gradient by a bottom heater (B) on a comb in a one-frame observation hive. A: *SH* = sealed honey cells, *H* = unsealed honey cells, *PO* = pollen cells, *SB* = sealed brood cells, *OL* = old larva cells, and *YL* = young larva cells. B: Temperature was measured on an empty comb after the observation. Measurement points are on the wooden frame (squares); on the cells (circles); 1 cm above the cells (hexagons); 4 cm above the cells (triangles). Small stars near the symbols indicate their range of temperature fluctuation. From these temperatures were calculated the average temperatures with their standard deviations independently in the south (S) side and the north (N) side.

labelled bees were photographed on May 13 (the numbers of 47 bees were unreadable: marked x in Fig. 5). Some of the youngest workers probably could not be shot because of the creaping into cells for nursing or cell cleaning. We can see low values (48.0 and 54.0 %) recapture rates within the photo of 5- and 6-d-old workers (cf. the bottom in Table 1). Considering the survivorship curve of workers by Sakagami and Fukuda (1968), their expected rates is as high as those of 8- or 9-d-old workers.

Sides of the comb faced north or south (Fig. 4). Because of the location of the hive entrance, the comb was placed nearer the south (S) wall glass so that the space between the comb surface and the north (N) wall glass doubled that between the other side and S wall glass. The discrepancy in the spaces seemed to have caused a difference in the number of eggs laid by the queen and in the amount of pollen and honey stored (Fig. 4A). In order to know the basic pattern of temperature distribution, hive temperature was measured on an empty and bee-free comb after the observations (Fig. 4B). The temperature of N side was somewhat higher and more unstable than on S side. The queen laid eggs more frequently on N side than on S side. The somewhat unstable feature was probably compensated by the worker cover.

The distribution of workers is given in Fig. 5 and Table 1 by 4 age groups. A glance at the figure shows that younger workers were found more on N side that on S side, while older workers behaved reversely. On S side were found 43.0 % of all workers. The

