

Behaviors of Adult Queen Honeybees within Observation Hives

I. Behavior Patterns

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Abstract

Based on observations of 6 virgin and 8 mated queens by the 'single-individual trailing' method for ca. 450 hrs, 46 behavior patterns were described, together with 32 worker behaviors closely related to queens, and some short comments, if any. After the difference between virgin and mated queens was compared, 'egg laying', 'royal court formation' and 'balling behavior' were particularly discussed. The last 2, which are worker behaviors and composed of 13 and 12 patterns respectively, may be based on 2 drives, fleeing and aggression.

Key words: balling, behavior repertoire, egg-laying capacity, focal-animal sampling, motivation analysis, royal court

Introduction

Social insects live in their colony together with many nestmates. Consequently, their life is divided into 2 parts: intranidal (inside the hive) and extranidal. Observation of the former is easier than the latter for us. Particularly with honeybees, their combs provide a nearly 2-dimension field for our observations.

Using my 'single-individual trailing' (SIT) method (described later), I could observe almost all behaviors of adult queens within the observation hives. Except for monotonous 'background' behavior, such as *resting*, *wandering*, *self-cleaning*, etc. that I could catch steadily on my observation sheets (cf. Fig. 2), queen behavior inside hives has been directly observed by many authors: *e.g.* Taranov and Ivanova (1946), Hammann (1957), Allen (1957, 1959a, b, 1960), Post *et al.* (1987) and Free *et al.* (1992) in *royal court formation*; Drescher (1968), Koeniger (1970), Fleig (1990) and De-

Grandi-Hoffmann and Martin (1993) in *egg laying*; Bruinsma *et al.* (1981), Robinson (1982) and Grooters (1987) in *aggressive interactions*; and Chauvin (1950), Solodkova (1958), Dietz (1969), Velthuis (1972) and Miida and Nakamura (1992) in various behaviors.

Many recent studies of social insects are seemingly undertaken with 'kin selection' or 'inclusive fitness' theories in mind (*e.g.* Hogendoorn and Velthuis, 1988; Inoue and Roubik, 1990; Oldroyd *et al.*, 1991; van der Blom and Verkade, 1991; DeGrandi-Hoffmann and Martin, 1993; etc.). However, there is a possibility that researchers committed to a particular 'paradigm' (Kuhn, 1970) may become blind to negative data while working mainly within a verification approach (cf. Wenner and Wells, 1990). Moreover, there is an inclination to scrap data which were collected under a certain paradigm as soon as another paradigm has been adopted. I feel a necessity for neutrality (*i.e.* resistance to 'paradigm hold' or 'paradigm shift') in

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data collection and behavior description. Then, I regard my SIT method, as published earlier in drone behavior (Ohtani, 1974), as one of the more neutral, and used it here.

Material and Method

Data collection

All observed bees belonged to hybrid strains of *Apis mellifera ligustica* Spinola. Several supply colonies were kept at a small apiary on the campus of Hokkaido University. Every day, 20-30 newly emerged workers were marked and introduced into 6 types of observation hives (Fig.1 and Table 1), where 6 virgin and 8 mated queens were individually observed from 1972 to 1978 (Table 1).

All or part of the bees in the observation hives were marked with numbered paper disks cut from the membranous surface of photo-printing paper. Each paper disk was bonded to the mesosomal dorsum of the bee with a binding agent (Bond G17, Konishi Inc.).

Basic observations were carried out by a sort of focal-animal sampling (Altmann, 1974), the 'single-individual trailing' (SIT) method: a newly emerged individual was released into an observation hive; all of its behaviors were followed and checked during a certain period; the data were registered on time-scaled data-sheet (Fig. 2). Observation was continued day after day until the observed bee died or disappeared from the hive. As the unit observation time is 5 s (cf. Fig. 2), 0 s means 2 or less seconds.

Two time-schedules were regularly adopted: (1) continuous one-hour sampling at intervals of 3 hours from 1972 to 1976; (2) continuous 8-hour sampling at intervals of 8 hours in 1977 and 1978. Continuous 24-hour sampling was sometimes interposed into the regular observation schedule.

Entry data on my original sheets were very fit for input to a compact digitizer (DT1000 type, Graphtec Ltd.). The digitized data were processed by personal computer.

The sound recording and analyzing system is

Table 1. Observed individuals, their biographies and related items of information.

<i>Individual code</i>	<i>Dates of observation</i>	<i>Total hrs observed</i>	<i>Age in days</i>	<i>Queen condition</i>	<i>Observation hive</i>	<i>Colony size</i>	<i>Observation ended by</i>
VIRGIN QUEENS							bees
V910	Sep. 14 — 28, 1973	19.0	4 — 18	FQ00, FQ212	OH1	600	missing
V7402	June 27 — July 2, 1974	33.0	1 — 6	FQ	OH2	600	flying off
V7410	July 23 — 29, 1974	41.0	1 — 7	FQ1417	OH2	600	natural death
V924	Sep. 25 — Oct. 10, 1975	115.0	1 — 16	LW	OH4	700	flying off
V6720	July 22, 1976	1.0	3	LW	OH4	600	flying off
V7774*	July 11 — 12, 1977	4.0	8,9	a mother queen	OH5×2	6000	mating flight
MATED QUEENS							
Q7200	July 20 — Oct. 30, 1972	95.2	-	-	OH1	500 — 100	natural death
Q7300	May 13 — 29, 1973	30.0	-	-	OH1	400 — 700	missing
Q7500	May 4 — 8, 1975	9.0	-	-	OH4	800	designed
Q7601	May 17 — 23, 1976	3.2	-	-	OH3	12000	designed
Q7602	May 17 — 20, 1976	2.1	-	-	OH3	12000	designed
Q7603	May 17, 30, Nov. 27, 28, 1976	7.5	-	-	OH3	12000	designed
Q7774*	July 14 — Sep. 19, 1977	48.0	-	-	OH5×2	15000	designed
Q7800	June 9 — 11, 1978	39.3	-	-	OH6	6000	designed

* The same individual. FQ: false queens; LW: laying workers.

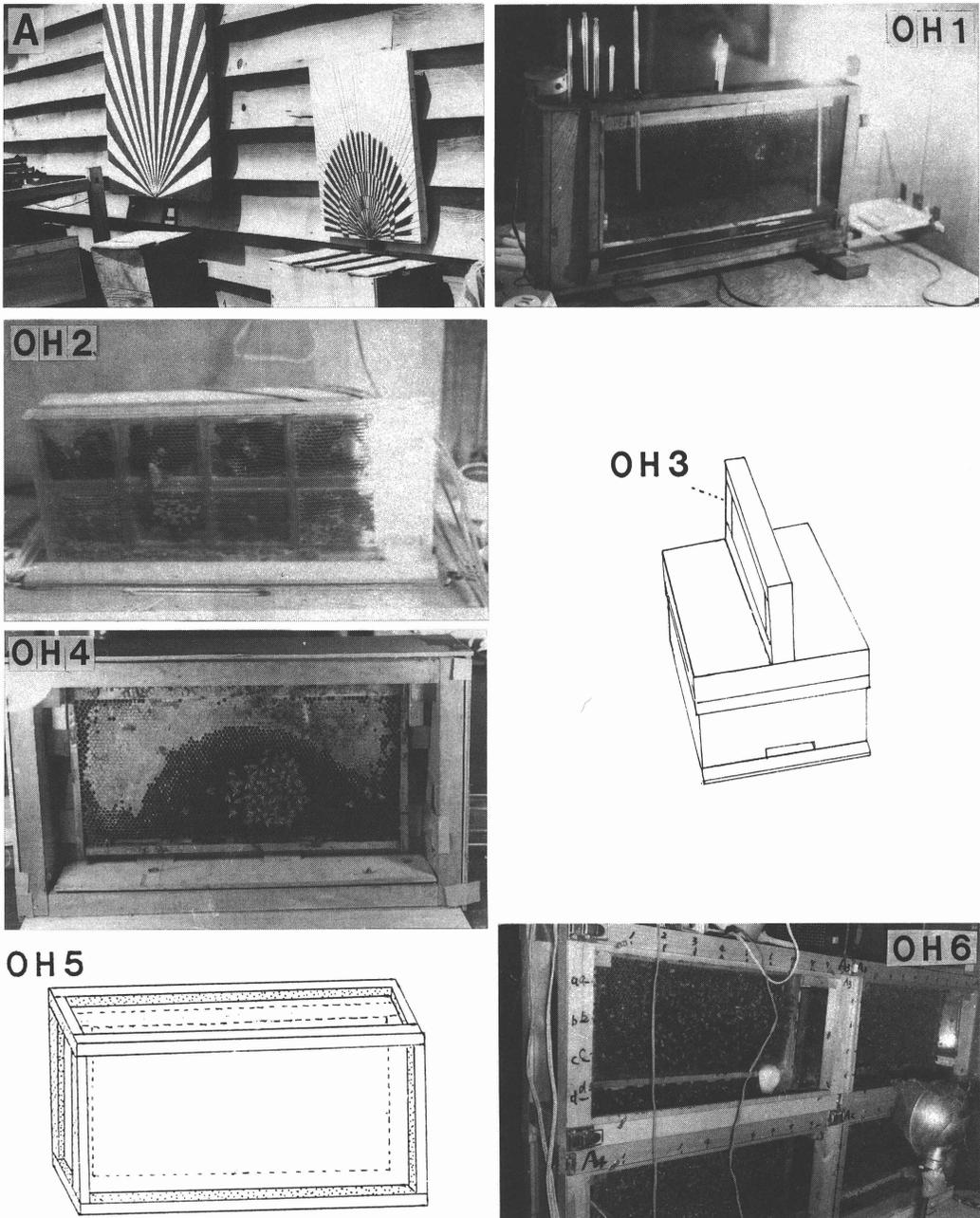


Fig. 1. Observation hives (OH1-OH6) used in this study. **A:** Radial marks at hive entrances of OH1 (left) and OH2 (right) for facilitating the orientation of foragers. The entrance marks are also adopted in OH4, OH5 and OH6. **OH1:** Designed and used by Sakagami (1953). Both sides of one Langstroth comb can be observed through 2 glass walls, with a nichrome-wire heater and thermostat. **OH2:** Entirely made of plastic. One side of single Langstroth comb was replaced by 8 small movable combs (10 x 10 cm) which was placed on a desk in a heating room. **OH3:** Two-side bottomless type temporarily put on the slit of the lid of a normal Langstroth hive and connected with the colony therein. **OH4:** One-side type of OH1, though without heater. Supplementary heat was given from an incandescent lamp (60W). **OH5:** Revised type of OH1; with 4 glass-walls; capable of jointing with other OH5. **OH6:** A large type, remodelled from OH5, capable of holding 4 Langstroth combs.

SP.NAME ()	IND.NO. ()	19__.	__.	__	WEATH. ()
TIME(:	~)		WIND()	TEMP.()	°C
↓	↓	↓	↓	↓	↓	↓	↓	↓
0	1	2	3	4	5			
↓	↓	↓	↓	↓	↓	↓	↓	↓
5	6	7	8	9	10			
↓	↓	↓	↓	↓	↓	↓	↓	↓
10	11	12	13	14	15			
↓	↓	↓	↓	↓	↓	↓	↓	↓
15	16	17	18	19	20			
↓	↓	↓	↓	↓	↓	↓	↓	↓
20	21	22	23	24	25			
↓	↓	↓	↓	↓	↓	↓	↓	↓
25	26	27	28	29	30			
30	31	32	33	34	35			
35	36	37	38	39	40			
40	41	42	43	44	45			
45	46	47	48	49	50			
50	51	52	53	54	55			
55	56	57	58	59	60			

Fig. 2. The data sheet for my single-individual trailing method. It is for one hour : minimum scale is 5 s. and numerals indicate min. First, as soon as the observed animal changed its behavior, the position of hands of an analog-type stop-watch was noted. Next, the abbreviated behavior code was given at the corresponding position on the sheet.

almost the same as that in Ohtani and Kamada (1980); see the caption of Fig. 11.

Behavior description

Ohtani (1974) described various drone behaviors using the combination of certain positions and mobile body part movements, divided into several grades or types. Similar description systems were used by Masatomi and Kitagawa (1975) for the Japanese crane, by Schleidt *et al.* (1984) for the blue breasted quail, and by Ohtani (1985) for the Japanese cabbage white. The description system of Ohtani (1974) was adopted in this study; the positions and movements of queen body parts are defined as shown in Fig. 3 and Table 2.

Queens mostly lead an intranidal life, and take to the air only for a short but important time. The mating flights of queens are briefly described below; swarming or absconding flights are not addressed in this study.

Two behavioral categories are covered in this study (cf. Ohtani, 1985): (1) *unit behaviors*, which can be treated separately, and (2) *temporally integrated behaviors*, which appear as a definite sequence of several patterns. The former is subdivided into (1a) behaviors by a queen (*solitary behaviors*) not related to other adult individuals and (1b) those between a queen and other bees, *i.e.* either workers, drones or other queens (*interindividual behaviors*).

Temporally integrated behaviors in queens are egg-laying behavior and *sexual behavior*, but only the former is treated in this study.

Codes for unit behaviors are given by a combination of capital and lower-case letters with bold italic (*e.g. Re, Wa*), and those of a group of some similar unit behaviors by a combination of 2 capital letters (*e.g. SC, AL*). Small letter(s) on the upper shoulder of some codes (*e.g. Sc^{hm}, Al^P*) mean(s) the sub-division of a certain behavior pat-

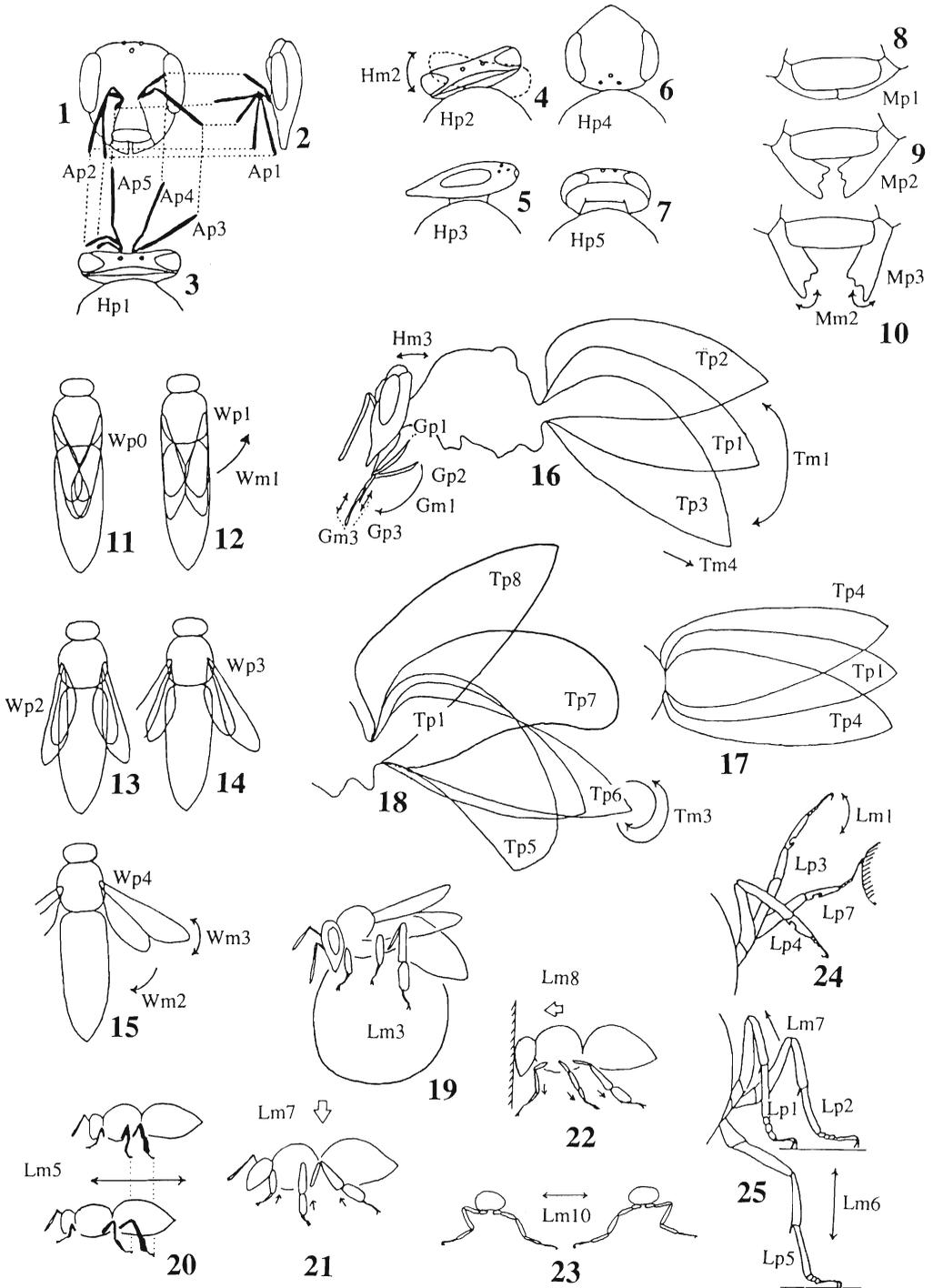


Fig. 3. Schematic presentation of mobile body parts of queens and/or workers. **1-3:** Antennae shown by frontal, dorsal and lateral views of head. **3-7:** Head by dorsal views. **8-10:** Mandibles by frontal views. **11-15:** Wings by dorsal views. **16:** Whole body, ignoring legs, by lateral view. **17:** Metasoma by dorsal view. **18:** Metasoma by lateral view. **19-22:** Movements of all legs by lateral view of body. **23:** Lateral movements of legs shown by cross-sectional view of body. **24 and 25:** Forelegs and midlegs by rear views. Each abbreviation corresponds partly to that given in Table 2.

Table 2. Terminology for mobile parts of the queen's body. Grades of their positions (p) and types of their movements (m) given here are used in Table 3. Numerals in parentheses correspond to those in Fig. 3. Some of positions and movements, shown by (#), were not used by drones (Ohtani 1974).

