

## A scientific note on the levels of polyandry in the black dwarf honeybee *Apis andreniformis* from Malaysia\*

Jun-ichi TAKAHASHI<sup>a,b</sup>, Sulaiman B. HASHIM<sup>c</sup>, Salim TINGEK<sup>d</sup>, Isamu SHIMIZU<sup>a</sup>, Tadaharu YOSHIDA<sup>b</sup>

<sup>a</sup> Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113, Japan

<sup>b</sup> Honeybee Science Research Center, Tamagawa University, Machida, Tokyo 194-8610, Japan

<sup>c</sup> Department of Agriculture, Jalan Damai, 35000 Tapah, Perak, Malaysia

<sup>d</sup> Agriculture Research Station Tenom, PO Box 197, 89908, Tenom, Sabah, Malaysia

Received 21 July 2007 – Revised 9 October 2007 – Accepted 17 October 2007

honeybee / *Apis andreniformis* / mating frequency / polyandry / relatedness

A majority of social Hymenoptera species such as ants, bees and wasps are monandrous or exhibit extremely low paternity frequency. Multiple mating by queens has independently evolved in some species of advanced social Hymenoptera (Boomsma and Ratnieks, 1996). The honeybee belongs to the genus *Apis*; they display extremely high levels of polyandry, resulting in very low genetic relatedness among workers and decreased potential inclusive fitness. Several researchers have attempted to explain the evolution and maintenance of extreme polyandry in honeybees by several plausible hypotheses such as sperm need, task specialization, sperm selection, reproductive conflict, resistance to parasites and diseases, and curious promiscuity of queens (reviewed by Palmer and Oldroyd, 2000; Tarpy and Page, 2001; Oldroyd and Wongsiri, 2006).

*Apis andreniformis* Smith is a small-sized honeybee that nests by constructing a single comb in open areas. Dwarf honeybees that occupy a basal position within the genus *Apis* comprise *A. florea* Fabricius and *A. andreniformis* (Oldroyd and Wongsiri, 2006). As compared to other *Apis* species, there is very little data regarding the paternity frequencies of this species. Additionally, mitochondrial DNA analysis reflected the presence of considerable genetic differentiation between the mainland Asia and Borneo populations of this species (Takahashi, 2006). We estimate that these 2 populations differ in the level of polyandry such as that observed between *A. mellifera* L. subspecies (Estoup et al., 1994; Franck et al., 2000). In this study, we analysed the levels of polyandry in the

black dwarf honeybee *A. andreniformis*; previously, only the Thai population of this species was analysed with regard to mating frequency (Oldroyd et al., 1997).

During 2002 and 2003, 6 *A. andreniformis* colonies were collected from the campus of Malaya University, Kuala Lumpur, Malaysia, and 4 colonies were collected from a forest in Tenom, Sabah, Malaysia. All the nests of the populations were located within a 5-km<sup>2</sup> area, respectively. By using DNA microsatellites, the mating frequency of 200 worker pupae and the queen for each colony was examined. Microsatellite DNA analysis was conducted using 4 microsatellite primers, namely, A76, A88, A107 and B124, designed by Estoup et al. (1994). The forward primer for each marker was 5'-end-labeled with a fluorescent phosphoramidite (NED, 6-FAM, VIC and PET, respectively). The polymerase chain reaction (PCR) products were visualized using an ABI 3100 genetic analyzer with the internal size standard GeneScan 500 LIZ<sup>TM</sup>. The fragments were analysed using the ABI GeneScan software (version 3.7) and ABI genotyper DNA fragment analysis software (version 3.7). The paternal allele in the worker bees was determined by the genotype of the queen alleles at 4 microsatellite loci. The effective mating frequency ( $m_e$ ) and the average coefficient of relatedness ( $g$ ) were weighted according to the relative proportions of each subfamily (Pamilo, 1993). The expected frequency of the particular non-detected patriline ( $d_p$ ) was calculated according to Boomsma and Ratnieks (1996).

The number of alleles at the 4 microsatellite loci was determined in all samples; 3, 4, 4 and 8 alleles were detected at loci A76, A88, A107 and B124, respectively. The number of observed patrilines ranged from 11 to 23, and the effective

Corresponding author: J. Takahashi,  
jit@ecology.kyoto-u.ac.jp

\* Manuscript editor: Klaus Hartfelder

**Table I.** The total number of worker samples ( $N$ ), number of observed fathers ( $k$ ), effective paternity frequency ( $m_e$ ) and average relatedness of workers ( $r$ ) in *A. andreniformis* colonies from Malaysia (this study) and Thailand (Oldroyd et al., 1997) and in *A. florea* from Thailand (Oldroyd and Wongsiri, 2006).