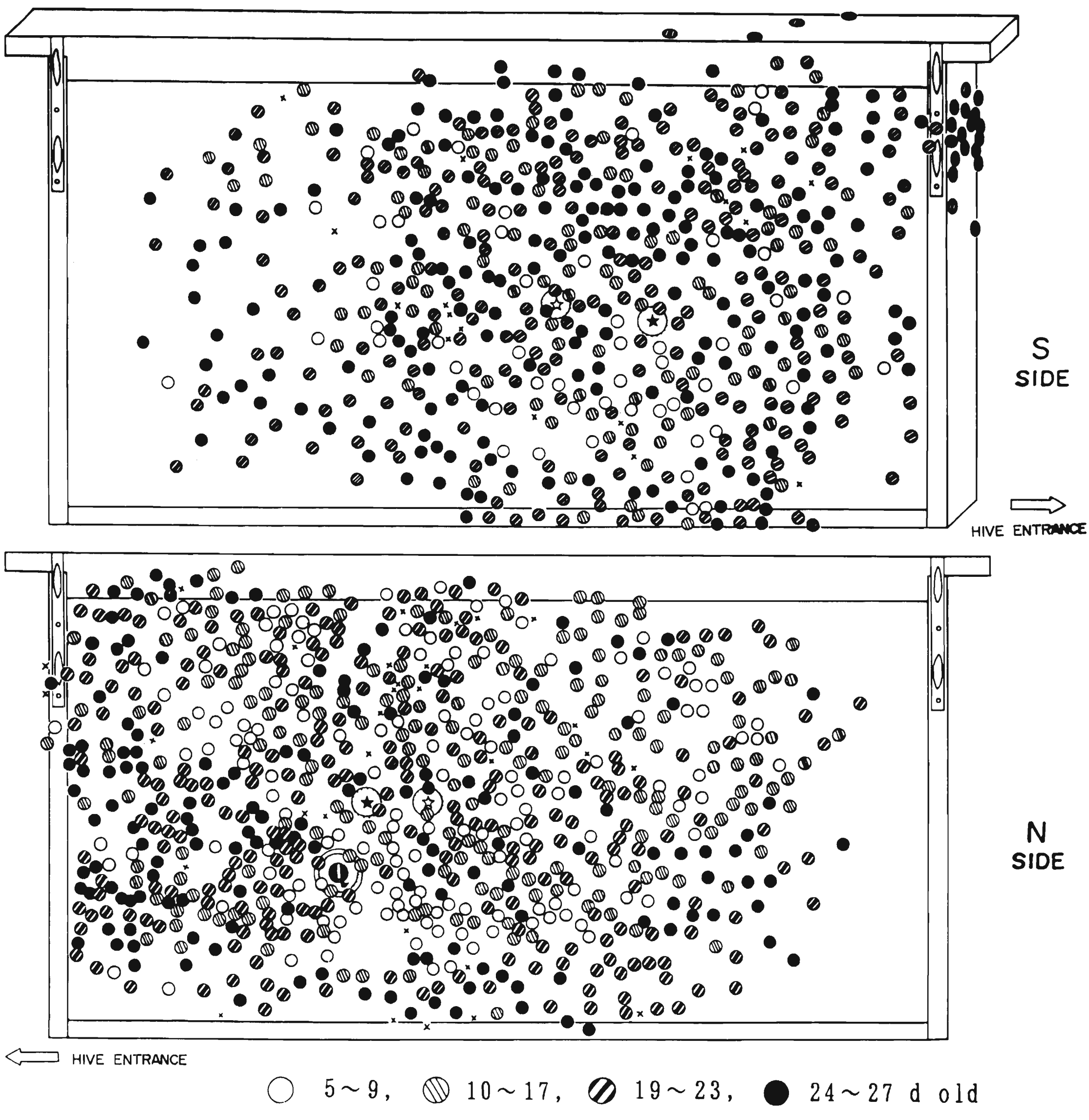

Fig. 5. Spatial distributions of workers on the comb in Fig. 4 from the photographs taken at 05.00 h on May 13 in 1979. The position of each worker is given by one of hatching circles of 4 age groups. Marks x denote the workers whose individual numbers could not be read from the photos. Black and white stars within circles are the center of the brood area and the worker cluster, respectively. Q within double circles is the position of a laying queen.

percentage represented by workers younger than 20 d old was lower than 43 % on S side (cf. the numerals with asterisks in Table 1). The correlation coefficient between the percentage and workers' age is high, and is significant ($r=0.9007$, $p<0.001$, d.f.$=18$).

With respect to distribution of workers and hive contents, N side resembles the central combs in apiary hives, while S side the peripheral combs with lower temperature, less brood, more honey stores and monopolized pollen stores, according to my long apiary experience.

S 3: *In a one-frame hive with brood and bottom heater, there are more workers, esp. younger workers, on N side than on S side, which has a peripheral aspect in apiary hive contents.*

In order to detect the centripetal tendency of younger workers, 2 centers were set up: the center of the brood area (black stars in Fig. 5) and that of the worker cluster (white stars). The distances from both centers were measured for each individual and summed up for each age group (Fig. 5; Table 1). There is no significant difference between mean distances from the 2 centers in each group (Mann-Whitney U-test). However, the correlations with worker ages were various and significant (3 out of 4) with degrees of freedom of 18, having no significant difference between 2 values in the worker center ($r=0.3920$; 0.6990) and the brood center ($r=0.5818$; 0.4852).

Correlation coefficients were also calculated with narrower ranges of age (Table 1). Only the ⟨3⟩ group on N side had statistical significance (A: $r=0.9532$; B: $r=0.9628$).

In ⟨1⟩ + ⟨2⟩ and ⟨3⟩ + ⟨4⟩ age groups, the significance was detected only in the A group of N side that suggests the relationship with different hive contents and the temperatures of both sides.

Therefore, performance of the bees on various conditions were summarized in Table 2. As 15 cm wider ranges were adopted in the boundaries of various hive contents, considering the body length of bees, some bees were counted within 2 or more ranges. Percentages of counted numbers on various conditions were tested for the discrepancy from whole distributions with age, adopting G-test by Sokal and Rohlf (1973). In order to detect an age discrepancy, individual values were checked with χ^2-test by very generous criterion ($p<0.5$, $p<0.1$, $p<0.05$).