<i>POSITIONS (Grade)</i>	<i>MOVEMENTS (Type)</i>
Antennae	
Ap	Am
1 Lowered (1-3)	1 <i>Tapping</i> (2): Repeated alternate touchings of some objects.
2 Stretched slightly (1-3)	2 <i>Exploring</i> : Repeated alternate touchings of substrate, sometimes without actual touching. Movement more slow and gentle than in Am1.
3 Outstretched (1-3)	
4 Protruded obliquely (1-3)	
5 Protruded straightforward (1-3)	
.....	
Mandibles	
Mp	Mm
1 Closed (8)	1 <i>Pinching</i> : Holding some objects between mandibles for compressing.
2 Opened slightly (9)	2 <i>Chewing</i> (10): Repeated opening and closing of mandibles either holding some soft or liquid objects or not.
3 Opened widely (10)	
.....	
Glossa	
Gp	Gm
1 Folded (17)	1 <i>Protruding</i> (17): Movement of glossa from Gp1 to Gp3.
2 Spread slightly (17)	2 <i>Lapping</i> : Brief searching with Gp3 accompanied by quick sucking movements.
3 Protruded (17)	3 <i>Sucking movements</i> [#] (17): Repeated protraction and retraction of proboscis with Gp3.
.....	
Head	
Hp	Hm
1 Disposed properly (3)	1 <i>Twisting</i> : Repeated turning head from right/left to left/right.
2 Tilted laterally (4)	2 <i>Rolling</i> (4): Quickly repeated movements with Hp2 from one side to the other. Often similar movements with Hp4.
3 Twisted (5)	3 <i>Nodding</i> [#] (17): Repeated movements between slightly raised position and slightly lowered position.
4 Raised (6)	
5 Lowered (7)	
.....	
Wings	
Wp	Wm
0 Tightly folded [#] (11)	1 <i>Spreading</i> (12): Movement from Wp0 or Wp1 to Wp4.
1 Folded (12)	2 <i>Folding</i> (15): Movement from Wp4 to Wp1 or Wp0.
2 Spread slightly (14)	3 <i>Fanning</i> (15): Vibration of widely spread wings (Wp4).
3 Spread obliquely (14)	4 <i>Vibrating</i> [#] : Fine vibration of wings with Wp0 - Wp3.
4 Spread widely (15) : Fore- and hind wings connected with hamuli.	

Table 2 (continued).

<i>POSITIONS (Grade)</i>	<i>MOVEMENTS (Type)</i>
Metasoma*	
<p>Tp</p> <ol style="list-style-type: none"> 1 <i>Disposed properly</i> (16-18) 2 <i>Raised</i> (16) 3 <i>Lowered</i> (16) 4 <i>Tilted laterally</i> (17) 5 <i>Contracted</i> (18) 6 <i>Extended</i> (18) 7 <i>Arched</i> (18) 8 <i>highly raised</i> (18) 9 <i>Nasanoff gland exposed</i> (18) 	<p>Tm</p> <ol style="list-style-type: none"> 1 <i>Dorsoventral motion</i> (16): Repeated motions from Tp2 to Tp3. 2 <i>Respiratory movements</i> : Repeated quick and small motions from Tp1 to Tp6, often accompanied with quick Tm1. 3 <i>Rotating</i> (18): Right, left or alternate rotations with Tp6. 4 <i>Extending</i> (18): Movement from Tp1 to Tp6. 5 <i>Sting-chamber opening</i> : Movement in Tp6. 6 <i>Stinging movements</i> : Repeated protruding and retracting of sting after Tm5.
<hr/>	
Legs	
<p>Lp</p> <ol style="list-style-type: none"> 1 <i>Contracted</i> (25): In contact with substrate and body sides. 2 <i>Outstretched</i> (25): In contact with substrate and detached from body sides. 3 <i>Protruded</i> (24): Detached from body sides. 4 <i>Detached[#]</i> (24): From substrate. 5 <i>Raised[#]</i> (25): With an obtuse angle between femur and tibia. 6 <i>Clawing[#]</i>: To other substrate by pretarsus. 7 <i>Touched[#]</i> (24): In contact with objects other than substrate. 	<p>Lm</p> <ol style="list-style-type: none"> 1 <i>Patting</i> (24): Repeated alternate touching of some objects with protruded forelegs (Lp3). 2 <i>Brushing</i>: Repeated wiping of various body parts with legs. 3 <i>Clinging</i> (19): Bestriding and grasping tightly a protrusive object or another bee body with all legs. 4 <i>Walking[#]</i> : Cooperative movements of 6 legs for advancing or stepping. 5 <i>Rocking[#]</i>(20): Repeated movements from Lp1 to Lp5 of mid- and hindlegs. 6 <i>Up-and-down motion[#]</i>(25): Quick and repeated motions from Lp2 to Lp5. 7 <i>Pressing[#]</i>(21, 25): Pressing body toward substrate with contracted legs. 8 <i>Pushing[#]</i>(22): Forcing against substrate to move upward or forward. 9 <i>Turning[#]</i>: Sudden turning back halfway on advancing. 10 <i>Swinging[#]</i>(23): Laterally quick-and-repeated movements of meso- and metasoma.
<hr/>	
General movements (Genm)	
<ol style="list-style-type: none"> 1 <i>Trembling</i> : Quick, vibrant movements of various body part. 2 <i>Twitching</i>: Short, sudden pull of a body part. 3 <i>Twisting[#]</i>: Repeated turning fore body from right/left to left/right. 	

* This term and *mesosoma* are used in this paper instead of abdomen and thorax, respectively, because of the abdominal petiole.

tern. Each component of temporally integrated behaviors is coded by 2 lower-case letters (*e.g.* **mi**, **ov**).

Codes for interindividual behaviors are used as follows: **cAb/d** (active behavior **Ab** by actor *c* to actee *d*), **c/Abd** (passive behavior **Ab** by actee *d* to actor *c*) and **c-Ab-d** (reciprocal behavior **Ab** between individual *c* and individual *d*). An unequivocal actor or actee is often abbreviated, such as **Ab/d** or **c/Ab**.

The paragraph heading **SITUATION** refers to the situation in which each behavior pattern appears and disappears. Patterns marked by (#) are those which did not appear in drones (Ohtani, 1974) or the cyclopic worker (Ohtani, 1977); that is, they are newly described in this paper.

Results and Discussions

Based on the definitions in Fig. 3 and Table 2, the behavioral information was condensed in Table 3. These are difficult to read and to construct because of their abstract by symbolization, but will have to be valuable for the reproduction of each behavior by some computer technic, *e.g.* computer graphics. It is thought that Table 3 and the following descriptions are complementary to each other.

1. Solitary behaviors

1.1. Resting (Re) (Fig. 4B; 5C): Respiratory movements (Tm2) faint or weak; wings usually tightly closed (Wp0), either fore- or hindlegs often away from substrate (Lp4).

SITUATION: Preceded by *wandering* (**Wa**, 43.4%), metasoma cleaning (**Sc'**, 20.4), *staying in motion* (**Sm**, 12.7) and *being fed* (**w/Fe**, 5.3) and followed by **Wa** (47.6), **Sc'** (17.3), **Sm** (11.2), *being attacked* by a worker (**w/At**, 5.5) and **w/Fe** (4.5) in virgin queens. Preceded by **Wa** (44.2%), **w/Fe** (12.9), **Sm** (9.7) and **Sc'** (8.8) and followed by **Wa** (43.8), **w/Fe** (14.9), **Sm** (11.5), **Sc'** (9.1) and *food begging* (**Bf/w**, 5.3) in mated queens.

Virgin queens more often self-cleaned (**Sc'**) during **Re** than mated queens, which more often were fed by workers (**w/Fe**) than virgin queens, which cannot yet lay eggs.

The circle of attendants or royal court around the resting queen is dense and complete for a mated queen but sparser and incomplete for a virgin queen (*cf.* Free *et al.*, 1992; also 2.1).

MEAN DURATION: 238.81 ± 342.34 (SD: standard deviation) s (n = 1412, virgin queens); 145.99 ± 223.77s (n = 2376, mated queens). Virgin queens rested longer than mated queens (p < 0.001, Mann-Whitney U-test). As data becomes incomplete at the beginning and ending of regular sampling or of missing data (if any), it is omitted.

A posture intermediately between **Re** and *staying alert* (**Sa**, *cf.* Ohtani, 1977) was found 2 times in V924 (550s; 30s): Ap3 or Ap4, Hp4, Wp1 or Wp2 (*cf.* Table 2), otherwise the posture was similar to **Re**.

At the peripheral brood area, laying queens often rested (**Re**), cleaned themselves (**SC**) and were fed (**w/Fe**). Out of resting sites of laying queens, that of Q7300 is selected and shown in Fig. 6.

1.2. Wandering (Wa): Walking (Lm4) with a speed in drones (1.2 cm/s), often faster (3.4 cm/s) and irregular in virgin queens; wings folded or often tightly folded.

SITUATION: Preceded by **Re** (29.7%), **Sc'** (16.4), **Bf/w** (11.3), **Sm** (9.0) and **w/At** (8.6) and followed by **Re** (28.9), **Sc'** (18.9), **Bf/w** (11.8), **w/Fe** (8.7), **Sm** (7.9) and **w/At** (5.8) in virgin queens. Preceded by egg laying (**El**, 35.5), cell inspection (**Ic**, 28.0), **Re** (14.4) and pausing (**Pa**, 7.3) and followed by **Ic** (47.4), **El** (26.8) and **Re** (15.2) in mated laying queens. **Wa** with several **Ic** per second were observed frequently in mated queens (n=94) but not in virgin queens (n=5).

'Searching' for a suitable site for **Re**, more cells for egg deposition in laying queens (Dietz, 1969), and suitable rivals to fight in virgin queens;

Table 3. Behavior patterns characterized by position (p) and movements (m) of main body parts. Grades of the positions and kinds of the movements are defined in Table 2 (some worker behaviors included as WB). Italic numbers are patterns rarely observed. Gothic numbers mean the main positions and/or movements which represent their characters.

Code	Behavior pattern	Antennae		Mandibles		Glossa		Head		Wings		Metasoma			Forelegs		Midlegs		Hindlegs	
		Ap	Am	Mp	Mm	Gp	Gm	Hp	Hm	Wp	Wm	Tp	Tm	Genm	Lp	Lm	Lp	Lm	Lp	Lm
<i>Re</i>	<i>Resting</i>	1	-	1*	-	1	-	1	-	0,4	-	1	2	-	1,4	-	1	-	1,4	-
<i>Wa</i>	<i>Wandering</i>	2-4	2	2,3	-	1	-	1,2	-	0,1	-	1,4	-	-	4	-	4	-	4	-
<i>Sm</i>	<i>Staying in motion</i>	2,3	2	2*	-	1	-	1,2	-	0,1	-	1,4	-	-	4,5	4	5	4	5	4
<i>(SC Self-cleaning)</i>																				
<i>Sc^h</i>	<i>Head cleaning</i>	1-3	-	2*	-	1,2	-	1	1 ^P	0,1	-	1,6	-	-	1,2	2	1,2	3	1,2	-
<i>Sc^s</i>	<i>Mesosoma cleaning</i>	1-3	-	2*	-	1	-	1	-	1	1,2	1,6	-	-	6	-	-	2	1,4	-
<i>Sc^t</i>	<i>Metasoma cleaning</i>	1-3	-	2*	-	1	-	1	-	1-3	1,2,3	3,6	3	-	2,6	3	1,2	-	1,2	2
<i>Sc^{hm}</i>	<i>Mouthparts cleaning</i>	1,2	-	2	-	3	-	1	-	2,3	-	1	-	-	2	2,4 ^a	-	2	-	
<i>Sc^{sl}</i>	<i>Legs cleaning</i>	1,2	-	2	-	1,2	-	1,5	-	2,3	-	3	-	-	1,2	-	1,2	2	1,2	2
<i>Sc^{hst}</i>	<i>Head and metasoma cleaning</i>	1,2	-	2	-	1,2	-	1	1 ^P	2,3	3	-	3	-	1,2	2	1,2	2	1,2	2
<i>Sc^{hst}</i>	<i>Head and mesosoma cleaning</i>	1,2	-	2	-	1,2	-	1	1 ^P	2,3	-	1	-	-	1,2	2	1,2	2	1,2	2
<i>Scst</i>	<i>Meso- and metasoma cleaning</i>	1,2	-	2	-	1,2	-	1	-	2,3	3	-	3	-	1,2	-	1,2	2	1,2	2
<i>(AL Alert-like posture)</i>																				
<i>Al^P</i>	<i>Pre-alert-like shaking</i>	3	-	2*	-	1	-	3,4	-	1	-	1,2	2	2	1,3	10 ^b	2	10 ^b	2	10 ^b
<i>Al^a</i>	<i>Proper alert-like posture</i>	3,4	-	2*	-	1	-	-	1	2	-	2	2	3	1,3	-	2	-	2	-
<i>Ic</i>	<i>Cell inspection</i>	-	2*	2*	-	1*	1*	5	-	1-3	-	3	-	-	2	-	2	-	2	-
<i>Hi</i>	<i>Honey intake from a cell</i>	-	2*	3*	-	3	3	5	-	1-3	-	3	1	-	2	-	2	-	2	-
<i>Er</i>	<i>Excited running</i>	3,4	2	2*	-	1*	-	1,2,4	-	2-4	3	1,6	-	1	-	2,4	-	2,4	-	2,4
<i>Ru</i>	<i>Running</i>	3,4	2	2*	-	1*	-	1,2	-	2,3	-	1,6	-	-	4	-	4	-	4	4
<i>EA</i>	<i>Excited advancing</i>	2-4	2	?	?	1*	-	2-5	-	0-3	3,4	2-6	-	1	-	4	-	4	-	4
<i>Es</i>	<i>Escaping</i>	1-3	-	3*	-	1*	-	4	-	3	3	2	-	1	-	4	-	4	-	4
<i>Pp</i>	<i>Proboscis protruding</i>	3,4	-	3	-	3	3	4	-	3	-	2	-	-	3	-	2	-	2	-
<i>Qd</i>	<i>Queen-cell destruction</i>	-	1*	-	1*-3*	1*	-	-	2*	0-2	-	3,6	-	1	-	3,4,8	-	3,4,8	-	3,4,8
<i>Fa</i>	<i>Fanning</i>	-	2	2*	-	1*	-	1,2	-	-	3	3,6	-	2	2,5	3,4	2,5	3,4	2,5	3,4
<i>Vb</i>	<i>Fine vibration</i>	1	-	1*	-	1*	-	1	-	0,1	-	1	2	1	1	-	1	-	1	-
<i>Go</i>	<i>Going-out</i>	-	2*	1*	-	1*	-	1*-5*	-	-	3	1*-4*	-	1*	4	-	4	-	4	-
<i>Wx</i>	<i>Wax manipulation</i>	-	2	-	1,2	1*	-	-	1-3	1,2	-	3,6	-	-	2,5	-	2,5	-	2,5	-

^a only one leg; ^b irregular movements; ^P passive appearance; * inference; Genm : general movements; WB: worker behavior.

"7+8": 7 and 8; "7,8": 7 or 8; "6-8": 6, 7 or 8.

Table 3 (continued).

Code	Behavior pattern	Antennae		Mandibles		Glossa		Head		Wings		Metasoma			Forelegs		Midlegs		Hindlegs	
		Ap	Am	Mp	Mm	Gp	Gm	Hp	Hm	Wp	Wm	Tp	Tm	Genm	Lp	Lm	Lp	Lm	Lp	Lm
w/RCq	Royal court being formed same as <i>Re</i> , <i>SC</i> , <i>Bf</i> and <i>Fe</i>																		
(wRC/q	WB, royal court formation)																			
RC-1	WB, examining	-	2	1*	-	1*	-	1,4	-	2,3	-	1,2	-	-	1,2,4	-	1,2	-	1,2	-
RC-2	WB, touching or licking see <i>Ma'</i> or <i>Ma'</i>																		
RC-3	WB, nibbling	-	1	-	2	1*	-	-	2,3	1-3	-	1,2	-	-	-	1,3	2	8	2	8
RC-4	WB, irritated rocking	-	2	2,3	-	1*	-	4	-	3	-	2	-	2	3	-	2	5	2	5
RC-5	WB, food offering	2-5	1	2,3	-	2	-	4	-	2,3	-	1,2	-	-	1,2	-	1,2	-	1,2	-
RC-6	WB, self-cleaning see <i>SC</i>																		
RC-7	WB, polishing-like rocking	-	1	-	2	1	-	-	2	3,4	-	1,2	-	-	-	1	-	5	-	5
RC-8	WB, proboscis protruding see <i>Pp</i>																		
RC-9	WB, shaking	-	2	1*	-	1*	-	4	-	1-3	-	-	1	-	2,7	-	2	6	2	6
RC-10	WB, staying alert	2,3	2	2	-	1*,2*	-	1,2	-	3	-	1,2	-	-	2,4,5	-	1,2	-	1,2	-
RC-11	WB, escaping see <i>Es</i>																		
RC-12	WB, chasing	2-4	2	2,3	-	1	-	1,4	-	2,3	-	1,2	-	-	-	4	-	4	-	4
RC-13	WB, head pressing	1,2	-	1*	-	1*	-	5	-	0,1	-	1	-	-	1,2,4	7+8	2	7+8	2	7+8
q-Ac-w	Antennal contact	-	1	2*	-	1	-	2,4	-	1-4	-	1-4	-	-	2,5	-	2	-	2	-
w/Wgq	Wing-base being gnawed	1	-	1*	-	1*	-	5	-	4,5	-	3	-	-	1	-	1	-	1	-
w/Shq	Being shaken same as <i>Re</i>																		
wSh/q	WB, shaking	-	2	1*	-	1*	-	4	-	1-3	-	-	1	-	2,7	-	-	6⇔4	-	6⇔4
(FT	Food transmission)																			
qBf/w	Food begging	2-5	1	3	-	3	1	4	-	1,2	-	1	-	-	1,2	1	1,2	-	1,2	-
d/Bfq	Being begged, or WB (q/Bfw) same as <i>Re</i> , or wRC/q (WB)																		
qOf/w	Food offering	2-5	1	2,3	-	2	-	4	-	2,3	-	1,2	-	-	1,2	-	1,2	-	1,2	-
q/Ofw	WB, being offered same as wRC/q																		
w/Feq	Being fed	2-5	1	2,3	-	3	3	4	-	1,2	-	1	-	1	1-3	-	1,2	-	1,2	-
qFe/w	Feeding, or WB (wFe/q)	-	1	3	-	2	-	4	-	0-3	-	1	-	-	1,2	-	1,2	-	1,2	-

^a only one leg; ^b irregular movements; ^p passive appearance; * inference; Genm : general movements; WB: worker behavior; ⇔: alternating.
 "7+8": 7 and 8; "7,8": 7 or 8; "6-8": 6, 7 or 8.

