OVARIAN DEVELOPMENT RELATED TO ACTIVITY LEVELS OF NURSE WORKERS IN *Melipona bicolor:* EVOLUTIONARY SIGNIFICANCE

Olga I. Cepeda Aponte ✉ ocepeda@yahoo.com
Vera Lucia Imperatriz-Fonseca vlifonse@ib.usp.br
Pérsio Souza Santos Filho pssantos@ib.usp.br
Laboratório de Abelhas – Depto. Ecologia - IB – USP - BRAZIL
Financed by FAPESP

INTRODUCTION

In numerous social insects, ovarian activity is correlated with hierarchies (West-Eberhard, 1978; Roseler *et al.* 1980; Sledge *et al.* 2001); additionally, oogenesis is a process limited by the availability of nutrients that an individual may acquire as larva or as imago (Wheeler, 1996).

Ovarian development in queenright colonies is found in all social Hymenoptera (Sakagami, *et al.,* 1963; Holldobler & Wilson, 1990; Edwards, 1980). In *Apis* bees, although queen and larvae highly inhibit ovarian development of the workers, some may escape control and lay (Bourke, 1988; Ratnieks & Visscher, 1989). In stingless bees, brood production is characterized by a complex, ritualized progression of behaviors denominated the provisioning and oviposition process, POP. Among the nurses participating in POP, ovarian development is very common (Sakagami *et al.,* 1963) with only 4 genera presenting totally sterile workers (Sakagami & Zucchi, 1968; Terada, 1970; Sakagami & Zucchi, 1974*;* Sakagami & Inoue 1990 *apud.* Crespi, 1992; Zucchi, 1993).

While most of the worker eggs laid by stingless bees are consumed by the queen (worker trophic eggs), workers of several species contribute significantly in male production (with worker reproductive eggs) illustrating the conflict at the individual reproductive level (Beig, 1972, Contel & Kerr, 1976; Koedam, *et al.* 1999; Sommeijer, *et al.* 1999; Toth, *et al.* 2002; Tóth et al., 2004). From an evolutionary outlook, "benefactor" behaviors may evolve if workers conserve the "hope" for reproducing (Lin & Michener, 1972), in addition it then would be possible that the principal function of trophic eggs is that of keeping the ovaries active (West-Eberhard 1981, *apud*. Crespi, 1993). These ideas are interesting bases upon which we can discuss our results.

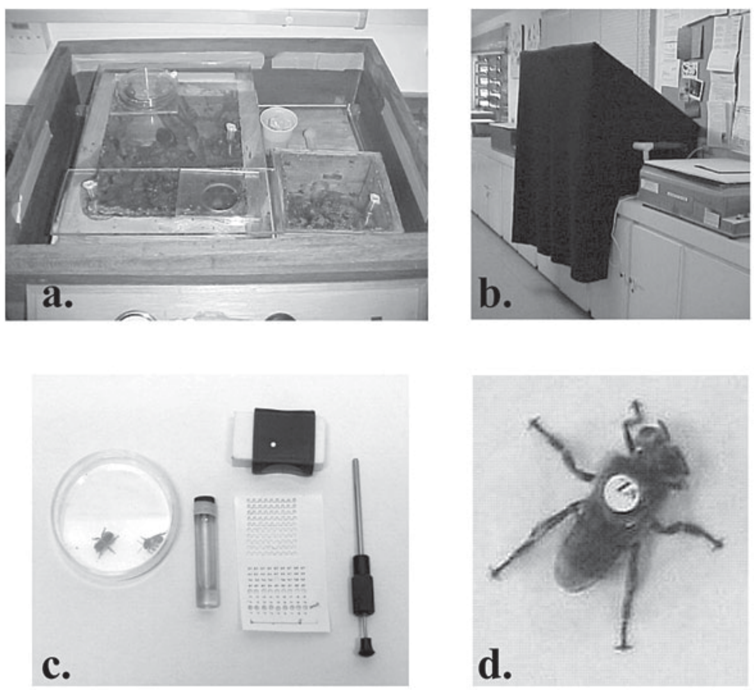
Our objective is to verify if those workers that participated more in the POP are heavier (indirectly representing the influence of food in oogenesis) and if they present higher levels of ovarian development (representing the "hope" for reproducing).