Workers on sealed brood cells are younger, but there is a statistical significance only within a small area on the S side. Similar tendency is found in workers on honey store cells, *i.e.* there is a significance only within a small area on the N side. This suggests that older workers seemingly avoid these areas rather than younger workers seek them. In the similar manner, older worker numbers on sealed honey cells, with significances in both sides, can be regarded as a passive result. This idea is supported by workers on young and old larvae cells. Younger workers did not seem to seek larvae cells. Perhaps, older workers (= foragers) prefer empty cells to other area for resting (cf. data in S side). In large empty range, however, younger workers must enter randomly (cf. data in N side). Wooden frames in both sides were preferred by older workers for resting. The wooden frame surface, except both sides, was also selected by older workers (cf. Table 1 and Fig. 5).

Younger workers seemed to seek a comfortable temperature rather than some hive contents to work. Higher temperature (33℃)

Table 2. Percentages of worker numbers in each age on cells with various contents and temperature. Values with underline are treated together (average used), avoiding 0-value or opposite tendency.

	WORKER AGE IN DAYS	5	6	8	9	10	11	12	13	14	15	16	17	19	20	22	23	24	25	26	27	TOTAL BEES	
	TOTAL BEES (sn)	4	8	28	10	13	15	19	16	17	5	12	8	13	12	134	69	59	48	40	21	551	
	sn/Σx100	0.7	1.5	5.1	1.8	2.4	2.7	3.4	2.9	3.1	0.9	2.2	1.5	2.4	2.2	24.3	12.5	10.7	8.7	7.3	3.8	100.0	G-test
	SEALED BROOD %	0.0	5.2^{+}	10.3^{+}	2.1	1.0	5.2^{+}	6.2^{+}	4.1	5.2^{+}	0.0	4.1^{+}	3.1^{+}	1.0^{-}	1.0^{-}	24.7	4.1^{---}	8.2	7.2	4.1^{-}	3.1	97	$p<0.05$
	HONEY STORE %	1.8^{+}	0.4^{-}	3.1	2.2	2.7	1.8	3.6	2.7	2.2	1.3	2.2	3.1^{+}	3.1	2.2	22.9	12.6	9.9	10.8	6.3	4.9	223	ns
S	SEALED HONEY %	0.0	0.0	2.2	6.7	6.7^{+}	2.2	4.4	4.4	8.9^{++}	0.0	0.0	0.0	4.4	2.2	26.7	11.1	4.4^{-}	2.2^{---}	8.9	4.4	45	$p<0.01$
SIDE	POLLEN STORE %	0.0	0.0	6.0	3.0	0.0	4.5	1.5^{-}	1.5^{-}	3.0	1.5	3.0	3.0^{+}	3.0	3.0	25.4	9.0^{-}	9.0	9.0	6.0	9.0^{+}	67	ns
	EMPTY CELLS %	0.0	1.6	6.6	0.0	2.5	2.5	2.5	2.5	0.0	0.8^{-}	2.5	0.0	0.8	1.6	20.5	15.6	18.0^{+}	8.2	12.3	1.6	122	$p<0.01$