Table 3 (continued).

Code	Behavior pattern	Antennae		Mandibles		Glossa		Head		Wings		Metasoma			Forelegs		Midlegs		Hindlegs	
		Ap	Am	Mp	Mm	Gp	Gm	Hp	Hm	Wp	Wm	Tp	Tm	Genm	Lp	Lm	Lp	Lm	Lp	Lm
<i>(AG Aggressive interactions)</i>																				
w/MAq	Being manipulated	1,2	-	2*	-	1*	-	1,2	-	1,2	-	1,4	-	-	1,2	4	1,2	4	1,2	4
(wMA/q	WB, manipulating)																			
wMa ^l /q	WB, touching	-	2	2*,3*	-	1*	-	4	-	1-3	-	1,2	-	-	7	-	2	-	2	-
wMa ^l /q	WB, licking	-	2	2*,3*	-	-	2	4	-	1-3	-	1,2	-	-	2,3	-	2	-	2	-
wMa ⁿ /q	WB, nibbling see RC-3																		
qAt/w	Attacking or WB(wAt/q,w)	-	1	-	1,2	1*	-	-	1-3	2,3	-	1-3	-	1	-	3,8	-	3,8	-	3,8
q,w/Atq	Being attacked	1-3	-	3*	-	2*	-	1,2	-	3	-	1,4	-	-	2	-	2	-	2	-
qSg/q	Stinging or WB(wSg/q)	-	1	-	1	1*	-	1	-	1-3	-	3,7	6	-	-	3	-	3	-	3
w,q/Sgq	Being stung same as w,q/At q																		
w/BAq	Being balled	1*	2*	1*	-	1*	-	1*-5*	-	1*-3*	-	1*-6*	-	-	1,2	-	1,2	-	1,2	-
(wBA/q	WB, balling behavior)																			
BA-1	WB, examining same as RC-1																		
BA-2	WB, touching or licking same as RC-2																		
BA-3	WB, food begging or feeding same as RC-5																		
BA-4	WB, immobile	1,2	-	1*	-	1*	-	1	-	0,1	-	1	-	-	1,2,4	7,8	2	7,8	2	7,8
BA-5	WB, guarding	2-4	2	2,3	-	1*	-	4	1	2,3	-	2	-	-	3,4	4	2,4 ^a	4	2	4
BA-6	WB, mounting	2-4	2	2,3	-	1*	-	5	-	2,3	-	3	-	-	3,7	-	3,7	-	3,7	
BA-7	WB, metasoma arching	2-4	2	2,3	-	1*	-	5	-	2,3	-	7	-	-	3	-	3	-	3	
BA-8	WB, metasoma inflecting	2-4	2	2,3	-	1*	-	5	-	2,3	-	7	5	-	-	3	-	3	-	3
BA-9	WB, attacking see At																		
BA-10	WB, stinging see qSg/q																		
BA-11	WB, alarm fanning	4,5	2	2*	-	1*	-	4	-	-	3	8+9	-	-	7	-	7	-	7	
BA-12	WB, excited fanning	4,5	2	2*	-	1*	-	4	-	-	3	8+9	-	-	4,7,9	-	4,7,9	-	4,7,9	
qTo/w	Tooting	2,3	-	1*	-	1*	-	4	-	0,1	4	2	-	-	7	-	7	-	7	
qSr/w	Shrieking	1-3	-	3*	-	2*	-	1,2	-	0,1	4	1-3	-	-	2	-	2	-	2	
<i>(EI Egg laying)</i>																				
mi	metasoma insertion	-	1	1*	-	1*	-	1,5	-	0-3	-	3,6	4	-	2	-	2	-	2	-
ov	oviposition	-	1	1*	-	1*	-	1,5	-	0-3	-	6	4,5	-	2	7	2	7	2	7
wd	withdrawal	-	1	1*	-	1*	-	5	-	0-3	-	3	-	-	4	-	4	-	4	
Pa	Pausing	1	- keeping the motionless posture during <i>Ic</i> , <i>mi</i> , <i>ov</i> or <i>wd</i>																

Queen behavior repertoire

^a only one leg; ^b irregular movements; ^p passive appearance; * inference; Genm : general movements; WB: worker behavior.
 "7+8": 7 and 8; "7,8": 7 or 8; "6-8": 6, 7 or 8.

otherwise functioning as a link between 2 behavior patterns (cf. Fig. 15).

MEAN DURATION: $74.22 \pm 86.25s$ ($n = 2312$, virgin queens); $25.14 \pm 33.58s$ ($n = 7438$, mated queens). Because of egg laying, mated queens wandered less ($p < 0.001$, U-test) than virgin queens, which often wandered around searching for rival sister queens.

1.3. Staying in motion (Sm): In motion (Lm4) without locomotion, often with changes of body axis; legs raised (Lp5) and forelegs often detached (Lp4); already described for a cyclopic worker (Ohtani, 1977).

SITUATION: Preceded by **Wa** (28.2%), **Re** (25.1), **w/Fe** (23.7) and metasoma cleaning (**Sc'**, 13.2) and followed by **Wa** (33.0), **Re** (31.7), **w/Fe** (14.5) and **Sc'** (13.7) in virgin queens. Preceded by **Re** (43.8),

w/Fe (21.2), **Wa** (9.0) and egg laying (**El**, 5.4) and followed by **Re** (38.0), **Wa** (29.4) and **w/Fe** (13.0) in mated queens.

MEAN DURATION: $42.84 \pm 46.98s$ ($n = 649$, virgin queens); $15.83 \pm 14.57s$ ($n = 651$, mated queens). There is a significant difference between them ($p < 0.001$, U-test). Longer **Sm** seems to indicate restlessness.

1.4. Self-cleaning (SC): Brushing own body (Lm2); 'Groom self' (Kolmes, 1984); 'self-grooming' (Nau- mann, 1991); 'autogrooming' (Moritz and South- wick, 1992:200).

Except for only *wing cleaning* (**Sc^w**), all sub- divisions observed for drones (based on the body parts cleaned) were also observed in queen behaviors. *Head cleaning* (**Sc^h**) includes only antennae (**Sc^{ha}**), only compound eyes (**Sc^{he}**) and

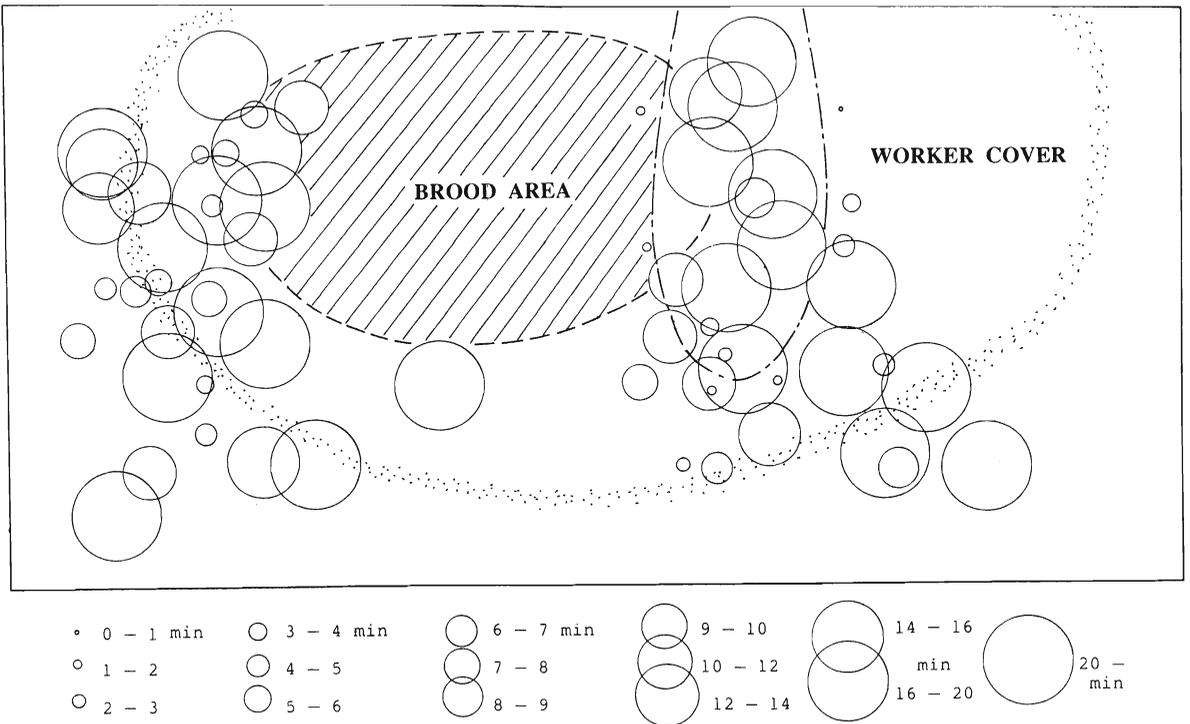


Fig. 6. Resting positions (circles) of a laying queen (Q7300) on the comb surface within a single-combed observation hive (OH1). The size of circles indicates the duration of *resting* (including *self-cleaning* and *being fed*) as ranked at bottom. The area within dot zone was covered by workers. A small piece of comb existed within chain-line area which was frequently used for *resting*. The observation made on May 13-29 in 1973.

only mouthparts (Sc^{hm}). *Mesosoma cleaning* (Sc^s) includes only *leg cleaning* (Sc^{sl}); mutual brushing of midlegs and/or hindlegs. *Metasoma cleaning* (Sc^t) (Figs. 4A; 5A, B; 7A); fanning inserted during ardent brushing.

SITUATION: *SC* appears before and after *Re* (*resting*), *Wa* (*wandering*) *Sm* (*staying in motion*) and *SC*. The last case frequently occurs in a combination pattern: $Sc^h \rightarrow Sc^s \rightarrow Sc^t$, or $Sc^h \rightarrow Sc^t$. Two sub-types of *SC* rarely appear synchronously ($Sc^h + t$, $Sc^s + h$ and $Sc^s + t$).

MEAN DURATION: Significant differences among Sc^h , Sc^s and Sc^t , but not among all sub-divisions in virgin and mated queens as indicated in Table 4:

1.5. Alert-like posture (AL).

This curious pattern was first called 'alert posture (AL)' in drone behavior (Ohtani, 1974), but is renamed here because there was no direct relation with 'alert'. Accordingly, 2 sub-types described for drones were also renamed 'prealert-like shaking (Al^p)' and 'proper alert-like posture (Al^a)'. Both were observed in 2 virgin queens (V7410; V910) and a mated queen (Q7800).

Al^p : Stiff-mannered irregular shaking (Lm10) at intervals, or more frequent twitching of body (Genm2), with appendages disposed as in *Re* (Tp1),

and wings spread slightly (Wp2); forelegs only protruded (Lp3) rarely; antennae usually outstretched (Ap3).

Al^a : Fore body raised with stiff-mannered, rhythmic twisting of head (Hm1) with protruded forelegs (Lp3) at intervals (Genm3), and metasoma raised slightly (Tp2) so that the body leans backward; mid- and hindlegs outstretched (Lp2), supporting the body; antennae outstretched (Ap3) or protruded obliquely (Ap4).

SITUATION: Out of 11 cases, preceded by *Re* (8 cases), *pausing* (*Pa*, 1), *food begging* (*Bf/w*, 1) and Al^a (1) and followed by *Re* (6), *Bf/w* (2), *Wa* (1), *being fed* (*w/Fe*, 1) and Al^p (1).

MEAN DURATION: $72.50 \pm 75.88s$ ($n = 8$, Al^a); $48.33 \pm 49.33s$ ($n = 3$, Al^p); no significant difference between them.

1.6. Cell inspection (Ic): Peeping into a cell; in worker cells, head and forelegs inserted, either partly or entirely; in drone or half-destroyed queen cells, often mesosoma also inserted partly or entirely.

SITUATION: Pre-phase of *honey intake* (*Hi*, cf. 1.7) or that of *metasoma insertion* (*mi*, cf. 3.2;3.1) in *egg cells* (*queen-cell destruction*, *Qd*, cf. 1.13); in *Hi* and *Qd*, however, no independent sampling. Preceded

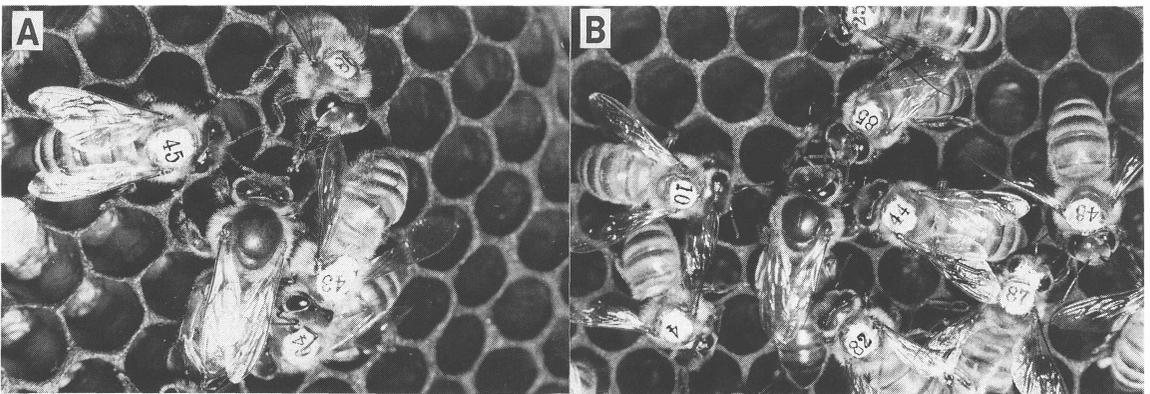


Fig. 7. A laying queen (Q7200) and her attendants. **A** : During *metasoma cleaning* by Q7200, worker, no. 45, was examining the queen (*RC-1*), nos. 43 and 64 *touching* (*RC-2*) and no. 98 *stropping tongue* (*RC-6*). **B** : Q7200 was fed by a worker, no. 85, soon after *food offering* (*RC-5*).

Table 4. Mean durations of subdivisions in **SC** (*self-cleaning*). Their differences between virgin and mated queens are not significant (ns), and those between some subdivisions are significant.

	<i>VQ</i> (virgin queens)		<i>MQ</i> (mated queens)		<i>Mann-Whitney U-test</i> <i>VQ - MQ</i>
Sc^{h*}	23.49 ±	22.20 (n=186)	20.43 ±	19.43 (n=207)	ns
Sc^{ha}	19.17 ±	9.69 (n=6)	28.35 ±	31.96 (n=109)	ns
Sc^{he}	60.00 ±	0.0 (n=1)	18.33 ±	11.46 (n=9)	ns
Sc^{hm}	19.62 ±	9.23 (n=13)	20.38 ±	18.13 (n=40)	ns
Sc^h (all)	22.94 ± 23.35 (n=571)				
Sc^{s**}	11.07 ±	7.25 (n=28)	17.89 ±	23.47 (n=19)	ns
Sc^{ti}	11.00 ±	6.52 (n=5)	25.71 ±	20.27 (n=14)	ns
Sc^s (all)	16.14 ± 17.11 (n=66)				
Sc^t	54.49 ±	66.19 (n=1036)	54.73 ±	72.17 (n=464)	ns
Sc^t (all)	54.15 ± 67.77 (n=1516)				
Sc^{h+t}	17.50 ±	6.45 (n=4)	43.75 ±	41.91 (n=4)	ns
Sc^{s+h}	28.23 ±	7.64 (n=3)	27.50 ±	10.61 (n=2)	ns
Sc^{s+t}	75.00 ±	0.00 (n=1)	25.00 ±	21.79 (n=3)	ns

*Excluded data of **Sc^{ha}**, **Sc^{he}** and **Sc^{hm}****Excluded data of **Sc^{ti}**

by **Wa** (90.8%) and **Re** (5.3) and followed by **Wa** (89.5) and **Re** (5.3) in virgin queens. Preceded by **Wa** (70.9), **El** (19.5) and **Pa** (4.2) and followed by **El** (46.9), **Wa** (42.0) and **Pa** (6.6) in mated queens.

MEAN DURATION: 2.37 ± 3.43s (n = 76, virgin queens); 3.90 ± 4.07s (n = 5004, mated queens). There is a significant difference between them (p < 0.001, U-test), which indicates that the **Ic** in egg laying is longer than the **Ic** in honey intake (cf. 1.7), probably because of requiring more time for inspection of the cell conditions and for decision as to whether an egg should be laid.

1.7. Honey intake from a cell (Hi): Same as **Ic**, but lasting longer.

SITUATION: Not observed just before and after flight, in contrast to drones. Preceded by **Wa** (8 cases) and **Re** (1) and followed by **Wa** (7), **Re** (1) and **Sc^h** (1) of V7402, V7410 and V924. Preceded by **Wa** (93), **Re** (6), **Wa** with **Ic** (3) and **El** (1) and followed by **Wa** (95), **Re** (7) and **Sm** (1) in mated queens.

According to Haydak (1950), near swarming,

workers cease to feed the queen, the queen feeds herself on honey (**Hi**) and deposits fewer eggs.