For *M. bicolor*, we correlated individual weight and extent of ovarian development with the levels of activity presented by each nurse bee. Our results lead us to believe that ovarian development is necessary for workers to assist effectively in brood production explaining the so called "idiosyncratic inclination to attend the brood" (Oster & Wilson, 1978). We demonstrate that in *M. bicolor*, behavioral differences divide nurses into *non layers* and *layers* (of trophic and/ or reproductive eggs), being egg layers the most interested in POP as demonstrated by their continuous presence (constancy) and contributing significantly to each process (assiduity). This separation indicates that ovarian development may play an important role in task partition in the colony and that it influences the degree of involvement presented by each worker.

MATERIALS AND METHOD

For filming: Two colonies (one polygynic, one monogynic) were kept in observation hives (Fig. a.) where workers were free to forage. All POP were filmed and mapped during 20 consecutive days. In order to avoid behavioral changes caused by exposing the comb to daylight, the colonies were kept in portable dark rooms (Fig. b.), illuminated by cold, red light.

For following individuals: Individual recognition of the workers was done by means of a tag glued to their thorax on the day of their emergence (Figs. c. & d.). For capturing the callow, mature combs were kept in a small annex connected to the colony (Fig. a.). Emerging callow were tag marked 3 times per day during 18 consecutive days.



Behavioral components: for each worker of known age participating in a POP was noted the occurrence and duration of the following behaviors: body insertion (partial or total), larval food discharge, egg laying (trophic or reproductive), and cell operculation. For each individual was calculated:

- * Assiduity: total frequency of each basic behavior monitored;
- * Constancy: total number of POP in which an individual participated;
- * Total time invested on each behavior.

Physical components: at the end of the monitoring period all marked workers in the monogynous colony were sacrificed, individually weighted and dissected. Ovaries were fixed and photographed; ovarian area was measured using the program SCION (www.scioncorp.com), special for analysis of medical images. Not all marked bees were recovered (some died or lost the tags) and not all dissections were successful, reducing the final size of the samples.

Statistical analysis included Kruskall Wallis, Mann Whitney, variation coefficient and Spearman correlation (Sokal & Rohlf, 1997).

RESULTS

As we observe on figure e, individual nurses born on the same day do not present ovaries of the same size. The coefficient of variation of the ovary area computed for workers within each age group (workers which emerged on a given day) was found to have a wide range of values between the age groups, from 29.2 to 172.6 in the monogynic colony. The high level of variation in ovary area is mainly due its growth and differentiation, which is, in turn, dependent on age, and also includes both within- and between-individuals' variation.

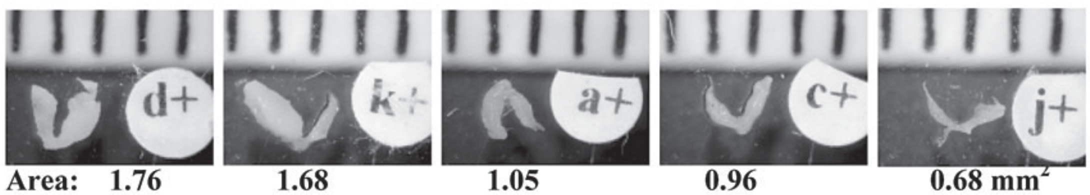


Figure e.: Ovaries of bees all 24 days old in *M. bicolor*.

The polygynic colony presented 11 individuals that laid from 1 to 3 reproductive eggs. As we can see in figure f, their participation in all behaviors demonstrated an outstanding, extraordinary performance: they were present in at least 7 times more POP (constancy), being active participants by discharging larval food.