Colony ID	$N$	$k$	$m_e$	$g$	Population
<i>A. andreniformis</i>					
K1	200	13	11.3	0.29	Kuala Lumpur <sup>a</sup>
K2	200	16	13.4	0.28	Kuala Lumpur
K3	200	11	10	0.30	Kuala Lumpur
K4	200	14	12.9	0.29	Kuala Lumpur
K5	200	12	9.9	0.30	Kuala Lumpur
K6	200	15	12.7	0.29	Kuala Lumpur
Mean + S.E.		13.5 ± 1.9	11.7 ± 1.5	0.29	
B1	200	18	15.6	0.28	Borneo <sup>b</sup>
B2	200	23	19.6	0.28	Borneo
B3	200	22	18	0.28	Borneo
B4	200	19	17.3	0.28	Borneo
Mean + S.E.		20.5 ± 2.4	17.6 ± 1.7	0.28	
Mean + S.E.		13.5 ± 4.5	10.5 ± 1.9	0.30	Thailand <sup>c</sup>
<i>A. florea</i>					
Mean + S.E.		11.6 ± 5.0	7.9 ± 3.3	0.31	Thailand <sup>d</sup>

<sup>a</sup> Wilayah Persekutuan Kuala Lumpur, Malaysia. <sup>b</sup> Tenom, Sabah, Borneo, Malaysia. <sup>c</sup> Oldroyd et al. (1997). <sup>d</sup> Oldroyd and Wongsiri (2006).

mating frequency of the queens ranged from 9.9 to 19.6 (Tab. I). All the workers analysed contained at least 1 of the 2 presumed queen alleles at all loci, suggesting that only 1 queen headed each colony. The effective mating frequency of the queen in the population from the Malay Peninsula is similar to that reported in the previously published report on the Thai population of this species (Oldroyd et al., 1997). In contrast, the effective mating frequency of the queen in the Borneo population of this species was higher than that of the mainland Asia population (Tab. I). The effective mating frequency significantly differed between the populations ( $P < 0.01$ ,  $t$ -test). The difference in the level of polyandry observed among subspecies such as *A. mellifera* (Franck et al., 2000) may result from the genetic variation in geographically isolated populations. The mean worker relatedness of the mainland Asia (Malay Peninsula) and Borneo populations were 0.29 and 0.28 (Borneo), respectively (Tab. I). The probability of not detecting patriline by using genetically identical inseminating drones ( $d_p$ ) was 0.0008 (Malaysia peninsula) and 0.002 (Borneo). Therefore, a rare paternity would remain undetected in these studies, suggesting that it would have no influence on our results. Our study demonstrates that the *A. andreniformis* colonies are characterized by high paternity and low relatedness between the workers – a colony kin structure similar to that observed in other *Apis* species (Palmer and Oldroyd, 2000; Oldroyd and Wongsiri, 2006). The mating frequencies of the queen in this species were slightly higher than those of *A. florea* queens but relatively lower than those of other honeybee species studied.

## ACKNOWLEDGEMENTS

We sincerely thank T. Takagi and A. Okamoto for kindly helping to collect samples for this study. This study was supported by JSPS research fellowships for J.T.

**Note scientifique sur les niveaux de polyandrie chez l'abeille naine *Apis andreniformis* de Malaisie.**

**Eine wissenschaftliche Notiz über die Anzahl der Paarungen der Zwerghonigbiene *Apis andreniformis* aus Malaysia.**

## REFERENCES

- Boomsma J.J., Ratnieks F.L.W. (1996) Paternity in eusocial Hymenoptera, *Philos. Trans. R. Soc. London B* 351, 947–975.
- Estoup A., Solignac M., Cornuet J.-M. (1994) Precise assessment of the number of patrilines and of genetic relatedness in honey bee colonies, *Proc. R. Soc. Lond. B* 258, 1–7.
- Franck P., Koeniger G., Lahner G., Crewe R., Solignac M. (2000) Evolution of extreme polyandry: an estimate of mating frequency in two African honeybee subspecies, *Apis mellifera monticola* and *A. m. scutellata*, *Insectes Soc.* 47, 464–470.
- Oldroyd B.P., Clifton M.J., Wongsiri S., Rinderer T.E., Sylvester H.A., Crozier R.H. (1997) Polyandry in the genus *Apis*, particularly *Apis andreniformis*, *Behav. Ecol. Sociobiol.* 40, 17–26.
- Oldroyd B.P., Wongsiri S. (2006) Asian honey bees, Harvard, USA.
- Palmer K.A., Oldroyd B.P. (2000) Evolution of multiple mating in the genus *Apis*, *Apidologie* 33, 553–561.
- Pamilo P. (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*, *Heredity* 70, 472–480.
- Takahashi J. (2006) Ecology of the dwarf honeybee *Apis florea* and *A. andreniformis*, *Honeybee Sci.* 27, 23–32.
- Tarpy D.R., Page R.E. (2001) The curious promiscuity of queen honey bees (*Apis mellifera*): evolutionary and behavioral mechanisms, *Ann. Zool. Fennici.* 38, 255–265.