MEAN DURATION: 19.44 ± 16.29s (n = 9, virgin queens); 9.85 ± 5.21s (n = 103, mated queens); no significant difference between them.

1.8. Excited running (Er): Rapid running (5-10cm/s) (Lm4) with body trembling (Genm1), wings usually spread obliquely (Wp3).

SITUATION: Pre-phase of flight activity. Preceded by *Metasoma cleaning* (**Sc^t**, 47.5%), *wandering* (**Wa**, 12.5), *going out* (**Go**, 10.0) *running* (**Ru**, 10.0) and followed by **Sc^t** (65.0), **Go** (12.5), w/**Fe** (7.5) and **Ac** (5.0) in 3 virgin queens, V7402, V924 and V7774; *self-cleaning* (**SC**) inserted frequently but fanning (Wm3) rarely during **Er** (2 cases: 10s in Q7300; 230s in V7410).

MEAN DURATION: 151.50 ± 194.43 s (n=40).

1.9. Running (Ru): Speed (5-10cm/s) same as **Er** (Lm4), but without body trembling.

SITUATION: Preceded by **Wa** (60.0%) , **Sc^t** (24.0), being fed (w/**Fe**, 8.0) and followed by **Wa** (40.0),

Er (20.0), **Wa** (16.0), *staying in motion* (**Sm**, 8.0) and *food begging* (**Bf**/w, 8.0) in virgin queens. Often observed on a link in V924: **Sc'** → **Ru** → **Wa**.

MEAN DURATION: 95.60 ± 127.24 s (n=25, 4 virgin queens); 146.67 ± 103.24 s (n=3, 2 mated queens); no significant difference between them.

1.10. Excited advancing (EA)[#]: Walking zigzag at speed 1-4 cm/s (Lm4) with wings obliquely spread (Wp3) and body trembling (Genm1).

Based on the difference in situation of occurrence, **EA** is divided into 2 sub-types, *excited walking* (**Ea^w**) on the comb surface and *excited creeping* (**Ea^c**) into the ball or on the surface of the ball.

SITUATION: Observed 14 times only in V7410; **Ea^w** and **Ea^c** related to each other and to *being attacked* (w/**At**, cf. 2.4.4), *being balled* (w/**BA**, cf. 2.4.7) and *attacking* (**At**/q, cf. 2.4.3).

MEAN DURATION: 59.62 ± 76.36 s (n=13, **Ea^w**); 507.92 ± 620.15 s (n=12, **Ea^c**); a significant difference between them ($p < 0.01$, U-test).

1.11. Escaping (Es)[#]: Running at speed 5-10 cm/s (Lm4); metasoma slightly raised (Tp2), wings obliquely spread (Wp3) and fanned (Wm3), rarely.

SITUATION: Usually observed upon stimulation by hive lid opening, etc. and when experiencing some aggressive behaviors. In regular sampling, **Es** was found only in V7410 (3 times) and Q7602 (twice) as follows:

V7410 (July 27, 14:41) → w/**Atq**(190s) → **Es**(15) → **Ea^w** (55)

V7410 (July 29, 14:25) → w/**BAq**(1055) → **Es**(15) → **Sc'**(50)

V7410 (July 29, 14:44) → w/**Atq**(50) → **Es**(10) → w/**Atq**(25)

Q7602 (May 20, 15:45) → w/**Maⁿ**(30) → **Es**(45) → **Wa** (120)

Q7602 (May 20, 15:49) → w/**Maⁿ**(15) → **Es**(25) → **Wa** (45)

MEAN DURATION: 22.00 ± 13.96 s (n=5); significantly shorter than the **Ru** in virgin queens ($p <$

0.05, U-test).

1.12. Proboscis protruding (Pp): Similar to *alert-like posture* (**AL**), but without irregular body shaking or head twisting as in workers, with slow back-and-forth movements of protruded proboscis (Gp3) ('protruding proboscis', Ohtani, 1977). May be 'vacant' sucking movements (Gm3). *Proboscis protruding* without Gm3 (**Pp'**) were also observed.

SITUATION: Observed in 3 queens, once each; probably very rare in queens though common in workers.

V7402 (June 28, 02:41) → **Sc^{hm}** (30) → **Pp** (20) → w/**Feq**(10)

Q7500 (May 3, 22:05) → w/**Feq** (10s) → **Pp** (90) → **Wa** (100)

Q7800 (June 11, 17:06) → **Re** (35) → **Pp** (60) → **Wa** (10)

Pp in Q7500 may be regarded as an inspection or an adjustment after utilization of proboscis. A worker responded by *food offering* (w/**Of**/q, cf. 2.3.4) to **Pp** in V7402 probably because **Pp** resembles *food begging* (**Bf**, cf. 2.3.1). Also in Q7800, **Of** (10s) by a worker was observed in **Pp** (60s), but ignored by Q7800.

Two cases of **Pp'** were observed only in Q7500 as follows:

(May 8, 07:51) → w/**Bfq**(15s) → **Pp'** (25) → **Re** (90)

(May 8, 07:55) → w/**Bfq**(10s) → **Pp'** (95) → **Wa** (20)

It may be the soft rejection to *food begging* of a worker (w**Bf**/q).

MEAN DURATION: 56.67 ± 35.12 s (n=3, **Pp**); 60.00 ± 49.50 (n=2, **Pp'**); no significant difference between them.

1.13. Queen-cell destruction (Qd)[#] (Fig. 8): Destruction of the wall of a sealed queen cell or damaging the queen larva in an open cell by pinching and chewing them with mandibles (Mm1, 2) and clinging with legs (Lm3).

According to Huber (cf. Haydak, 1950), as soon

as the queen made a larger opening in the cell, she turned about to push her metasoma into it and stung the pupa involved. Fletcher (1978b) reported that the queen made a small hole at the base of sealed queen cells to sting a newly-emerged queen but did not sting the pupae in queen cells. Caron and Greve (1979) observed **Qd** by workers response to holes cut in the cell wall by queens. Avitabile and DeSomma (1984) described **Qd** by a mated foreign queen introduced artificially into queenless observation hives.

SITUATION: When a wandering queen discovers a queen cell.

Such a queen is often prevented from **Qd** by many workers. This prevention relates to swarming, queen supersedure, queen piping or queen fighting (cf. Haydak, 1950). Grooters (1987) reported that **Qd** was interrupted by replaying recorded tooting sounds.

Qd was not observed in the regular sampling schedule, but 'Drone-cell destruction' was observed in V7410. Two drone cells in the process of 'lid construction' were slightly damaged once (90s) and twice (40s, 15s) by V7410 which possibly misidentified them as queen cells.

1.14. Fanning (Fa)[#] : Motionless except fanning by wings (Wm3) without sound and clinging (Lm3)

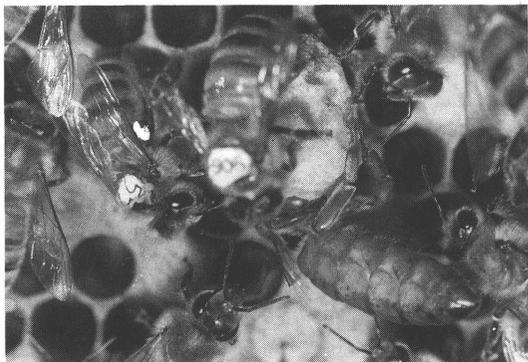


Fig. 8. A mated queen (Q7800) was destroying an emergency queen cell which was made on a place rarely visited by her.

with outstretched or raised legs (Lp2, 5), or **Fa** with *wandering (Wa)* (Lm4).

SITUATION: Preceded by **Wa** (68.8%) and followed by **Wa** (50.0), *metasoma cleaning (Sc^t, 18.8)* and *resting (Re, 12.5)* in V7402, V7410, Q7602 and Q7774; often (7/16) with **Wa**.

MEAN DURATION: 21.56 ± 20.47s (n=16).

1.15. Fine vibration (Vb)[#] : Resting or pausing (cf. 3.5, **Pa**) posture with fine vibration of the whole body, without sound.

SITUATION: 3 times only in V924.

(Sept. 25, 14:15) → **Sc^t** (175s) → **Vb** (130, *Re*-posture) → **Sc^t** (50)

(Sept. 28, 18:52) → **Sc^t** (50) → **Vb** (20, *Re*-posture) → w/**Fe** (35)

(Oct. 7, 22:33) → **Sc^t** (45) → **Vb** (150, *Re*-posture) → **Pa** (525)

MEAN DURATION: 100.00 ± 70.00s (n=3)

1.16. Going-out (Go)[#] : Taking-off from the hive entrance for nuptial flight and 'circling' orientation flight (cf. Wenner and Wells, 1990:322).

SITUATION: 5 times only in V7774.

(July 11, 11:12) → **Wa** (110s) → **Er** (165) → **Go** (315) → **Er** (185) → **Go** (700) → **Er** (45) → **Go** (285) → **Er** (10) → **Go** (255) → **Er** (95) → **Sc^t** (55) → **Er** (115) → ... → **Er** (20) → **Go** (1825) → **Wa** (60) → **Sc^h** (15)

The last **Go** was a successful flight for mating.

MEAN DURATION: 388.75 ± 208.94s (n=4, unsuccessful or orientation flight).

1.17. Wax manipulation (Wx)[#] : Any treatments by pinching or chewing bee wax with mandibles (Mm1, 2), and by twisting, rolling or nodding head (Hm1-3) except 'comb building' and 'worker cell capping'.

SITUATION: Only once in V924 which was gnawing inner wall of a cell:

(Sept. 26, 10:41) → w/**Fe** (15s) → **Wx** (35) → **Wa** (50)

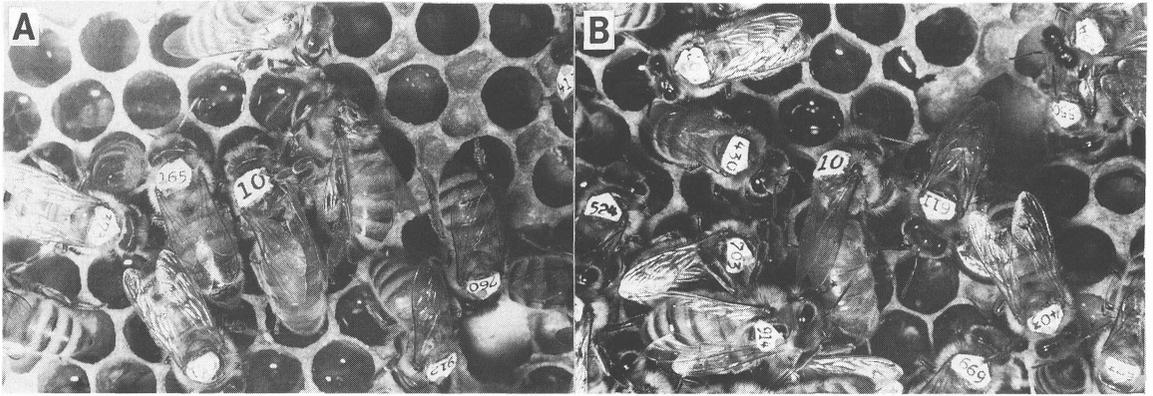


Fig. 9. A virgin queen (V910) and her attendants. **A** : V910 was attacked by a monopolist attacker, taking the flinching posture. **B** : Four workers were attracted to the metasoma of V910. No. 914 was touching the queen.

2. Interindividual Behaviors

Out of 3 possibilities, (1) queen - queen, (2) queen - drone, and (3) queen - worker, the last is mainly described here, since the first 2 were scarcely observed.

2.1. Royal court being formed (w/RCq)[#] : Encircled by worker attendants. The queen's behavior is not distinguished from **Re**, **SC**, *food begging* (**Bf**, cf. 2.3.1) or *being fed* (w/**Feq**, cf. 2.3.3). Virgin queens have several attendants mainly around her metasoma (cf. Fig. 5). The number of attendants tends to increase with the age of virgins, as Solodkova (1958) reported, at first 5-7, later 10-15 workers. For laying queens, Taranov and Ivanova (1946) observed 10-12 workers at the beginning of swarming preparation but 22 during so-called swarming-impulse time. Most laying queens are and can be fully encircled by ca. 12 workers (cf. Fig. 4). If there are more than 12 workers, some must climb on others. Virgin queens may have queen substances only on the metasomal surface (Velthuis, 1970), while mated queens have them both on metasoma and in mandibular glands (e.g. Vierling and Renner, 1977). In stingless bees, interestingly, virgin queens attract more workers than mated queens do (Sakagami *et al.*, 1977).

Worker Behavior, royal court formation

(w**RC/q**)[#] : 'Forming a court', 'retinue formation' (Velthuis and Es, 1964; Winston *et al.*, 1989); 'retinue behaviour' (Velthuis, 1985) or 'court behavior' (Free *et al.*, 1992); involving the next 13 observed patterns.

RC-1, examining (Allen, 1956, 1957) (cf. Fig. 9B, nos. 430, 611 and 703): Motionless, only antennae slowly moved toward the queen; 'palpating only' (Free *et al.*, 1992). Similar to resting (**Re**) or staying alert (**Sa**, cf. Ohtani, 1977); head raised (Hp4), forelegs often detached (Lp4) and wings slightly or obliquely spread (Wp2, 3).

This is probably the same as Velthuis' (1972) 'retinue 1': "At some distance the antennae are directed towards the queen, so that the very tips of the antennae just reach the queen's cuticle".

RC-2, touching or licking (Ma^l or Ma^l), cf. 2.4.4) (Fig. 7A, no.43; Fig. 9B, no. 914): Touching a queen with forelegs or proboscis (Lp7, Gm2).

Allen (1956, 1957) reported only licking. Verheijen-Voogd (1959) observed both. Velthuis (1972) reported tongue-rubbing of the queen's metasoma as his 'retinue 2'. Free *et al.* (1992) distinguished 'licking and palpating' from 'licking' only.

RC-3, nibbling[#] (Maⁿ), cf. 2.4.2): Rolling or nodding (Hm2, 3), tapping (Am1), chewing (Mm2) and patting (Lm1). All were mostly directed to

the neck of the queen (Lm8), often intervened with *Sc^{ha}* and *Sc^{he}*.

This is probably the same as Velthuis' (1972) 'retinue 3': "While the worker is still closer to the queen and may even have mounted the abdomen, this part of the queen is repeatedly touched with the mandibles and a given place is inspected thoroughly. At the same time the bee persists in moving the antennae all over the abdomen of the queen."

RC-4, irritated rocking [#] (cf. Hammann, 1958, 'rocking movements'): Forelegs slightly raised (Lp3), mid- and hindlegs anchored to substrate (Lp2), and whole body irritatedly repeating back and forth movements (Lm5); wings obliquely spread (Wp3); mandible opened (Wm2 or Wm3); posture resembling a mild 'shock reaction' (Sakagami, 1958) and 'cradle movement' of 'threatening', as defined by Yadava (1970). **RC-4** has 2 deformed type as follows:

RC-4', deformed type I: Forelegs and forebody highly raised (Lp5, Tp2), soon followed by dropping down, and forelegs touch substrate (Lp7); regularly repeated as incomplete rocking movements (Lm5).

RC-4'', deformed type II: Forelegs and a midleg detached from substrate (Lp4) and usually twitching (Genm2); rocking movements not inserted.

RC-5, food offering (wOf/q, cf. 2.3.4), consequent **feeding** (wFe/q, cf. 2.3.3) (Fig. 7B, no. 85) or reciprocal **food offering** (wOf-w).

Taranov and Ivanova (1946) observed wOf/q at the first stage of swarming preparation. Verheijen-Voogd (1959) observed wOf/q, wOf/w and wBf/w in the royal court. Velthuis (1972) observed and described wOF/q and wFe/q.

RC-6, self-cleaning (*SC*, cf. 1.4): Mouthparts cleaning (*Sc^{hm}*), especially 'stropping of tongue' (Butler and Free, 1952; Sakagami, 1954) (Fig. 7A, no. 98), and *Sc^{he}* (Fig. 5C, no. 4) more frequent.

A report that rectal contents from queens released grooming behavior suggested that **RC-6** is caused by 'the possible existence of a faeces

pheromone' (Post *et al.*, 1987).

Naumann (1991) suggested that 'self-grooming' by workers resulted in the translocation of queen pheromone from the head to the metasoma.

RC-7, polishing-like rocking (*PI*) [#]: Forelegs patting (Lm1) and mid- and hindlegs rocking (Lm5).

Somewhat resembling a 'washboard movement' (Root *et al.*, 1980; Ru, 1982) and 'Hobeln' (Freundstein, 1932; Sakagami, 1953; Milum, 1955), but probable combination among **RC-2**, **RC-3** and **RC-4**. Once observed in V7410 (3 days old).

RC-8, proboscis protruding (*Pp*, cf. 1.12) (Fig. 4B, no. 18).

RC-9, shaking (wSh/q, cf. 2.6): Metasoma dorsoventral motion (Tm1) and up-down motion (Lm6) with outstretched legs (Lp2).

Taranov and Ivanova (1946) observed wSh/q lasting 3-4 s at swarming time. Milum (1955) mentioned that queen shakers (wSh/q) were not 'general wandering shakers' (wSh/w). Other authors observed and studied wSh/q (Hammann, 1957, 1958; Allen, 1958, 1959a, b; Gahl, 1975; Fletcher, 1978a, b; Schneider, 1991).

RC-10, staying alert (*Sa*): "Without locomotion; forelegs usually raised but sometimes lightly touching substratum; metasoma lowered; wings spread obliquely; whole body rather well detached from substratum; antennae lowered or moved slowly" (in a cyclopic worker, Ohtani, 1977).

RC-11, escaping (*Es*, cf. 1.11).

Probably the same to Velthuis' (1972) 'avoidance': "The worker bee shrinks back when still a few cm away from the queen, refuses to enter the part of the cage where the queen is located, and moves around in an agitated way as if in search for an opening allowing escape".

RC-12, chasing (*Ch*) [#]: Some workers chase the queen which begins to walk.

RC-13, head pressing (wHp/q) [#]: Pressing and pushing frons against the queen's mesosoma (Lm7 +8); motionless.