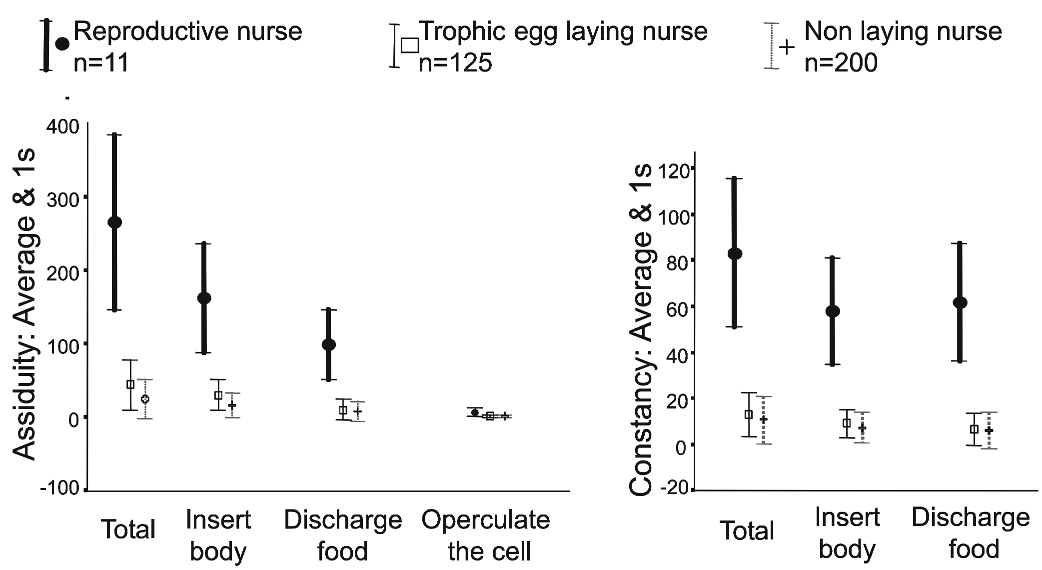


Figure f.: Comparison between the constancy and assiduity of the behavioral performance of the three types of nurses participating in POP in *M. bicolor*.

As for trophic egg laying, when comparing mature nurses (older than 10 days: min. age for laying trophic eggs) that laid or not, we find that in both monogynous (Mann Whitney p< 0.05 for all variables and behaviors) and polygynous colonies (Kruskall Wallis p<0.05 for all variables and behaviors), layers of trophic eggs differ significantly from non layers. Trophic egg layers present averages of activity that double fold the activities of non laying bees as we can see in Figure g.

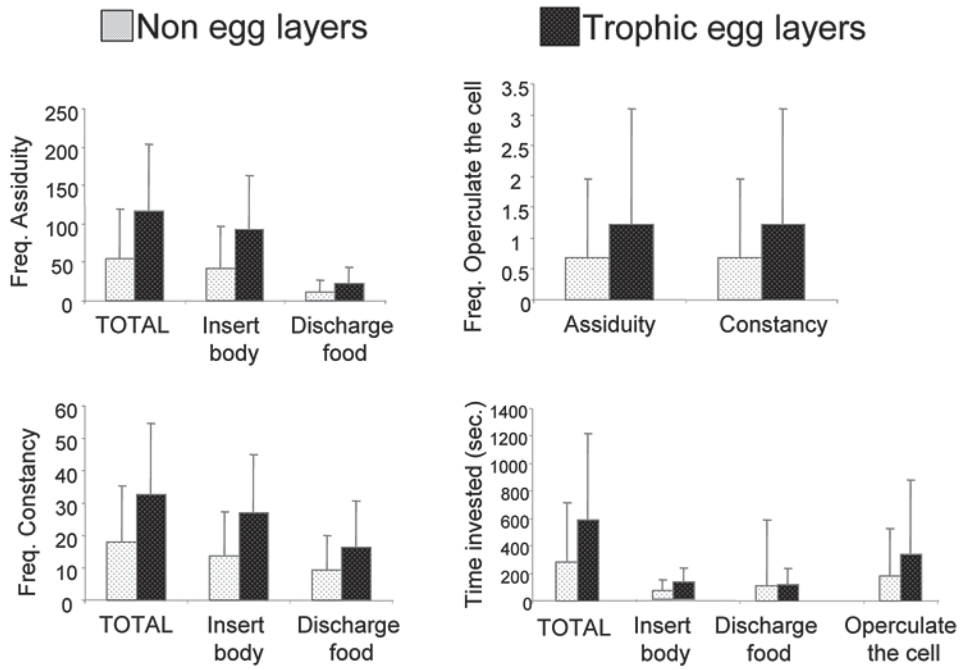


Figure g.: Comparison between the constancy, assiduity and time invested for the behavioral performance in POP between trophic egg layers and non egg layers in *M. bicolor*.

Another way to perceive the great contribution of egg layers in the POP is by calculating an average participation per bee, seen in the following table:

Table I. Average participation in POP per individual in polygynous and monogynous colonies of *M. bicolor*.