	WOODEN FRAME %	0.0	0.0	0.0^{---}	0.0	2.4	0.0^{-}	2.4	0.0^{--}	4.8	0.0	0.0^{-}	0.0	0.0^{-}	4.8^{+}	23.8	23.8^{++}	11.9	14.3^{+}	7.1	4.8	42	$p<0.01$
	TOTAL BEES (nn)	20	19	69	38	30	32	28	25	30	8	22	12	19	21	146	60	48	50	28	24	729	
	nn/Σx100	2.7	1.5	9.5	5.2	4.1	4.4	3.8	3.4	4.1	1.1	3.0	1.6	2.6	2.9	20.0	8.2	6.6	6.9	3.8	3.3	100.0	G-test
	SEALED BROOD %	5.4^{+}	4.2^{+}	14.4^{+}	5.4	3.0	3.6	3.6	4.8	6.0	1.8	3.6	0.6	4.2	2.4	15.6^{-}	4.8^{-}	6.0	3.6	3.6	3.6	167	ns
	HONEY STORE %	0.0	3.3	8.2	9.8^{+}	6.6^{+}	9.8^{+}	9.8^{+}	0.0	6.6	1.6	0.0	3.3	0.0	4.9	13.1^{-}	4.9^{-}	4.9	4.9	4.9	3.3	61	$p<0.01$
N	SEALED HONEY %	4.3	0.0	4.3	4.3	8.7^{+}	8.7^{+}	8.7^{+}	4.3	8.7^{+}	0.0	4.3	0.0	4.3	4.3	21.7	8.7	0.0^{---}	0.0^{--}	0.0^{--}	4.3	23	$p<0.01$
SIDE	YOUNG LARVAE %	0.0^{-}	0.0	6.0^{-}	3.0^{-}	4.4	4.4	3.5	1.8^{-}	6.1	3.5	0.9	0.9	4.4^{+}	3.5	20.2	7.0	4.4	4.4^{-}	1.8^{-}	1.8	114	ns
	OLD LARVAE %	9.1	0.0	9.1	9.1	0.0	3.6	0.0^{--}	1.8^{-}	0.0^{---}	1.8	1.8	1.8	3.6	3.6	23.6	5.5	9.1	5.5	5.5	5.5^{+}	55	$p<0.05$
	EMPTY CELLS %	1.6	2.6	9.2	3.7	4.0	3.7	3.4	4.2	2.4	0.5	4.0	2.1	2.6	2.9	20.3	9.0	8.7	8.2	4.0	2.9	379	ns
	WOODEN FRAME %	0.0^{-}	0.0	4.8^{-}	4.8	9.5	14.3	4.8	0.0	4.8	0.0	0.0	0.0	0.0	4.8	0.0	14.3^{-}	9.5^{+}	14.3^{+}	4.8	9.5^{++}	21	$p<0.01$
	TOTAL BEES (sn+nn)	24	27	97	48	43	47	47	41	47	13	34	20	32	33	280	129	107	98	68	45	1280	
	(sn+nn)/Σx100	1.9	2.1	7.6	3.8	3.4	3.7	3.7	3.2	3.7	1.0	2.7	1.6	2.5	2.6	21.9	10.1	8.4	7.7	5.3	3.5	100.0	G-test
	○ 35℃ %	0.0	0.0	5.3	0.0^{-}	10.5	5.3	0.0	0.0	5.3	0.0	0.0	0.0	0.0	0.0	36.8	5.3	0.0	5.3	10.5	15.8^{+++}	19	$p<0.0$
	◎ 33℃ %	2.5	0.0	17.5^{++}	7.5^{+}	2.5	0.0	5.0	0.0	5.0	0.0	0.0	5.0	7.5	5.0	22.5	5.0	7.5	5.0^{-}	0.0	2.5^{-}	40	$p<0.01$
	◬ 33℃ %	0.0	3.7	7.4	3.7	0.0	11.1	11.1^{++}	3.7	7.4	0.0	3.7	0.0	0.0	3.7	14.8^{-}	3.7^{--}	11.1	11.1	0.0	3.7	27	$p<0.01$
BOTH	◉ 31℃ %	0.0	1.1	9.2	6.9^{+}	1.1	5.7	4.6	3.4	3.4	0.0	1.1	2.3	3.4	3.4	24.1	10.3	10.3	3.4	3.4	2.3	87	ns
SIDES	◬ 31℃ %	0.0	2.9	2.9^{-}	2.9	2.9	0.0	8.6	2.9	14.3	0.0	0.0	0.0	2.9	8.6^{++}	20.0	11.4	8.6	8.6	2.9	0.0^{-}	35	$p<0.01$
	● 29℃ %	0.0	2.4	2.4^{-}	4.8	0.0	4.8	4.8	0.0	9.5	0.0	0.0	2.4	4.8	0.0	21.4	9.5	7.1	16.7^{++}	4.8	4.8	42	$p<0.01$
	▲ 29℃ %	2.4	0.0	2.4^{-}	4.9	0.0^{--}	0.0^{--}	4.9	2.4	2.4	0.0	7.3	0.0	2.4	0.0	24.4	4.9	19.5^{+++}	4.6	2.4	4.9	41	$p<0.01$
	⭘ 27℃ %	0.0	0.0	8.6	5.7	0.0	8.6	2.9	5.7	0.0	2.9	5.7	0.0	2.9	2.9	25.7	5.7	8.6	5.7	0.0	8.6^{+}	35	$p<0.05$