SITUATION (*head being pressed*, w/Hpq): w/MA"

(2 cases) and **Re**(1) of preceding patterns; **Re**(2) and **w/MAⁿ**(1) of following patterns only in V6720.

MEAN DURATION: 25.00 ± 13.23s (n=3).

SITUATION (all **RC**): Irregularly and individually occur at low activity of queens. In non-swarming time, **RC-1** is the most frequent pattern (76/164), and **RC-10** (29/164) and **RC-4** (27/164) follow it.

When the queen begins **Wa**, most attendants are left behind (cf. Solodkova, 1958), though a few workers sometimes follow her for a while (*i.e.* **RC-12**). **RC-5** to **RC-10** may both be displacement behaviors due to a state of conflict between 2 drives, fleeing and aggression (cf. Fig. 17). **At** the swarming time attendants becomes closer to the queen and the number increases to more than usual (Taranov and Ivanova, 1946). Though **RC-13** only 3 times in V6720, the same patterns were observed on the shaft of the thermostat set on the observation hive instead of queen's mesosoma.

2.2. Antennal contact (w, d-**Ac**-q) [#] : Reciprocal contact with antennae of 2 individuals *vis-a-vis*; mostly 2 or 3 contacts by tapping (**Am1**) within 1 s, rarely more than 5 s; once **Ac** between queen and drone (d-**Ac**-q) was observed in V7402 (June 27, 14:34); same as 'antennation', the subject bee and another bee contacting each other through their antennae (Juska *et al.*, 1981).

SITUATION: Preceded by *wandering* (**Wa**, 82.6%) and *resting* (**Re**, 10.9) and followed by **Wa** (78.3) and **Re** (10.3) in 5 virgin queens. Precede by **Re** (41.4), **Wa** (39.5), *being fed* (**w/Fe**, 7.0) and *staying in motion* (**Sm**, 5.7) and followed by **Wa** (43.9), **Re** (41.4) and **Sm** (8.9) in 6 mated queens. Lasting **Ac** (more than 5 s, max. 49 s in Q7200) relates to *food begging* (**Bf**; cf. 2.3.1), mostly occurring after short **w/Feq** or before long **w/Feq**.

Ac shorter than 5 s occurring mostly (27/29 in V7402) or mainly (30/86 in Q7200) during **Wa** might be 'electro-communication' via discharge

current if the antennae have different polarities (Warnke, 1976).

MEAN DURATION: 0.98 ± 3.27 s (n=46, virgin queens); 2.90 ± 6.26 (n=157, mated queens); significant difference between them (p < 0.05, U-test).

2.3. Food transmission (FT) [#]

2.3.1. Food begging (q**Bf/w**, d): Protruding glossa (**Gm1**, **Gp3**) toward an attendant nearby or a wandering drone; head raised (**Hp4**), antennae with positions variable (**Ap2-5**), tapping slowly (**Am1**); rarely, forelegs patting (**Lm1**).

SITUATION: Preceded by **Wa** (86.1%) and **Re** (5.6) and followed by **Wa** (84.0) and **Re** (5.9) in 4 virgin queens. Preceded by **Re** (48.2), **Wa** (26.6), **Sm** (9.3) and **Sc^f** (4.7) and followed by **Re** (46.0), **Wa** (26.3), **Sm** (9.9) and **w/Fe** (9.5) in mated queens. In the last case (**Bf/w** → **w/Fe**), only the lasting **Bf/w** over 3 s were registered, because the shorter **Bf/w** than 3 s were belonged in **w/Fe**.

MEAN DURATION: 2.38 ± 3.90 s (n=324, virgin queens); 1.82 ± 3.25 (n=274, mated queens); no significant difference between them.

Worker Behavior, being begged (q**Bf/w**): Most workers reacting with *food offering* (**wOf/q**; cf. 2.3.4), and some workers in **RC-1** reacting with **RC-5**, but a few individuals ignoring q**Bf/w**.

Drone behavior, being begged (q**Bfd**): Only 2 cases in V7402, (1) ignoring and (2) counter-begging (d**Bf/q**; cf. 2.3.2).

2.3.2. Being begged by workers (w**Bf/q**): 3 responses to begging workers, (1) ignoring, (2) counter-begging and (3) flinching posture (cf. Figs. 5D, 9A) response to ardent begging.

SITUATION: Usually during **Wa** or **Re**. Rarely reacted with *food begging* (q**Bf/w**), which often went to *reciprocal begging* (w**Bf-q**).

Szabo and Smith (1973) observed *reciprocal begging* between queens (q**Bf-q**) under an experimental condition. Velthuis (1985) adopted the term of 'asking / being asked' among workers.

MEAN DURATION: 3.02 ± 5.70 s (n=86, 3 virgin

queens); 7.50 ± 6.45 ($n=4$, 3 mated queens); no significant difference between them.

Worker behavior, food begging (wBf/q): Same as qBf/w.

2.3.3. Being begged by drones (d/Bfq): Same as w/Bfq (cf. 2.3.2).

Counter-begging is always followed by *reciprocal begging* (d-Bf-q), because drones are devoid of *food offering* (Of); observed only in V7402.

SITUATION: Preceded by *Wa* (68.2%) and *Re* (18.2) and followed by *Wa* (77.3) and *Re* (18.2).

MEAN DURATION: 14.09 ± 12.21 s ($n=22$); significantly longer ($p < 0.001$, U-test) than the w/Bf in virgin queens (3.02 ± 5.70 s).

Drone behavior, food begging (dBf/q): Same as dBf/w or dBf/d. "Protruding glossa (Gm1) towards a worker or rarely a drone nearby; Head raised (Hp4), antennae protruded straight forwards (Ap5) and tapping (Am1); Forelegs occasionally patting (Lm1) and wings disposed as in *Wa* (Wp2)" (Ohtani, 1974).

2.3.4. Being fed (w/Feq) (Fig. 7B): Same as Bf (*food begging*), only more prolonged with actual sucking with glossa (Gm3); much slower than in drones (w/Fed), neither foreleg patting nor head rolling. Sometimes forelegs protruded (Lp3) and whole body trembled (Genm1).

SITUATION: After qBf/w. Always cut off by cessation of food acceptance by the queen. Preceded by *wandering* (*Wa*, 46.3%), *staying in motion* (*Sm*, 21.5), *resting* (*Re*, 15.2) and *metasoma cleaning* (*Sc^f*, 10.2) and followed by *Sm* (35.4), *Wa* (33.6), *Re* (19.0) and *Sc^f* (5.0) in virgin queens. Preceded by *Re* (40.1), *Wa* (23.0), *egg laying* (*EL*, 9.9) and *pausing* (*Pa*, 9.9) and followed by *Re* (36.3), *Wa* (31.6) and *Sm* (15.1) in mated queens.

MEAN DURATION: 35.18 ± 39.00 s ($n=437$, virgin queens) and 43.70 ± 36.16 s ($n=910$, mated queens). Laying queens received significantly longer feedings than non-laying queens ($p < 0.001$, U test).

Worker Behavior, feeding (wFe/q): As in food transmission from workers to drones (wFe/d), but slower in most cases; without movements characteristically appearing near the end of feeding to the drones (wings spread obliquely, metasoma raised, forelegs patting and mid- and hindlegs stepping backward; Ohtani, 1974).

2.3.5. Food offering (qOf/w): Opening and protruding mandibles (Hp4, Mp3) holding a food drop; Glossa spread slightly (Gp2); Head raised (Hp4). Same as *food offering* between workers (wOf/w).

SITUATION: Only in virgin queens, sometimes attacked by workers; in alien queens approached by workers for balling. Only second response were observed in the regular sampling.

Worker Behavior, being offered (q/Ofw): 3 responses, (1) ignoring, (2) protruding glossa (Gp2) and accepting food (q/Few), and (3) *attacking* (wAt/q, cf. 2.4.3).

Of by attacked workers to attackers in queenless colonies were reported by Sakagami (1954). *Of* in an aggressive situation may be an 'appeasement display' (Manning, 1972). According to Sakagami (pers. comm.), far more specialized *food offering* by virgin queens is observed in some stingless bee species.

Velthuis (1985) studied 'offering / being offered' among 5 workers.

w/Ofq (being offered by a worker) was once observed during *Re* in Q7774, which responded with ignorance.

2.3.6. Feeding (qFe/w, d): Similar to food transmission from a worker to the queen (wFe/q) or to a worker (wFe/w).

SITUATION: As response to qOf/w. Preceded by *Wa* (50.0%) and *Sc^f* (18.8) and followed by *Wa* (37.5) and *Re* (25.0) in 4 virgin queens (V910, V7402, V924 and V6720).

These *feedings* (qFe/w, d) are probably regarded as postphases of *food offerings* (qOf/w, d); that is, the bees simply responded to the *food*

offering by queens that perhaps performed the appeasement display mentioned above.

MEAN DURATION: 26.25 ± 23.77 s ($n = 16$, qFe/w); 90.0 s ($n=1$, qFe/d)

2.4. Aggressive interactions (AG)

Social wasps and bees are fairly aggressive toward other animals, because their nests are attractive to many animals as desirable food sources. On the other hand, they must suppress aggression toward nestmates. Nevertheless, it is difficult to inhibit the aggression completely within the hive. Nine patterns of **AG** were observed inside the colony.

2.4.1. Manipulating (qMA/q , w): Same as *touching* (Ma') and *licking* (Ma') by workers to drones (Ohtani, 1974).

SITUATION: **MA** once observed between 2 virgin queens (May 25, 1973); *licking* a worker (qMa'/w) once observed during **Wa** in V7402, and *touching* a worker (qMa'/w) 6 times usually during **Wa** in V924 and V6720.

Gary *et al.* (1967) and Szabo and Smith (1973) also reported qMA/q in their experiments on the aggressive relationship among many queens or between pairs of queens. Short **MA** before *attacking* (**At**, cf. 2.4.3) was observed by Skirkevicius (1965) and Szabo and Smith (1973) in certain experimental conditions.

MEAN DURATION: 5.0 s ($n = 1$, Ma'/w); 6.67 ± 2.58 s ($n=6$, Ma'/w).

2.4.2. Being manipulated (w/MAq) (Fig. 5B): *Being touched* with forelegs by worker(s) or being ridden on ($w/Ma'q$), *being licked* with proboscis by worker(s) ($w/Ma'q$), or *being nibbled* (w/Ma'')# (cf. 2.1, **RC-3**); usually not followed by particular responses, though by flinching posture and *escaping* (**Es**) when manipulated frequently and violently.

SITUATION: Preceded by *resting* (**Re**, 37.5%), *wandering* (**Wa**, 30.7), and *metasoma cleaning* (**Sc'**,

10.2) and followed by **Wa** (40.9), **Re** (21.6) and **Sc'** (13.6) in 5 virgin queens. Preceded by **Re** (36.8%), *egg laying* (**El**, 15.8), and **Wa** (15.8) and followed by **Wa** (47.4) and **Re** (15.8) in 7 mated queens.

MEAN DURATION: 30.49 ± 30.64 s ($n = 41$, w/MA'); 26.45 ± 30.98 s ($n=55$, w/Ma'); 32.73 ± 30.53 s ($n=11$, w/Ma''); no significant difference among them. 27.84 ± 30.75 s ($n = 88$, virgin queens); 32.37 ± 30.48 s ($n=19$, mated queens); no significant difference between them.

Worker Behavior, manipulating (wMA/q): Similar to that toward drones (*licking and touching*), but *nibbling* only in the royal court.

2.4.3. Attacking (qAt/q): Legs clinging (Lm3) to aggressee, mandibles pinching (Mm1) and pulling appendages and wings. "Biting movements" (Mm2) "directed toward the abdomen of the anterior queen" (Gary *et al.*, 1967).

Queen attacks on drones (qAt/d) have never been observed inside the hive. V7774 was once observed seizing a drone at the hive entrance (July 11, 1977), when she successfully returned from a mating flight.

Queen attacks on workers (qAt/w) were not observed, but Solodkova (1958) reported antagonistic behavior in a virgin queen toward her workers. Skirkevicius and Vaitkeviciene (1969) also reported virgin queens attacking and *stinging* (**Sg**, cf. 2.4.5) some alien workers. Weaver and Weaver (1980) found that young virgin queens physically dominated the workers.

SITUATION: Observed only in V924 (3 times) and V7410 (twice) as follows:

V924 (Sept. 25, 18:01) → **Wa** (45s) → **At** /q(5) → **Wa** (40)

V924 (Sept. 25, 18:20) → **Wa** (150) → **At** /q(10) → **Wa** (25)

V924 (Sept. 25, 22:46) → **Re** (670) → **At** /q(10) → **Sm** (80)

V7410 (July 27, 14:13) → **Wa** (30) → **At** /q(350) → **EA''** (75)

V7410 (July 27, 14:44) → **EA**^w (55) → **At** /q(105)
→ **EA**^c (360)

MEAN DURATION: 96.00 ± 148.05s (n=5).

2.4.4. Being attacked (q/Atq): “The ‘defending’ queen, *i.e.* the queen which at the commencement of the engagement was not successful in taking up a good position, also tried to grip with her mandibles the legs of the attacking queen by way of warding her off, or disengaging” (Skirkevicius, 1965). Once the attacked queen (V7411) removed her legs from the comb, and fell down, together with the attacking queen (V7410).

w/Atq: Responses to worker attacks; motionless but with flinching posture (Hp2 + Tp4, to one side) or sometimes food offering (qOf/w, cf. 2.3.4), often followed by *shrieking* (**Sr**, cf. 2.4.9) or trying to escape, dragging attackers (**Es**, cf. 1.11) and *being balled* (w/BAq, cf. 2.4.7).

SITUATION: Preceded by **Wa** (48.9%), **Re** (29.3) and **Sc**^t (11.1) and followed by **Wa** (72.9), **Sc**^t (10.0) and **Re** (7.1) in 5 virgin queens. Most frequent case: **Re** → w/At → **Wa** → w/At → **Wa** → w/At → **Wa** → **Sc**^t.

In mated queens only Q7800 was attacked by workers: **Wa** (25.0%), w/Fe(25.0), **Sc**^t (25.0) → w/At → **Wa**(75.0)

MEAN DURATION: 37.16 ± 60.71 s (n=278, virgin queens); 24.38 ± 18.02s (n=8, mated queens); no significant difference between them.

Worker Behavior, attacking (wAt/q): Same as attacking drones (wAt/d), including 3 patterns, *pulling*, *biting* and *seizing*, defined as follows:

“Pulling movements, clinging movements and rolling the queen over” (Hammann, 1958). “Biting - A worker uses its mandibles to clamp onto a leg or wing. In addition, a worker may attempt to pull the queen” (Robinson, 1982). “Pulling: consisted of seizing plus a locomotory element, the worker making an effort to drag the queen”, and “Seizing: consisted of grasping part of the attacked queen with the mandibles” (Yadava, 1970).

Attackers were monopolists. Queen’s ‘retaliate’

(Hammann, 1958) has never been observed.

2.4.5. Stinging (qSg/q)[#] : “Abdomen arching” (Tp7) and “sting protrusion”(Tm6) (Gary *et al.*, 1967). “Stinging: consisted of the actual extrusion of the sting and an attempt to drive it into the body of the attacked queen, particularly in the region of her thorax or abdomen” (Yadava, 1970). **Sg** is accompanied by pinching with mandibles (Mm1).

wSg/q: “A fully mounted worker with its abdomen sharply arched ventrally attempts to insert the sting into the body of the queen, usually in the intersegmental membranes between the first few abdominal segments” (Robinson, 1982).

2.4.6. Being stung (w, q/Sgq)[#] : Motionless and all legs contracted (Lp1) or often detached from substrate (Lp4), metasoma lowered (Tp3) or contracted (Tp5); toward death because of the poison of successful stinging. “Usually the first attacker injured or killed her opponent” (Szabo and Smith, 1973).

SITUATION: Often observed in *being balled* (w/BAq, cf. 2.4.7). No **Sg** was observed in the regular sampling schedule.

Worker Behavior, stinging (wSg/q)[#] : Same as qSg/q.

Velthuis (1972)’s ‘aggression’ seems to include wSg/q as well as wAt/q.

2.4.7. Being balled (w/BAq)[#] (Fig. 10): Probably same as w/Atq or w/Sgq, but concealed by many workers forming the ball.

SITUATION: Preceded by *being attacked* (w/At, 40.0%), *excited walking* (**EA**^w, 20.0) and *shrieking* (**Sr**/w, 20.0) and followed by **Sr**/w (40.0), **EA**^w (30.0), **Wa** (20.0) and *escaping* (**Es**, 10.0) in V7410.

MEAN DURATION: 546.25 ± 387.48s (n=8); significantly longer than that of the w/At in virgin queens (p < 0.001, U-test).

Robinson (1982) perceived 4 patterns in queens

balled: 'defensive posture' (legs tucked in and metasoma pressed against substrate), 'immobile posture' (for packing by workers), 'food begging' (but not offered any food) and 'sting-sheath exposing' (intermittent opening of sting chamber, and exposing the sting and bi-lobed sting sheath). The last response is very interesting because the protruding sting sheath attracted workers and they licked it.

I also observed a similar behavior once in V924, after short w/At and short *tooting* (*To*, cf. 2.4.8): *Wa* → w/At (5 s) → *Wa* (5) → *To* (10) → *Sc*'(20) → *Re* (60) → *Wa* (105) → *Re* (45) → 'sting reversal' (10) → *Re* (Sept. 28, 18:41). 'Sting reversal' is the behavior in which the sting chamber is more widely opened than as photographed by Robinson, and the sting seems to be reversed when the first basal rami of the sting protrude, as if it is the start of egg laying. 'Sting-sheath exposure' may be a prephase of 'sting reversal'. In relation to both behaviors, the queen's Koschewnikow's glands are known to be near the sting sheath (cf. Grandperrin and Cassier, 1983). Recently, it was found that ethanol extracts of Koschewnikow's glands induced typical balling (Lensky *et al.*, 1991, discussed later).