	Monogynous colony Nurses n=214		Polygynous colony Nurses n=353	
Total number of events in 20 consecutive days	15,380	Total %	13,689	Total %
Non egg layers	154 nurses participated in average in 50 events	50	217 nurses participated in average in 24 events	38
Trophic egg layers	60 nurses participated in average in 128 events	50	125 nurses participated in average in 44 events	40
Reproductive egg layers			11 nurses participated in average in 271 events	22

In the monogynous colony we see that of the total number of nurses, 1/3 of the trophic laying nurses participate in 50% of the events; in the polygynous colony we see the extraordinary efforts of only 11 reproductive nurses participating in almost ¼ of the total number of recorded events.

When we correlated individual physical characteristics of each nurse type with their behaviors, we obtained the following results:

EGG LAYERS: we find little or no correlation between ovarian area and behavioral variables. This is explicable once we consider that within egg layers, there were two subgroups present: bees with developed ovaries that if they had lived longer would had laid more eggs, but among these were also older bees who presented ovaries degenerating after oviposition. However, we do see that the heaviest laying nurses were more constant and assiduous in the POP. We also find a correlation of the constancy presented by laying bees as they age (Table II). Because of space limitations, correlations with the basic behaviors were not included here, though some may be mentioned in the discussion.

YOUNG NON EGG LAYERS: among non layers we also find subgroups: old bees that never laid eggs and young bees, many of which demonstrated that as they age, ovaries mature and grow, their levels of activity increase, as well as their weight (Table II). If these were left to live longer they would had become egg layers. Young non laying workers were then the most adequate group to follow.

Table II: Significant correlation coefficients between physical and behavioral variables in *M. bicolor*

			Trophic egg layers	n	Young non egg layers n=51
AGE		Body weight			0.27
		Ovarian Area			0.42
	TOTAL	Assiduity			0.40
		Constancy	0.33	36	0.43
		Time invested			0.33
OVARIAN AREA		Body weight			
		Age			
	TOTAL	Assiduity			0.44
		Constancy			0.48
		Time invested			0.45
BODY WEIGHT		Ovarian Area			
		Age			
	TOTAL	Assiduity	0.34	48	0.36
		Constancy	0.32	48	0.37
		Time invested			0.38

DISCUSSION

When we analyzed nurses in both colonies, we find great variability in individual behavior for each age group. By dividing nurses into egg layers and non layers, we find significant differences between the three types of nurses for all variables measured. From the analysis on the average individual participation, we see that even though there were only 11 reproductive nurses, they presented such high levels of performance that competitively reduced the participation other nurses in the POP. In the monogynous colony where there were no reproductive nurses, the reduced number of trophic egg layers (n=60) worked 2.5 times more than non laying nurses (n=154) in order to participate in 50% of the events. We clearly perceive the great interest laying nurses have in the POP. There is no information in the literature where to compare our results.

Egg laying has been related to division of labor. In several social species, behavioral changes of aging workers parallel with changes in the development of their ovaries: young individuals that work in the nest present developed ovaries, while older ones forage and present degenerated ovaries (Wilson, 1985). Inoue & col. (1996) found in *Trigona (Tetragonula) minangkabau*, that the population of a nest could be divided into nurses dedicating all their lives to care for the brood, and foragers that hardly remained with the brood. However, it is worth noting that these bees are totally sterile. It is possible that in some species ovarian development evolved as a mechanism to divide work in Hymenoptera (Bourke, 1988). Another approach suggests that laying workers would prefer to remain in the nest, close to their reproductive interests while not exposing themselves to predators (Franks & Scovell, 1983).

Our results indicate that laying nurses are behaviorally very different from non laying ones. A general comparison demonstrates that they are more constant, more assiduous and invest more time in the POP. Due to age differences within the complete group of egg layers, we found no correlation between ovarian area and behavioral variables. However, we do see that the heaviest laying nurses were more constant and assiduous in the POP, inspecting the cells and laying their eggs; these behaviors were found to be correlated with age. As for the young non layers, there were clear correlations showing how as they age and their ovaries mature and grow, their levels of efficient interest in the POP increases, as well as their weight. Since significant correlation coefficients were never higher than 0.59, we conclude there must exist intricate feedback relations between weight, ovarian development, hormonal levels, social interactions and even learning capacity.