$^{-}$, $^{+}$ $0.1<p<0.5$ $^{--}$, $^{++}$ $0.05<p<0.1$ $^{---}$, $^{+++}$ $p<0.05$ (X^2 test to each value of sn/Σx100, nn/Σx100 or (sn+nn)/Σx100) was preferred by younger workers, while lower temperatures (31℃, 29℃) by older workers. Moreover, highest (35℃) and lowest (27℃) temperature were selected by the oldest workers (27 d old).

S 4: *In the one-frame hive, younger workers incline to congregate nearer the cluster center and warmer areas, while older workers often rest at the periphery on empty cells or wooden frame and within the range with unfavorable temperatures.*

3. Comparison between the results in 2 observation hives

The previous results were got under the different conditions, as shown in Table 3. The favorable conditions to workers in 1979 seem to dim the clear tendency of thermal reaction in 1975. Presence of many workers, and the existence of a laying queen and consequent brood, must maintain a higher and stabler hive temperature. Moreover, in the more natural condition, as the apiary hive is more spherical, the cluster could homeostatically regulate hive temperature. The spherical structure without bottom heater, however, must cause a sharp gradient in hive temperature. Further discussion on this point will be carried out later.

S 5: *There is an age-specific thermal reaction above different conditions, favorable ones of which incline to dim the thermal tendency of workers.*

Some Speculations

1. The ability of endothermy in older workers

In most endothermal insects the source of thermogenesis is in flight muscles (Heinrich, 1974; May, 1979). In honeybees

Table 3. Comparison between 2 results with their conditions.

YEAR	1975	1979
THERMAL GRADIENT	sharp	gentle
QUEEN	virgin	laying
BROOD	without	with
WORKER NUMBER	289	1300
Correlation Coefficient between distribution and	0.4253[a] (d.f.=287)	0.1274[c] (S dide) (d.f.=549) 0.1120[c] (N side) (d.f.=727)
worker age (r=)	0.7402[b] (d.f.=15)	0.6101[c] (d.f.=18)

[a] cf. p.12 [b] cf. p.12 and Fig.2 [c] cf. Table 1

wing beat or a raise of action potential with concealed muscle movements leads to warm up the muscles (Esch, 1964). The ability to produce heat in *Apis cerana* and *A. mellifera* is better than that in *A. dorsata* and *A. florea* (Dyer and Seeley, 1987).

From the measurement of oxygen consumption, Heusner and Stussi (1964) concluded that foragers were able to produce heat in the daytime within the range measured for homeothermic animals by their basal metabolism, but they became poikilothermic animals at night. This was supported by Southwick and Mugaas (1971) who measured temperatures and oxygen consumption at the cluster core and the shell.

Kronenberg and Heller (1982) found in their cooling experiment that the threshold of an increase in metabolic rate was significantly lower at night (18.2±3.8℃) than in the daytime (26.1±3.5℃). This suggests that the bees can endure the cold better at night than during the day.

According to Allen (1959), newly emerged workers were unable to raise their body temperature at a lower air temperatures until they became 2 d old. Stussi (1972) concluded that the diurnal rhythm of metabolism and thermogenic reaction against cold begin between the 1st and the 4th day after emergence. Cahill and Lustick (1976) proved that workers maintain mesosomal temperature at 34-36℃ during a change of ambient temperature from 15℃ to 40℃.

The thermogenic reaction to cold is associated with the function of the corpora allata. Lukoschus (1956) found a positive correlation between the volume of the corpora allata of workers and the production of their body temperature. Volume of the corpora allata gradually increases with worker age (Lukoschus, 1956; van Laere, 1971; Sasagawa, 1988). The increase curves by van Laere and Sasagawa resemble very much that of Fig. 2.