Worker Behavior, balling behavior (wBA/q): "May be defined as the holding, abdomen inflecting, or stinging behavior of a clustered group of workers" (Walton and Smith, 1969). Based on my fragmentary observations and the intensive study by Robinson (1982, 1984), **BA** can be subdivided into the following 12 patterns:

BA-1, examining: Same as **RC-1**; 'antennating' by Robinson (1982, 1984).

BA-2, licking and/or **touching:** Same as **RC-2**; 'licking and/or stroking' by Robinson (1982, 1984).

BA-3, food begging or **feeding:** Same to **RC-5**; 'feeding' by Robinson (1982, 1984).

BA-4, immobile: Remaining motionless while pressing tightly (Lm7, 8) against the queen (Robinson, 1984).

BA-5, guarding: Facing the queen with head

raised (Hp6), antennae exploring (Am2) and mandibles opened widely (Mp3); 'Threatening' is also exhibited by bees that guard the nest entrance (Robinson, 1982).

BA-6, mounting: Climbing partially or fully onto the dorsal surface of the queen (Robinson, 1982); antennae exploring (Am2), head lowered (Hp5), metasoma lowered (Tp3) and legs clinging (Lm3, 7).

BA-7, metasoma arching: Mounting fully (Lm3) and metasoma arched (Tp7) slightly ('abdomen arching' by Robinson, 1982).

BA-8, metasoma inflecting: Mounting fully (Lm3), metasoma arched (Tp7) and often touching the dorsal surface of the queen, and frequently sting chamber opening (Tm5) but not sting protrusion ('abdomen inflecting' by Robinson, 1982).

BA-9, attacking (*At*, cf. 2.4.4): 'Biting' by Robinson (1982, 1984) includes 'seizing', 'pulling' and 'clamping'.

BA-10, stinging (*Sg*, cf. 2.4.2).

BA-11, alarm fanning (Fig. 10C): Metasoma highly raised (Tp8) with fanning wings (no sound) and with exposed hairy membrane (Tp9) above the bulb of the sting (Maschwitz, 1964). This pattern was frequently found in disturbed workers.

BA-12, excited fanning: Excitedly walked or stepped (Lm4) with fanning (Wm3) and sometimes suddenly reversed direction (Lm9) (cf. Ghent and Gary, 1962); often observed on the ball surface. Fanning wings sometimes produce the bipping sound (Wenner, 1964). This pattern is caused by various intruders. Shearer and Boch (1965) observed alerted and agitated guard bees which can step jerkily in circles or short zigzags preferentially toward filter paper treated with isoamyle acetate or 2-heptanone.

SITUATION (all wBA/q): Appeared when a colony was invaded by an alien queen, or when a queen's laying capacity was slowed down, as in the case of the mother queen of V7774 (cf. Fig. 10), an important observation that is detailed below:

The balling of the mother queen was observed outside the regular observation. On May 25, 1977, the mother queen was found with several attendants on the floor of an observation hive (OH5). Her egg-laying capacity had been slowing down for several days. Workers on the comb began to construct several emergency queen cells, from one of which a new virgin (V7774) emerged on July 6. The mother seemed to be unable to go out to swarm with her workers. To observe the fighting between mother and daughter, I picked up the mother queen and placed her on the surface of the comb. Nearing her mother, V7774 passed her without interest. A few minutes later, the attendants around the mother suddenly became excited, and commenced balling (Fig. 10A). This ball dropped slowly (Fig. 10B, C) to the floor (Fig. 10D). The next morning, the corpse of the mother was found outside the hive.

In reference of the introduction to an alien queen to a colony, many beekeepers observed balling: "Very often when the bees decide they will not accept the queen left loose among them they will begin to pull at her, piling on her in such numbers that they form a ball around her" (Root *et al.*, 1980). Allen (1956) also observed 2 cases of balling of a queen by workers.

In the balling of an alien virgin queen, V7410 approached the balling with *excited walking* (EA^w , cf. 1.10) and often crept on and into the ball (EA^c : *excited creeping*).

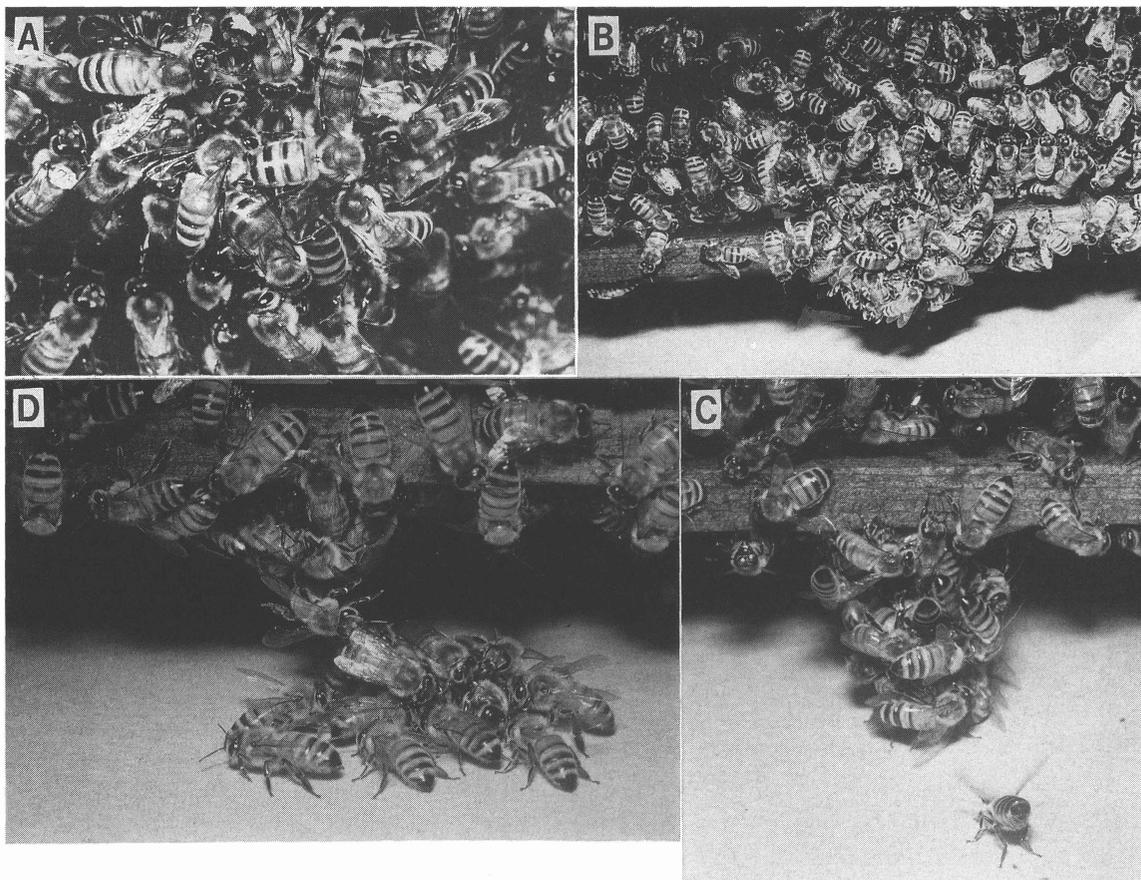


Fig. 10. The balling of a mother queen that coexisted with her daughter queen, V7774 (from the color slides by Satoshi Kuribayashi). **A:** A few minutes after V7774 passed near the mother, her attendants formed a ball. **B:** The ball dropped down slowly. **C:** When the ball reached the floor, a worker began to perform *alarm fanning*. **D:** The ball on the floor. The mother queen can be seen in the center.

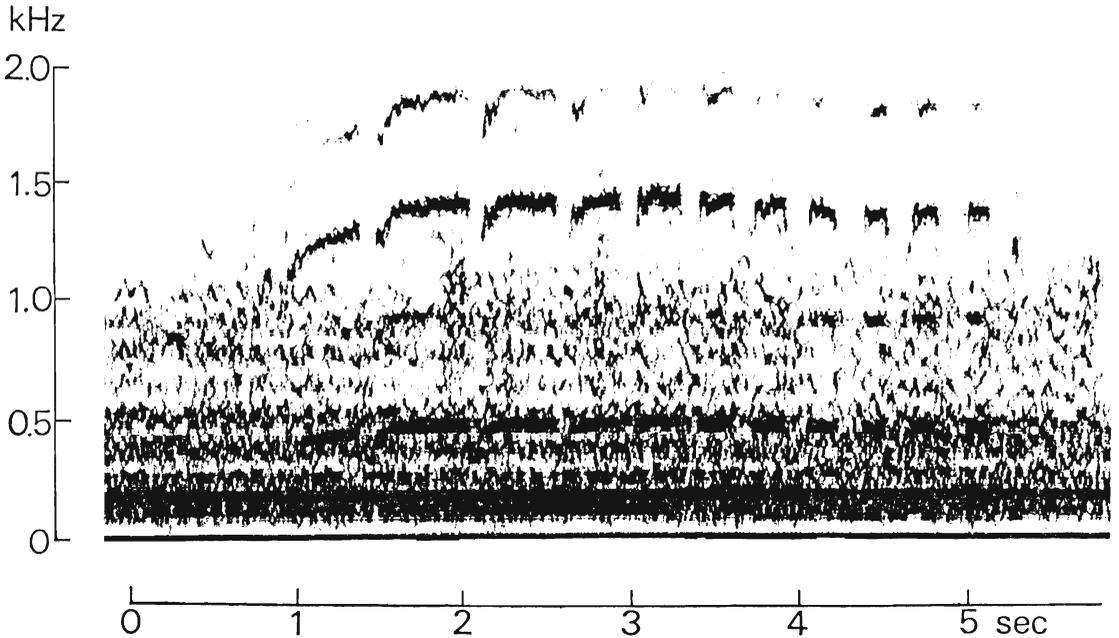
tooting

Fig. 11. The sound spectrogram of *tooting* (*To*). The sound was recorded by an Aiwa Model TPR-602 cassette tape recorder at the speed of 4.8 cm/s. The analyzer was a Sonagraph Model 7029 (Kay Electronic Co.) in Department of Oral Physiology, Hokkaido University School of Dentistry.

2.4.8. Tooting (*To*/q, w)[#] (Fig. 11): Mesosoma pressed on the comb (Lm7) and metasoma raised (Tp2), wings folded (Wp0 or Wp1) and vibrated (Wm4) synchronously with tooting rhythm (cf. Wenner, 1964).

Tooting posture fairly resembles the posture in 'worker piping' (Ohtani and Kamada, 1980). It was observed that V924 pressed her mesosoma on cells after getting her body into the small and shallow hollow of the comb.

SITUATION: Preceded by *wandering* (*Wa*, 29 cases), *being attacked* (w/*At*, 3), *metasoma cleaning* (*Sc'*, 3), *shrieking* (*Sr*/w, cf. 2.4.9) (1), *resting* (*Re*, 1) and *excited walking* (*EA''*, 1) and followed by *Wa* (28), *Sc'* (5), w/*At* (3), *head cleaning* (*Sc^h*, 1) and *being fed* (w/*Fe*, 1) in V924 (35 cases) and V7410 (3), which emitted the tooting sound when they became free from some attackers by which they were forced to emit the shrieking sound (cf. 2.4.9).

MEAN DURATION: 8.29 ± 2.67 s (n=38).

Tooting sounds caused a delay in emergence of rival queens with relation to *shaking* (*Sh*, cf. 2.6) on queen cells (Bruinsma *et al.*, 1981), or to *queen-cell destruction* (*Qd*) (Grooters, 1987).

2.4.9. Shrieking (q*Sr*/w)[#] (Fig. 12): Same as w/*At*q but emitting a shrieking sound during w/*At* or w/*BA*; sometimes short *Sr* emitted between w/*At* or w/*BA* without attackers.

Queen piping, except *To*, has been known as *quacking* emitted within the queen cell (Armbruster, 1922; Hansson, 1945). Having not observed quacking, I reserve its description here. Instead, I observed another piping sound, q*Sr*/w, which might be 'quacking emitted outside the queen cell'.

Robinson (1982) also reported queen 'crying' during w/*At* and w/*BA*. Robinson's sonagram was

not the same as, but was similar to the sonagram in Fig. 12.

SITUATION: Preceded by w/*At* (10 cases), *Wa* (5), w/*BA* (4), *Sc'* (1), *Bf*/w (1), *Sm* (1) and *Re* (1) and followed by *Wa* (7), w/*BA* (5), *Re* (3), *Sc'* (3), w/*At* (2), w/*Fe* (1) and *To* (1) in V924 (9 cases) and V7410 (14).

To and q*Sr*/w were observed in V924 when introducing into the observation hive where some laying workers and several attackers were observed in a queenless condition (cf. Sakagami, 1954); and in V7410 when the neighbor queen (V7411) erroneously tried to enter the hive after her unsuccessful mating flight. The 2 queens emitted the shrieking sound when they were attacked by one of some particular workers, and did the tooting sound when they caught the attacker off her aggressive behavior.

MEAN DURATION: 10.00 ± 2.50s (n = 9, V924); 215.36 ± 220.37s (n = 14, V7410); significant difference between them (p < 0.01, U-test).

2.5. Wing-base being gnawed (w/*Wgq*): Immobile state with wings spread widely (Wp4) against gnawing worker (cf. Ribbands, 1953). 'Being gnawed wing-bases' (Ohtani, 1974).

SITUATION: Preceded by *Sc'* (4 cases), *Re* (3), *Wa* (1) and w/*MA'* (1) and followed by *Sc'* (4), *Re* (3), and *Wa* (2) in V924 (8 cases) and Q7774 (1).

V924 maintained widespread wings for 35-110 s when a worker rode on her back without gnawing (4 cases), and when a worker touched her metasoma (3 cases), but once for 10s without any workers. The same pattern was once observed for 30 s in Q7774 after her metasoma was licked by a worker (w/*Ma'*).

MEAN DURATION: 57.22 ± 32.03s (n=9).

Worker Behavior, wing-base gnawing (w/*Wg*/q): Probably same as w*Wg*/w (Milum, 1947) and w*Wg*/d ('gnawing wing-bases') (Ohtani, 1974): "Head raised (Hp4) or disposed and tapping (Am1) slowly, mandibles chewing (Mm2) and pecking wing-bases or constriction between mesosoma and

metasoma, and legs clinging (Lm3) to metasoma of drone."

According to Ribbands (1953), w*Wg*/q is similar to that toward workers (w*Wg*/w) and toward drones (w*Wg*/d). In my observation, however, the 4 workers which rode on V924's back did not perform anything.

It is often observed in w-*Wg*-w that an actee performs 'grooming dance' (Milum, 1947; Sasaki, 1993) before she is gnawed by an actor, but neither in w*Wg*/q nor in w*Wg*/d.

Other 4 cases of the above w/*Wgq* seemed to be released erroneously by touching or licking by workers. Grozdanic (1951) succeeded in inducing w/*Wgw* and w/*Wgq* by a gentle stroke with the point of a straw on the mesosoma between the wings, and laying queens reacted even during actual laying, though it was unsuccessful with virgin queens and drones. Whenever endogenous conditions are fulfilled, 'being gnawed posture' seems to be released by simple mechanical stimuli, or even without any stimuli. Workers' q/*Wg*-posture was also observed in this study when V924 during *Sc'* or *Wa* mechanically touched each of 2 workers

2.6. Being shaken (w/*Shq*)[#]: A momentary halting of wandering (*Wa*) or ignorance to a shaking worker at resting (*Re*).

SITUATION: Preceded by *Wa* (42.0%), *Re* (20.0), staying in motion (*Sm*, 16.0) and *metasoma cleaning* (*Sc'*, 14.0) and followed by *Wa* (42.0), *Re* (20.0), *Sc'* (16.0) and *Sm* (14.0) in V924 (45 cases), V7774(1), Q6720 (1), Q7774 (1) and Q7800 (2).

MEAN DURATION: 0.30 ± 1.20s (n=50).

Worker Behavior, shaking (w*Sh*/q)[#]: "Moves in an apparently methodical way over the combs, from time to time grasping a bee with its forelegs and then rapidly vibrating its body in a vertical plane for approximately one second" (Allen, 1959b). Such vibration is often observed on the comb next to the queen (Fletcher, 1978a,b). "Split tapping or DVAV (dorsoventral abdominal

vibration)" (Milum, 1955). 'Trembling movements' (Hammann, 1958) (cf. Nomenclature in Fletcher, 1978a). 'Vibration dance' as an activating factor (Schneider, 1991). No sound can be heard.

Bruinsma *et al.* (1981) suggested that tooting sounds suppressed *wSh/* on queen cells, but Grooters (1987) could not demonstrate it, and

asserted that *Qd* (*queen-cell destruction*) was suppressed by tooting sounds. In this study, it is only suggested that *wSh/* is related to the queen piping (*To/w* and *Sr/w*) because of the intensive occurrence of 3 behaviors in V924 (cf. 2.4.8 and 2.4.9).