Correlation between dominance and ovarian development has been demonstrated since 1948 on wasps (Pardi, *apud.* West-Eberhard, 1978). Proximal mechanisms involve the concentration of hormones that change with age, probably acting best on well fed individuals. However our results permit us to discuss the meaning of why workers have retained ovarian development. What we see in *M. bicolor* are females trying to reproduce. It has been proved that among social bees persists the ancient conflict for reproducing. Even among *Apis* bees where queen and larvae highly inhibit ovarian development in workers, some may escape control and lay (Bourke, 1988; Ratnieks & Visscher, 1989) and isolated workers of the same age present individual differences in ovarian development (Velthuis, 1970). As for behavioral differences, genera that present totally sterile workers, are characterized by POP considered as “simple”, lacking the typical excited behavior observed in species where nurses produce eggs (e.g. Sakagami & Zucchi, 1968, 1974). The simplicity of these POP suggests that the ritualized interactions queen-workers are associated to the conflict over male production and that ritualization partially resolves conflict leading to cooperation (Crespi, 1992).

Lin & Michener (1972) argue that a benefactor behavior with no altruism involved may develop, provided there is a significant contribution to male production by workers. Whenever a female conserves the “hope” for reproducing, her participation in colony tasks may be considered as an investment in her future reproduction. In many Meliponine species there is a percentage of workers that lay reproductive eggs, contributing significantly in male production. In the *Melipona* genera we have: *M. subnitida* (Contel & Kerr, 1976), *M. favosa* (Sommeijer, *et al.* 1999), *M. marginata, M. scutellaris* and *M. quadrifasciata* (Toth, *et al.* 2002). Furthermore, the same individual may produce both trophic and reproductive eggs (e.g. Koedam, *et al.* 1999). This evidence may indicate that there is selection to keep the production of trophic eggs in an individual that can produce both types of eggs (Kukuk, 1992); it may also indicate that males sons of reproductive workers have a high reproductive success. Trophic eggs may have the function of deceiving the queen, but West-Eberhard (1981, *apud* Crespi, 1993) proposes a more interesting hypothesis, stating that the function of trophic eggs is to maintain ovaries active. This would indicate that the role of trophic eggs as the principal source of nutrients for the queen (Sakagami, 1982) is a subproduct of the cooperative interaction between worker and queen.

We conclude then what we see in *M. bicolor* is that the best fed workers keep their ovaries activated probably in "hope for reproducing", and that they participate effectively in as many POP as possible. In *M. bicolor* is recreated in each POP, although hidden within their ritualized behaviors, the ancestral war between all females for the privilege of reproducing.

REFERENCES

BEIG, D. The production of males in queenright colonies of *Trigona (Scaptotrigona) postica.* Journal of Apicultural Research, v.11, p.33-39, 1972.

BOURKE, A.F.G. Worker reproduction in the higher eusocial Hymenoptera. Quaternary Review of Biology, v.63, p.291-311, 1988.

CONTEL, E.P.B. & KERR, W.E. Origin of males in *Melipona subnitida* estimated from data of an isozymic polymorphic system. Genetica, v.46, p.271-277, 1976.

CRESPI, B.J. Cannibalism and trophic eggs in subsocial and eusocial insects. In: M.A. ELGAR & B.J.CRESPI (Eds), Cannibalism, ecology and evolution among diverse taxa. Oxford Science Publication, U.S.A., Cap. 9, p.176-213, 1992.

EDWARDS, R. Social wasps: their biology and control Great Britain The Rentokil Library, 1980, p 201-206.

FRANKS, N.R. & SCOVELL, E. Dominance and reproductive success among slave making worker ants. Nature, v.304, p.724-725, 1989.

HOLLDOBLER, B. & WILSON, E.O. The Ants The Belknap Press of Harvard University Press USA 732 pp, 1990.

INOUE, T.; SALMAH, S. & SAKAGAMI. S.F. Individual variations in worker polyethism of the Sumatran stingless bee *Trigona (Tetragonula) minangkabau* (Apidae, Meliponinae). Journal of Entomology, v.64, n.3, p.641-668, 1996.

KOEDAM, D. CONTRERA F.A.L. & IMPERATRIZ-FONSECA, V.L. Clustered male production by workers in the stingless bee *Melipona subnitida* Ducke (Apidae, Meliponinae). Insectes Sociaux, v.46, p.387-391, 1999.