This slow increase probably results from the inhibition of neurosecretory cell activity until the 15th day after emergence by a pheromone from the queen (Gast, 1967). The activity of neurosecretory cells inhibits the corpora allata activity (Gast, 1967; Herrmann, 1969).

Recently, Fahrenholz (1989) reported that heat production increased with increasing age to a maximum at 21 d old, and that the metabolic rate for heat production was reduced by about 50 % in the presence of a laying queen. There is a probable inhibition course: queen substance → neurosecretory cells → corpora allata → heat production.

In other words, workers acquire the diurnal rhythm of metabolism and thermogenic ability at most by 4th day, but as these are inhibited by the queen, workers younger than 2 or 3 weeks are unsuitable to participate in the cluster shell.

Combining our 5 results (S1-S5) with the above information, it is assumed that younger workers prefer only the area of higher and stabler temperature, while older workers have an ability to tolerate lower and less stable temperature, and the middle aged workers push out the olders to the periphery of the hive and move about within the whole hive surrounding the youngers.

Younger workers selected N side which had somewhat higher temperature (0.71℃ in average) than S side (S3). As workers can respond to the change of 0.25℃ (Heran, 1952), it is probable that our younger workers actively chose the warmer areas.

2. Thermoregulation in the core and shell structure of cluster

At a low ambient temperature, workers diminish heat loss by condensing the cluster which decreases its cooling surface (Free and Spencer-Booth, 1958; Simpson, 1961; Heinrich, 1981). Recording temperature of the cluster core and shell, Southwick and Mugaas (1971) suggested a shift of position by the bees between shell and core during the day and a maintenance of position in the core or shell at night. Taskless foragers become the cluster shell at night. While the "bee curtain" is drawn during the night, the "cluster room" is guarded against ambient cold temperatures. Foragers may enter "circadian hibernation" every night as hummingbirds do (Pearson, 1950).

Craiciu (1965) reported that a winter colony with young workers can maintain a higher temperature than that without young workers. When the structure of the cluster core and shell is established, the conservation of the cluster heat will be easier. Being guarded by older workers in the shell, younger workers in the core move freely and produce metabolic heat.

Workers in the shell are characterized by the centripetal posture (Simpson, 1961).

According to Heinrich (1981), this posture occurred in the swarm cluster only at low ambient temperatures, while at 25 ℃ the shell workers began to space widely from each other and most of them direct their heads centrifugally. In this study, the youngest workers (0-3 d old) directed their heads toward the heat source, the electric bulb (cf. S 2). They may desire to get heat more ardently than older workers do. The orientation of bees' heads seems to depend on the fact that workers have many pit pegs on their antennae classified as cold receptors (Lacher, 1964).

There is a guarding device against heat loss in the body of the individual worker, which has a highly convoluted aorta in the petiole (Snodgrass, 1956). Heinrich (1976) considered that the increased surface of the vessel in the petiole should ensure counter-current heat exchange, resulting in little heat shunting into the metasoma. Even if the metasoma is numb with cold, an individual can move about because of legs on the mesosoma. Moreover, short and long forms of mesosomal hair provide an efficient insulating layer against heat loss (Southwick, 1985). Accordingly, it is reasonable to infer that the shell workers direct their heads to the center, contact their mesosomata to each other and expose their metasomata to the cold.

From our 5 results (S 1-S 5), it is inferred that the youngest workers remain in the area where they emerged, foragers rest in the vicinity of the hive entrance or peripheral parts and the middle-aged workers move throughout the cluster. This core-shell structure prevents the colony from losing heat by alternation between the chilled workers and the warmed ones ("a biotic convection current"). This resembles the above mesosoma-metasoma relation in the individual body.

3. Process determining the nest pattern of cell utilization

There is a characteristic nest pattern but no differentiation with respect to the utility of worker cells in honeybees, which are different from stingless bees with larger honey pots. In an undisturbed apiary colony, the brood is kept in the cells of central combs and surrounded by the cells used for pollen and honey storages. The pollen is usually concentrated in the vicinity of the brood area. As this pattern forms a sphere, one comb is reflected as a sectioned structure of the sphere. How is that nest pattern of cell utilization, or hive contents, determined ?