Hammann (1957) and Allen (1959b) reported an increased number of *wSh/q* or *wSh/* on queen

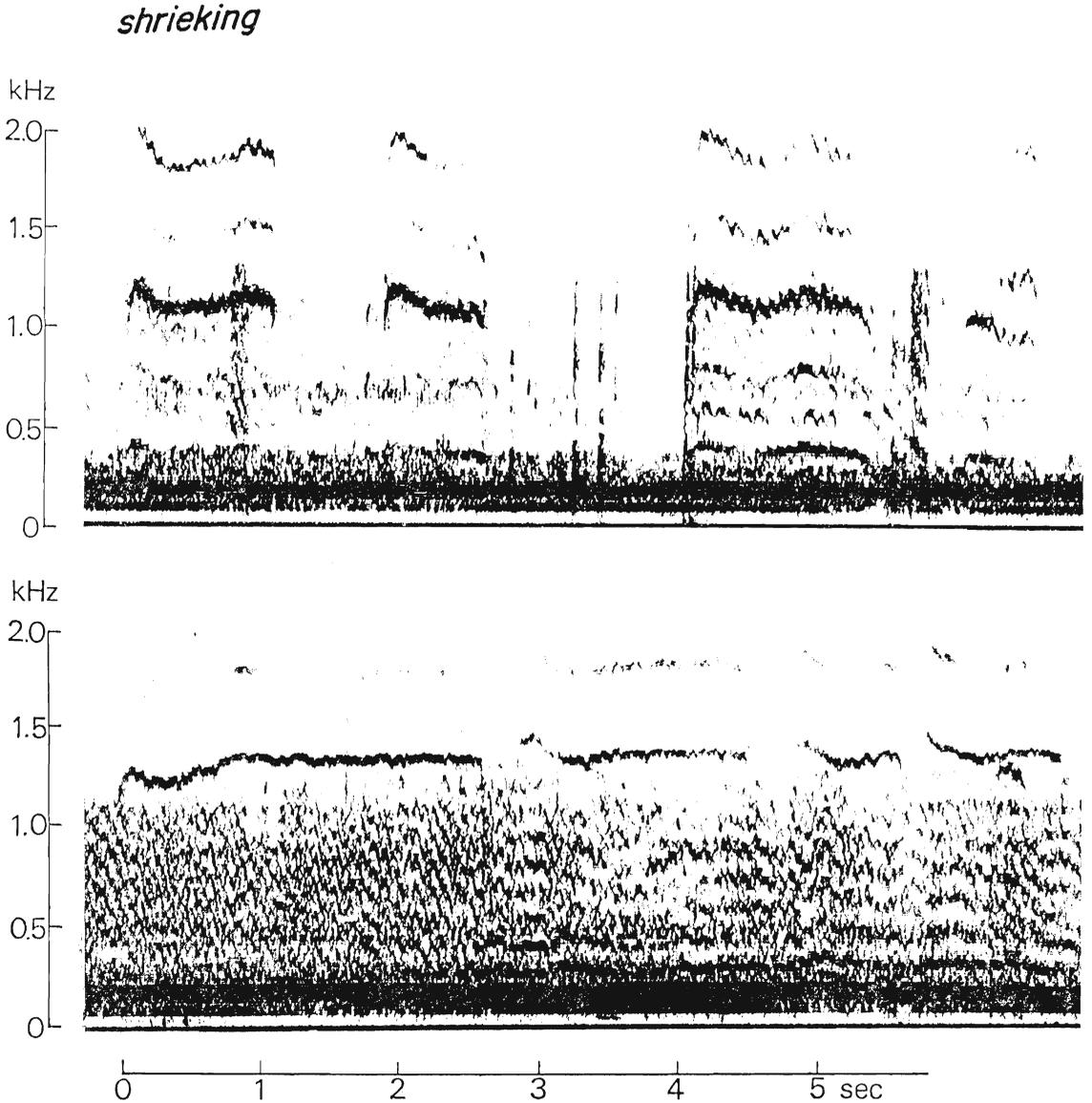


Fig. 12. The shrieking sounds by V7410 (upper) and by V924 (lower). The recording and analyzing systems for their sound spectrograms were the same as those in Fig. 10.

cells at the time of mating and swarming flights. Fletcher (1978a) observed that the number of wSh/q in mated queens declined to zero.

3. Egg laying (*El*)[#]

This behavior is a sequence of 5 component patterns temporally linked together by the laying drive. The patterns shown by only lower-case letters (*mi*, *ov* and *wd*) appear always in *El*, never independently.

3.1. Cell inspection (*Ic*) (Figs. 13A, B; 14B, C): Aforementioned (cf. 1.4); duration variable, 1-10 s; head and forelegs and often also a part of the mesosoma inserted into the cell, probably to avoid cells (i) already occupied by immatures or stored food or (ii) not yet cleaned after emergence.

The queen sometimes lays 2 eggs in a cell or an egg on a stamped pollen mass. Surplus eggs are probably eaten by workers (Sakagami and Fukuda, 1968).

3.2. Metasoma insertion (*mi*)[#] (Figs. 13C, D): After *Ic*, metasoma drawn toward head and inserted in the cell; duration ca. 2-5 s; rarely misdirected to the nearest cell by stumbling of the metasomal tip over the cell edge, often causing a 2-egg cell.

3.3. Oviposition (*ov*)[#] (Fig. 14A): Motionless posture with all legs straddled (Lp5) for ca. 5 s followed by a sudden further insertion, probably for adherence of egg to cell bottom.

Jordan (1933) directly observed the process of *ov* by using an observation hive made of 2 sheets of glass: the vulva widely opened, the sting lifted at right angles to the cell bottom, the metasoma pushed toward the cell bottom without touching it, and the egg laid.

Duration of *ov*: ca. 10-15 s. According to other authors, 6-12 s (Dresher, 1968), 9-12 s (Haydak, 1950), and 16.2 s in a normally mated queen and 17.2 s in 2 artificially inseminated queens (Dietz,

1969). These probably include a part of *Ic*, *withdrawal* (*wd*, cf. 3.4) and *pausing* (*Pa*, cf. 3.5).

3.4. Withdrawal (*wd*)[#]: Short motion (ca. 2-5 s) to withdraw metasoma soon after *ov*, often followed by the next *Ic* before completion of *ov* and *wd* (Figs. 14B, C).

Because of the queen's size, the next oviposition is usually made not in one of the 6 cells which surround the oviposited cell but in one of the 12 cells outside them (Fig. 14D). On empty combs, however, Fleig (1990) found that normal queens laid 96.2% of eggs (n=3724) within $\pm 60^\circ$ downward.

3.5. Pausing (*Pa*)[#]: Keeping a motionless posture usually during egg laying for a few seconds to several minutes; rarely in virgin queens.

The 'arch' posture by Taranov and Ivanova (1946) is probably *Pa* which is stopped on the way from *wd* to *Ic*. Such *Pa* was frequently observed.

SITUATION: Preceded by *oviposition* (*ov*, 65.9 %) or *cell inspection* (*Ic*, 29.3) and followed by *wandering* (*Wa*, 48.4), *Ic* (18.8), *ov* (13.6), *withdrawal* (*wd*, 5.7) or w/*Fe* (4.4).

Major cases: *ov* + *wd* → *Pa* → *Wa*; *Ic* → *Pa* → *mi* + *ov*; *ov* + *wd* → *Pa* → *Ic*.

Minor cases: *ov* → *Pa* → *wd*; *ov* + *wd* → *Pa* → w/*Fe*; *mi* → *Pa* → *ov*.

Four cases in V924 were observed independent of egg laying: *Sc*^t (140s) → *Pa* (45) → *Re* (90); *Vb* (150) → *Pa* (525) → *Ac* (15); *Sm* (50) → *Pa* (200) → *Sm* (50); *Ic* (5) → *Pa* (390) → *Re*

MEAN DURATION: 290.00 ± 210.83s (n = 4, V924); 34.22 ± 37.42s (n=1124, 8 mated queens); significant difference between them (p < 0.01, U-test).

3.6. *El* (egg laying) as a temporally integrated behavior

The mean duration of *El* (short *mi* + *ov* + short *wd*) to a worker cell is 17.92 ± 8.06s (n=4631), significantly longer than to a drone cell

(12.69 ± 3.78 s, $n=39$) ($p < 0.001$, U-test) probably because in wider cells it is easier to issue an egg and/or drone eggs do not need the sperm transfer. The 2 observed cases (55 and 25s) probably resulted from the difficulty in egg passage through the oviduct. The interrupted oviposition is significantly shorter than normal *El* with duration 5.90 ± 2.88 s ($n=111$, $p < 0.001$). Another abnormal *El* is *oviposition (ov)* without *metasoma insertion (mi)*. This non-inserting *El* was observed in Q7774 (14 cases) and in Q7800 (1). While it was advancing (7.14 ± 3.78 s, $n=14$), stepping back on cells was

observed 3 times. Non-inserting *El* was also recorded twice even in a virgin queen (V7774). DeGrandi-Hoffmann and Martin (1993) often observed that virgin queens were walking on frames with an egg protruding from tip of the metasoma.

Fe or *SC* were sometimes observed while the queen's metasoma was in the cell, and *wd* occurred slowly during the *Fe* or *SC*.

SITUATION: *El* basically follows the sequence of *Ic* → *mi* → *ov* → *wd*, expressed by the following formula and Fig. 15.

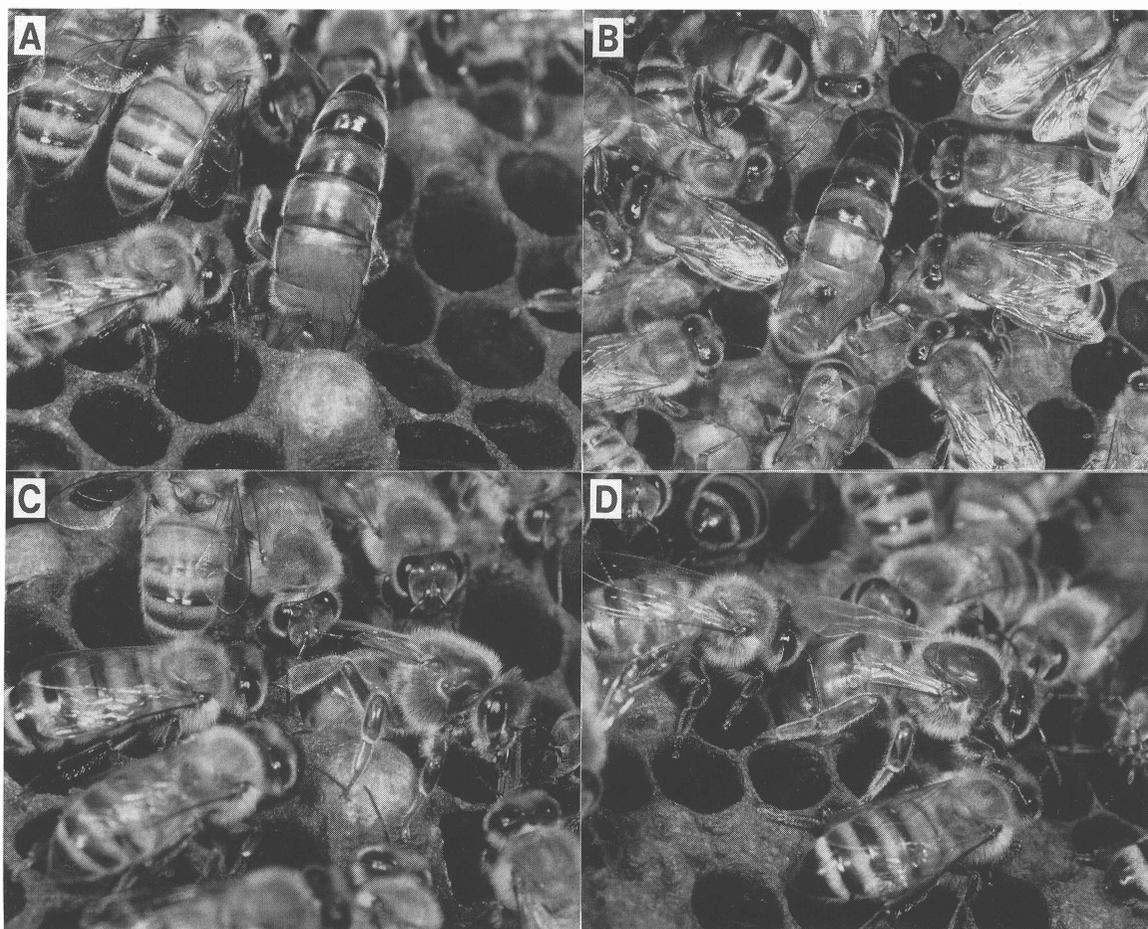


Fig. 13. Egg laying in drone cells (A, C) and worker cells (B, D). In cell inspection (A, B), mesosoma was also inserted into the drone cell but not into the worker cell, otherwise virtually no difference in laying posture (C, D). (Printed from the color slides by S. Kuribayashi)

$$El = k [m \{n \langle Ic + (Pa) + mi + (Pa) + ov + (Pa) + wd \rangle + (Pa) \} + Wa]$$

where *n*, *m* and *k* mean each optional repetition in the sequence.

Fig. 16 is given as an example of a mated queen (Q7200) that was laying eggs as in the above formula, including both egg-laying and resting phases reported by Miida and Nakamura (1992).

General Discussion

1. Behavioral comparison between virgin and mated queens

Some of the above behavior patterns were selected on the basis of their mean durations (MD)

and/or their incidences (ICD). The ICD-data of virgin queens are revised, based on the total observation times (213.0 h vs 226.8 h = 1 vs 1.065) of virgin queens (VQ) vs mated queens (MQ).

		VQ	MQ	U- or χ^2 -test
<i>Resting (Re)</i>	MD	long	short	p < 0.001
	ICD	1504	2376	p < 0.001
<i>Wandering (Wa)</i>	MD	long	short	p < 0.001
	ICD	2462	7438	p < 0.001
<i>Staying in motion (Sm)</i>	MD	long	short	p < 0.01
	ICD	691	651	ns

Virgin queens rest longer, but once they have begun to move, are more restless than mated

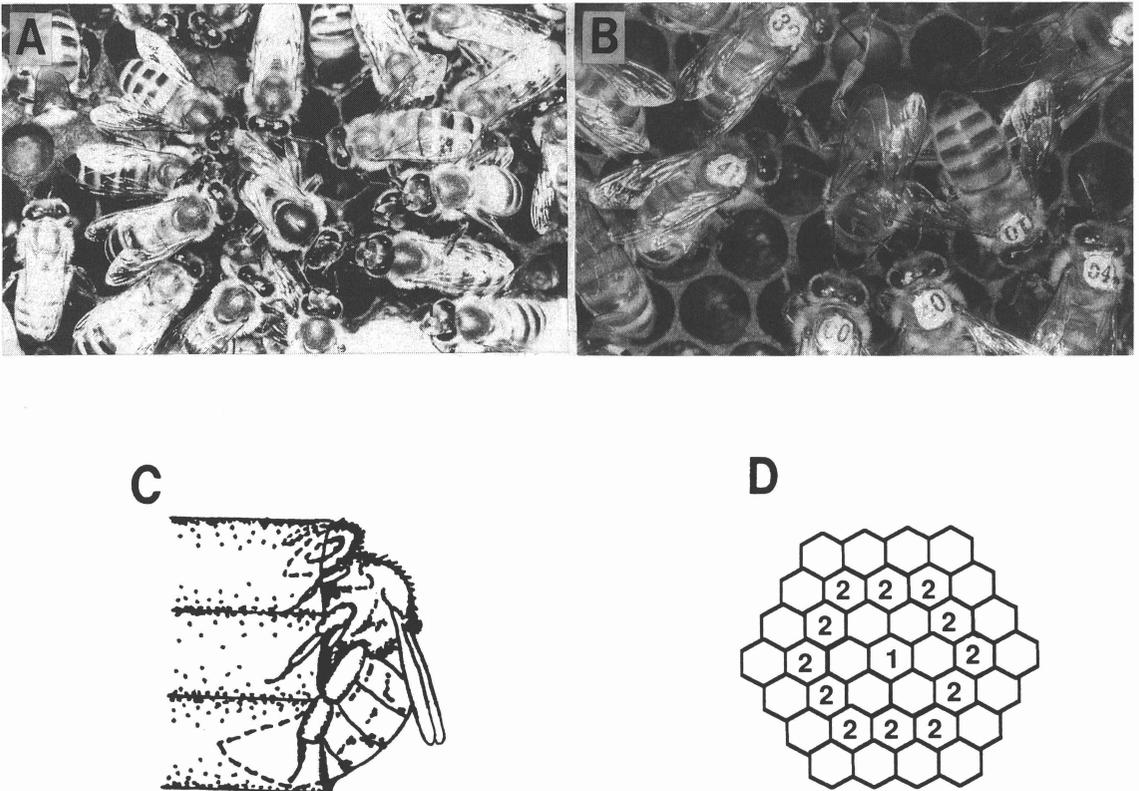


Fig. 14. Egg laying of mated queens. **A:** At oviposition (*ov*). **B** and **C:** Inspection of the next cell (*Ic*) before withdrawal (*wd*) of metasoma was finished. **D:** The cell (1) where an egg was laid and the cells (2) in one of which the next egg will be laid.

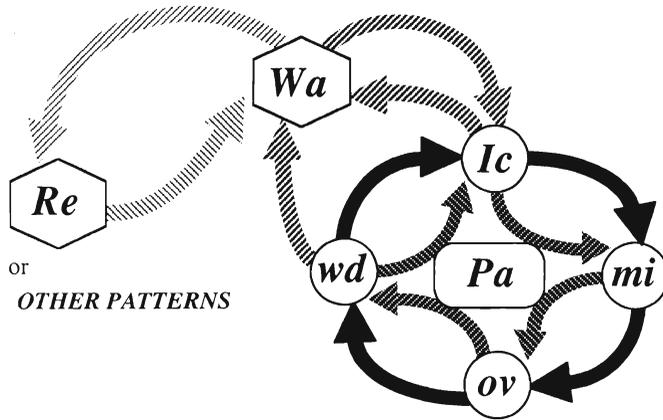


Fig. 15. An egg laying cycle. Main cycle on the comb with many cells to be laid proceeds according to black arrows, but sometimes the sequence ceases (*Pa*) for a while (dark-hatched arrows). The case *wd-Pa-Ic* was most frequent. When the queen cannot find any cells to be laid, she moves to the other place in search of cells (i.e. *Wa*, middle-hatched arrows). If the 'laying drive' disappears, the queens behavior changes from *Wa* to *Re* or other pattern (light-hatched arrows). For behavioral codes see Fig. 16.