KUKUK, P.F. Cannibalism in social bees. In: M.A. ELGAR & B.J.CRESPI (Eds), Cannibalism, ecology and evolution among diverse taxa. Oxford Science Publication, U.S.A., Chap. 10, p.214-237, 1992.

LIN, N. & MICHENER, C.D. Evolution of society in insects. Quarterly Review of Biology, v.47, p.131-159, 1972.

OSTER, G.F. & WILSON, E.O. Caste and ecology in social insects. Monographs in population biology-12. Ed. By R.M. May Princeton University Press Inc. USA, 1978, 352 pp.

RATNIEKS, F.L.W. & VISSCHER, P.K. Worker policing in the honeybee. Nature, v. 342, p.796 – 797, 1989.

ROSELER, P.F.; ROSELER, I. & STRAMBI, A. The activity of corpora allata in dominant and subordinate females of the wasp *Polistes gallicus.* Insectes Sociaux, v. 27, n.2, p.97-107, 1980.

SAKAGAMI, S.F. Stingless bees. In: Social insects Vol III. H.R. Hermann (Ed.) Academic Press Inc. 1982, p.272-423.

SAKAGAMI S.F. & ZUCCHI, R. Oviposition behavior of an Amazonic stingless bee *Trigona (Duckeola) ghilianii.* Journal of the Faculty of Science of Hokkaido University Series VI, Zoology, v.16, p.564-581, 1968.

SAKAGAMI, S.F. & ZUCCHI, R. Oviposition behavior of two dwarf stingless bees, *Hypotrigona (Leurotrigona) muelleri* and *Hypotrigona (Trigonisca) duckei,* with notes on the temporal articulation of oviposition process in stingless bees. Journal of the Faculty of Science of Hokkaido University Series VI, Zoology, v.19, p.361-421, 1974.

SAKAGAMI, S.F.; BEIG, D.; ZUCCHI, R. & AKAHIRA, Y. Occurrence of ovary developed workers in queen right colonies of stingless bees. Revista Brasileira de Biologia, v.23, n.2, p.115-129, 1963.

SLEDGE, M.F.; BOSCARO, F. & TURILLAZZI, S. Cuticular hydrocarbons and reproductive status in the social wasp *Polystes dominulus*. Behavioral Ecology and Sociobiology, v.49, p.401-409, 2001.

SOKAL, R.R. & ROHLF, F.J. Biometry. The principles and practice of statistics in biological research. 3rd Edition, W.H. Freeman and Company, New York, USA, 887p. 1997.

SOMMEIJER, M.J.; CHINH, T.X. & MEEUWSEN, F.J.A.J. Behavioral data on the production of males by workers in the stingless bee *Melipona favosa* (Apidae, Meliponinae). Insectes Sociaux, v.46, p.92-93, 1999.

TERADA, Y. Estudos sobre a regulção social em colonias de Hypotrigona e Frieseomelitta. Ciência e Cultura, v.12, p.289, 1970.

TÓTH, E.; STRASSMANN, J.E.; NOGUEIRA-NETO, P.; IMPERATRIZ-FONSECA, V.L. & QUELLER, D.C. Male production in stingless bees: variable outcomes of queen-worker conflict. Molecular Ecology, v.11, p.2661-2667, 2002.

TÓTH, E.; QUELLER. D.C.; DOLLIN, A.; STRASSMANN, J.E.- Conflict over male parentage in stingless bees. Insectes Sociaux, v.51, n.1, p. 1-11.

VELTHUIS, H.H.W. Ovarian development in *Apis mellifera* worker bees. Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society, v.13, p.377-394, 1970.

WEST-EBERHARD, M.J. Polygyny and athe evolution of social behavior in wasps. Journal of the Kansas Entomological Society, v.51, n.4, p.832-856, 1978.

WHEELER, D. The role of nourishment in oogenesis. Annual Review of Entomology, v.41, p.407-431, 1996.

WILSON, E.O. The sociogenesis of insect colonies. Science, v.228, p.1489-1495, 1985.
ZUCCHI, R. Ritualized dominace, evolution of queem-worker interactions and related aspects in stingless bees (Hym., Apidae). In: Evolution of Insects Societies. Tokyo, Hakuhin-Sha Publishing Co., p. 207-249