In our S 3, S side of the comb had a lower temperature, fewer brood cells and more honey stored cells than the N side, and all of the pollen stored cells. This suggests the relationship between temperature and distribution of hive contents.

The following is a speculation on the process determining the nest pattern of cell utilization. A queen lays eggs only in the cells within the warmest area of the colony (brood area), where hatched larvae are reared by younger workers. The metabolic heat produced by brood (Lukoschus, 1956; Zhdanova, 1961) further attracts younger workers which warm the brood, and probably reduces the burden of heat production by workers. According to Fahrenholz (1989), metabolic rate of workers was reduced by about 30 % in the presence of brood. In this way, the central brood area is finished only in the warmest area.

Though the brood need maintain heat for development, stored foods need not. Nevertheless in stored honey, the highest temperature possible is used for evaporating excessive water from half-ripened honey. Therefore, the 2nd warmest areas are selected for the stored honey. They are the upper peripheries of the colony. Furthermore, there is a merit that the upper peripheries are able to support heavy ripened honey storage. (The caps of sealed honey cells may become supporters of the heavy weight.)

Pollen stores are allocated to the areas free of brood and stored honey, which are often the sub-peripheral areas surrounding brood. As pollen stored areas are not scattered (cf. Fig. 4A), the existence of pollen cells probably attracts other pollen foragers.

If the nest pattern is determined by thermal gradient as discussed above, the disturbance of the gradient will have a great influence on distribution of the hive contents. The contents of our observation hive was irregular, as shown in Fig. 4A. This must be derived from the thermal conditions being disturbed by the bottom heater. The honey store seems to be sealed first in areas having higher temperature (cf. Fig. 4 A and B) for evaporation.

4. Age poly ethism interpreted with nest pattern and thermal reaction

As mentioned above, once the nest pattern is achieved, it must affect intranidal behaviors of workers. Many reports on age polyethism have arranged some tasks with worker's age, and synchronously from central duties toward peripheral ones with fine difference of their age sequence (cf. Kolmes, 1985).

Free (1977) adopted the structure of younger and older workers as a mechanism of task allocation. Moreover, Seeley (1982) proposed 4 age subcastes among workers, and his speculation that the labor schedule for intranidal tasks reflects a compromise between selection for efficiency in performing tásks and selection for efficiency in locating tasks. A speculation by selection is seemingly reasonable, but supported only by a "large black box" (*e.g.* lack of genetic bases).

Age polyethism can be interpreted without adopting hypothetical selection pressures. First of all, we set up the premise that all workers are capable of all tasks and never loafers. New workers emerge everyday from the brood area located where the temperature is warmest. Older workers are gradually driven out by the newly emerged workers toward the nest periphery within the core area, which is surrounded by resting foragers as the shell of the colony. The workers which have taken their position as Figs. 1 or 5 may perform at random all the tasks that they encounter.

The above simple model of age polyethism is compatible with Wenner (1961)'s foresighted idea that division of labor is governed by a Markov process. Our mechanical idea will be elaborated in the future studies, and its non-genetical aspect will challenge Seeley's speculation, that age polyethism is not an illusion and is explained by natural selection, even though that speculation has seemingly overcome a recent controversy (cf. Seeley and Kolmes, 1991) between Kolmes (1985; 1986) and Seeley (1986).

Postscript: After finishing this manuscript, I became aware of an excellent work by Camazine (1991) on formation process of the nest pattern. Camazine (1991) proposed the self-organizing hypothesis: the nest pattern emerges mechanically from only interactions among the filling and emptying process of brood, pollen and honey cells, without any other assumptions. This hypothesis offers clearer explanation for the reason why pollen cells concentrate surrounding a brood area. However, his computer simulation did not provide with us a characteristic ellipse of the brood area. Our temperature factor may operate positively on the self-organizing process. Anyhow, Camazine's viewpoint of self-organization in insect societies encourages and stimulates our model on age polyethism.

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