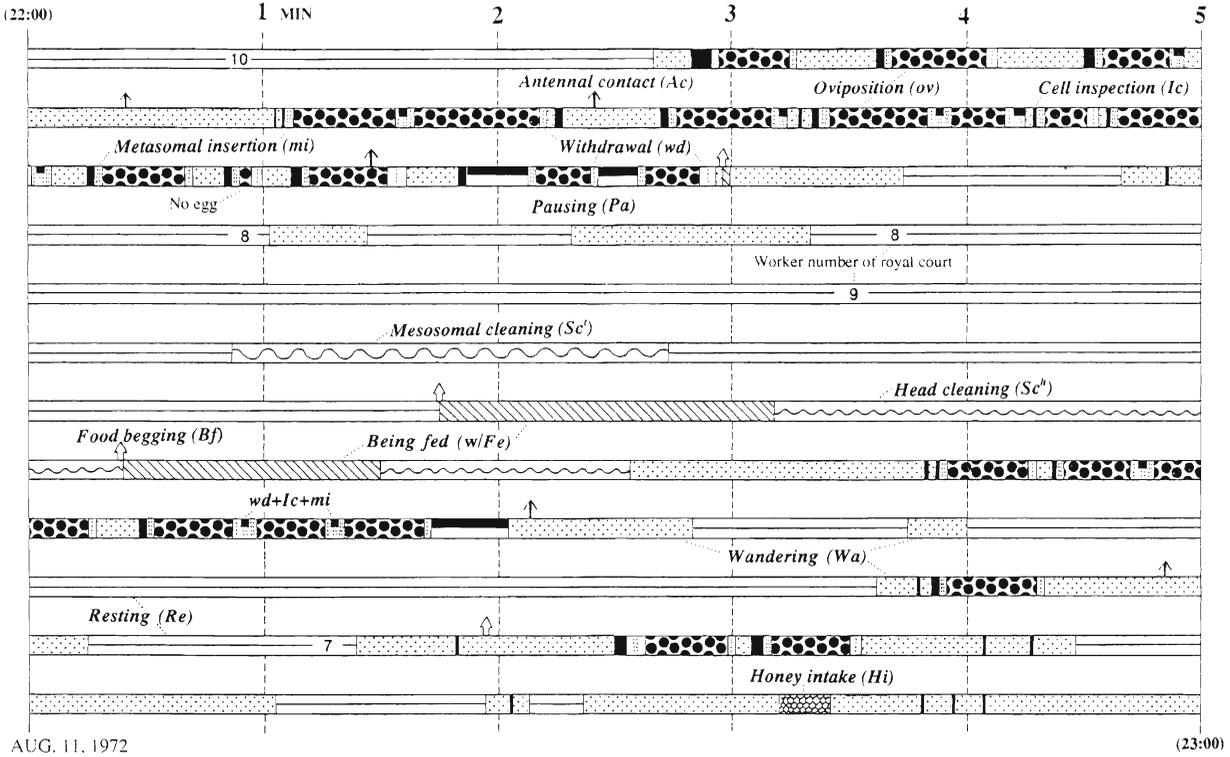


Fig. 16. One-hour continuous observation of a mated queen (Q7200) on Aug. 11, 1972. Q7200 laid 13 eggs (*EL*), somewhat wandered (*Wa*) and rested (*Re*) on the periphery of an observation hive (OH1). After ca. 7 min, she began metasomal cleaning (*Sc'*), and was fed (*w/Fe*) and cleaned her head (*Sc^b*). Then, after *Wa*, she laid 6 eggs, and an egg, and then 2 eggs.

queens. As described earlier, virgin queens wander at a higher speed than mated queens.

		VQ	MQ	U- or χ^2 -test
<i>Head cleaning (Sc^h)</i>	MD	(long)	(short)	ns
	ICD	219	365	p < 0.001
<i>Mesosoma cleaning (Sc^s)</i>	MD	(short)	(long)	ns
	ICD	35	33	ns
<i>Metasoma cleaning (Sc^t)</i>	MD	same	same	ns
	ICD	1103	464	p < 0.001

The mean durations of self-cleaning (**SC**) may be specific, regardless of the physiological condition of a queen, because **SC** is a basic behavior pattern. The incidences of **Sc^h** and **Sc^t**, however, differ significantly between virgin and mated queens, a fact probably related to **Ic** and **Ac** (see below), and to spreading of queen substances from virgin's metasoma, or displacement behavior.

		VQ	MQ	U- or χ^2 -test
<i>Cell inspection (Ic)</i>	MD	short	long	p < 0.001
	ICD	81	5004	p < 0.001
<i>Antennal contact (Ac/w)</i>	MD	short	long	p < 0.05
	ICD	49	157	p < 0.001
<i>Food begging (Bf/w)</i>	MD	(long)	(short)	ns
	ICD	345	274	p < 0.001
<i>Being fed (w/Fe)</i>	MD	short	long	p < 0.001
	ICD	465	910	p < 0.001

Mated queens inspected cells more frequently and for longer times than virgin queens, probably because they sought cells for egg laying. Also, they were fed more frequently and for longer times, which is apparently for egg laying. More and longer **Ac/w** seems to indicate the catching of workers' attention, and **Bf/w** may indicate the rejection of feeding.

		VQ	MQ	χ^2 -test
<i>Being manipulated (w/MA)</i>	ICD	94	19	p < 0.001
<i>Being attacked (w/At)</i>	ICD	296	8	p < 0.001
<i>Being shaken (w/Sh)</i>	ICD	51	2	p < 0.001
<i>Manipulating (MA/w)</i>	ICD	7	0	p < 0.01

<i>Excited advancing (EA)</i>	ICD	15	0	p < 0.001
<i>Feeding (Fe/w)</i>	ICD	18	0	p < 0.001
<i>Shrieking (Sr/w)</i>	ICD	24	0	p < 0.001
<i>Tooting (To/w)</i>	ICD	40	0	p < 0.001

Higher values of the above 8 patterns in virgin queens seem to indicate unstable interaction between themselves and workers. The virgin queen must be a less important member until the start of egg laying.

A more detailed comparison will be discussed in a second paper (II. A quantitative analysis).

2. **El (egg laying) in worker cells and drone cells**

The queen can lay either inseminated eggs (females) or uninseminated eggs (males) according to the cell size. Koeniger (1970) mentioned the importance of information received from the forelegs for laying of inseminated eggs in worker cells and rejected the so-called pressure theory (cf. Jordan, 1928), which explained squeezing out of sperm from the spermatheca to the oviduct by compression of the queen's metasoma. After confirming that both forelegs were put into the cell at **Ic**, Koeniger (1970) attached tiny paper flags to the forelegs or amputated the forelegs differently, which caused laying of inseminated eggs in drone cells. He concluded that insemination was prevented by a specific stimulus from a drone cell, but could not explain its nature.

This specific stimulus may relate to a pressure given to the forelegs. Koeniger (1970) mentioned no behavioral difference in **El** between drone cells and worker cells. I observed frequently that a queen inserted her whole metasoma into a drone cell (cf. 3.1; Fig. 13), but not into worker cells, during the same laying sequence. I also found longer mean duration of **El** in a worker cell than in a drone cell (17.93 vs 12.69). The blocking of foreleg insertion by flags would impart pressure at or near the fore trochanters because the queen tries to make **Ic** nevertheless. Such pressure would not be produced in normal laying into

drone cells. Difference that resulted from amputation may have been caused by the fact that operated forelegs come in contact abnormally with the edge of drone cells.

Only Huber (cf. Haydak, 1950) and Allen (1956) observed **El** in the queen cups, but it may be similar to **El** in worker cells because the openings of the queen cups are almost the same size as those of worker cells (queen cups: 5.4 mm, diam.; worker cells: 5.1 mm; cf. drone cells: 7.1 mm) according to Koeniger (1970).

According to a recent study, queens do not seem to lay automatically in drone or worker cells provided by workers. Sasaki *et al.* (1993) found that queens preferred worker cells to drone cells for **El**.

3. Egg-laying capacity

“For many years it has been believed that the maximum capacity of a good queen under favorable conditions would be about 3000 eggs in a single day” (Root *et al.*, 1980:108), which means that the queen successively lays at intervals of 28.8 s without resting and feeding during one day. Without comment on the upper limit of anatomical egg-laying capacity, a famous anatomist wrote: “... it has been estimated that a queen in the prime of her reproductive vigor may produce as many as 3000 eggs in a single day. The usual number, however, is probably closer to 1500 eggs a day, and even at this rate the queen produces more than her own body weight in eggs every 24 hours.” (Snodgrass, 1956:302)

Duration of **lc**, **mi**, **ov** and **wd** are 1-10, 2-5, 10-15 and 2-5s, respectively. Consequently the entire sequence of **El** lasts 15-35s. Haydak (1950) mentioned a few seconds of **lc** and ca. 9-12s of **ov**. Koeniger (1970) reported ca. 3s (min. 1.5s; max. 10s) of **lc** and ca. 25s (min. 16s; max. 42s) of **mi** → **wd**. As a short **Wa** is usually inserted between 2 successive **El**, the duration of a unit **El** in successive laying may attain 30-40s. If a unit **El** lasts 35s and successively goes on, 2469

eggs could be laid during a day. As the queen obviously must rest and feed herself, it may be difficult for her to lay more than 2000 eggs a day.

As the egg-laying rate of queens is influenced by the populations of their colonies (Free and Williams, 1972), the number of eggs laid in a day becomes smaller in observation hives with small worker cover (Table 5, bottom). Because of the definite duration of laying (17.9 ± 8.4 s), however, there is an upper limit of about 2000 eggs, even in the strongest colony under the most favorable condition (Table 5, top).

4. **El** (egg laying): Fount of nest structure

There is a definite structure inside the honeybee colony: central brood area, pollen area surrounding it, and upper peripheral honey stock area. Recently, Camazine (1991) demonstrated that the basic pattern of the nest structure is defined by cell-occupation time of brood, pollen and honey, respectively. He called the process ‘self-organization’.

Independently, Ohtani (1992) explained the process determining 3 areas of the nest structure: central brood area because of queen lays eggs only within the warmest area; upper peripheral honey area because of evaporation from half-ripened honey and the heavy weight of ripened honey stock; and peripheral ring-like pollen area free of brood and stored honey. This idea which regards **El** as the fount of nest structure is partially supported by the definite region where a queen rests (cf. Fig. 6).

Each explanation may complement the other, though it is unknown for the present which is mainly responsible for the nest structure.

5. Postulation of 2 drives, fleeing and aggression in RC (royal court formation)

The process of **El** in honeybees is a solitary behavior and its sequence is relatively simple. On the other hand, the oviposition of stingless bees is an interindividual event, its process being much

Table 5. Mean and maximum of eggs laid per day under the most favorable condition and within observation hives.

<i>Source (author)</i>	<i>Mean</i>	<i>Maximum</i>	<i>Material</i>
Berlepsch (1860)*	1855	3021	uc
Dufour (1901)*	1627	-	uc
Brünnich (1922)	1460	1750	Swiss black
Merrill (1924)	1720	2030	Italian
Nolan (1925)	1232	1587	Italian
Krasnoplev (1949)	1498	2230	uc
Chauvin (1950)	1262	1697	uc
Nelson and Jay (1972)	-	1625	uc
Free and Williams (1972)	1663	1987	uc
Gerig and Wille (1975)**	1277	2065	uc
Fukuda (1983)	986	1246	hybrid Italian
Harbo (1986)	922	1278	unknown
.....			
Average \pm SD	1409.3 \pm 300.8	1865.1 \pm 495.6	<i>Apiary hives</i>
.....			
Drescher (1968)	539	973	cig
Dietz (1969)	778	1103	uc
Adlakha (1972)	1180	1368	uc
Ohtani (this study)	794	1141	Q7774, Q7800
.....			
Average \pm SD	822.8 \pm 265.2	1146.3 \pm 164.4	<i>Observation hives</i>

* Cited from Ribbands (1953)

** From 10 maximum data of eggs laid a day

uc: Unknown, but may be Carniolan or *A. mellifera mellifera*

cig: Used Carniolan, Italian and gynandromorph producing strains

more complicated than that of the honeybees (cf. e.g. Sakagami and Zucchi, 1963; Sakagami, 1971; Sakagami *et al.*, 1977; Sakagami, 1982).

Sakagami and Zucchi (1977) assumed that the oviposition process and other queen-worker coactions in stingless bees were all linked to the basic drives of fleeing and aggression. As for oviposition behavior, Sakagami and Zucchi (1974) and Sakagami *et al.* (1977) tried to interpret the mechanism by postulating 3 drives, 'flee away the queen', 'attack on the queen' and 'food discharge'. As to the royal court in honeybees, they assumed that the 'retreating backward' of **RC** in honeybees can be regarded as an avoidance response, but it is difficult to judge whether 'pushing forward' is entirely released by attractive stimuli or involves aggression. In the present result (cf. 2.1 and 2.3), *nibbling* (**RC-3**) clearly includes aggressive movements, which were seldom differed little from *attacking* (**At/q**).

Subsequently, Sakagami *et al.* (1983) abandoned

their interpretation of 3 drives, particularly in the part called 'post-discharge escape' by workers before queen's oviposition in stingless bees. Sakagami still believes, however, that the first 2 antagonistic drives govern queen-worker relationships (pers. comm.), which may be similar in the royal court of honeybees, although the eusociality of honeybees and stingless bees probably evolved independently (Winston and Michener, 1977).

I also observed an increase of worker attendants in an aggressive situation, *i.e.* the emergence of balling. Therefore, both drives, fleeing and aggression, are postulated here, and their relative relations are presented as in Fig. 17, based on the classical study in domestic cats by Leyhausen (1956).

As mentioned above, **RC-5** to **RC-9** are both assumed to be displacement behavior due to a state of conflict between fleeing and aggression. They are each given tentative positions in Fig. 17. In any event, experimental analyses are needed to

INCREASING AGGRESSION →

INCREASING FLEEING ↓

RC-1 (examining) BA-1	RC-2 (touching) (licking) BA-2	RC-3 (nibbling)	At (attacking) BA-9	Sg (stinging) BA-10
RC-4 (irritated rocking)	RC-7 (polishing-like rocking)	RC-12 (chasing)	BA-7 (metasoma arching)	BA-8 (metasoma inflecting)
RC-5 (food offering) BA-3 (feeding)	RC-8 (proboscis protruding) RC-6 (self-cleaning)	RC-9 (shaking)	BA-6 (mounting)	
RC-11 (escaping)	RC-10 (staying alert) BA-5 (guarding)	RC-13 (head pressing) BA-4 (immobile)	BA-11 (alarm fanning)	BA-12 (excited fanning)

Fig. 17. Conceivable relationships among worker behaviors in *royal court formation* (**RC-1** to **RC-13**, cf. 2.1) **and in balling behavior** (**BA-1** to **BA-12**, cf. 2.4.7), postulating 2 antagonistic drives, aggression and fleeing. Definite position of each box should be confirmed experimentally. A broken line means an obscure boundary.

separate and measure the degrees of fleeing and aggression.

6. Two drives in BA (balling behavior)

Wallis (1964) arranged 5 aggressive behavior patterns of *Formica* ants in the direction of increasing aggressive motivation: licking → threat posture → seizing → dragging → stinging. As mentioned above, however, the *balling behavior* (**BA**) in honeybees is too complicated to be arranged unidirectionally. The duration of **BA** was from 2.5 to 8 hrs (median 5 hrs), and the process of balling break-up was gradual (Robinson, 1982). I also directly observed the process transforming **BA** from **RC** in the mother queen of V7774 (cf. Fig. 10). That is, the transposition between **BA** and **RC** is possible, rather than the difference being very slight. As in **RC**, therefore, the worker

behaviors in **BA** are also arranged according to the 2 directions postulating fleeing and aggression. All 12 patterns of **BA** are also shown in Fig. 17 with no experiments. Precise positions of each of the patterns of **RC** and **BA** remain for some future study.

With respect to **BA**, Yadava and Smith (1971) proposed the 'stress pheromone hypothesis', that balling was caused by chemicals from the queens' metasoma when they suffered 'stress'. The observation of **BA** by Robinson (1982, 1984) did not always support that hypothesis. In a recent study, however, 'stress' pheromone was found from the Koschewnikow's glands (Lensky *et al.*, 1991), that are located on the sting apparatus of queen honeybees (Grandperrin and Cassier, 1983). If the above 'motivation analysis' is correct, the chemicals from Koschewnikow's glands would be

a cause of the 'attacking drive'. Moreover, as the faecal material of virgin queens repelled workers and stimulated grooming behavior (Post *et al.*, 1987), it may be one of the causes of the 'fleeing drive'

Motivation analysis must also be conducted for queen behavior in the colony just preparing for swarming. Observations by Taranov and Ivanova (1946), Hammann (1957), Allen (1959b) and Fletcher (1978b) suggest some relation between the swarming mechanism and aggressive aspects in the honeybee colony. For instance, in the so-called swarming impulse, if the suggestion is a fact, the queen would be more frequently attacked, manipulated and shaken by workers, would have a 'thick' royal court, would emit the tooting sound, and would lay eggs into queen cups.

The data presented and discussed here were collected by the 'single-individual trailing' (SIT) method, independent of any particular theory; they are as theoryneutral as possible. They may be used to generate new ideas, as have been done in the above discussion, and/or as an unbiased data bank against which predictions of current or future theories can be tested. The power of the SIT method is in its objectivity backed with quantity.

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観察巣箱内のミツバチ女王蜂成虫の行動

I. 行動パターン

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「1 個体追跡法」により未交尾女王 6 匹と既交尾女王 8 匹を約 450 時間観察したデータに基づき、46 種の行動パターンとそれに関連する働きバチの行動パターンを 32 種記載した。体各部の位置と動きの定義およびそれらの組み合わせに基づいて行動パターンを記載し、各パターンには短いコメントと発生前後の状況を加えた。各パターンは「単発行動」「個体間行動」「行動環行動」に大別したが、最後に属する配偶行動については巣箱内で観察されないため、記載されていない。論議では、未交尾女王と既交尾女王との比較、産卵行動、ロイヤルコート、ボーリングについて取り上げ、後二者については、「動因分析」的な視点から攻撃と逃走の 2 つの動因による統一的説明を試み、両者が同一線上の行動パターン群である点を論じた。産卵行動については、雄蜂巣室と働蜂巣室での行動の違いを論じた後、1 日の産卵数を 1 卵の産下時間から最大 2000 個以内と推定した。最後に 1 個体追跡法の客観性を強調した。