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Scientific note

A scientific note on levels of polyandry of 2 queens of the Himalayan giant honeybee, *Apis laboriosa*

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***Apis laboriosa* / polyandry / mating number / microsatellites / relatedness**

Honeybee queens mate with more males and have the lowest coefficient of genetic relatedness among workers among all eusocial Hymenoptera (Tarpy and Nielsen, 2002). In particular, the recently discovered Asian cavity-nesting honeybee *Apis nigrocincta* F. Smith exhibits the highest degree of polyandry in the genus *Apis* L. (Palmer et al., 2001). The evolution of multiple mating by honeybee queens is central to the issue of kin selection and social evolution in the Hymenoptera. Therefore, much attention has been paid to the evolution and maintenance of multiple mating in the genus *Apis* in concentration with kin selection or gene diversity, for which several hypotheses have been proposed (Palmer and Oldroyd, 2000).

The Himalayan giant honeybee *A. laboriosa* F. Smith is found in the mountainous regions of Vietnam, Bhutan, China India and Nepal. The first specimen of *A. laboriosa* was reported in Yunnan, China. More recently, Sakagami et al. (1980) reported that *A. laboriosa* shows many morphological differences from *A. dorsata* Fabricius. Furthermore, Underwood (1986) was the first to report on certain behaviors of *A. laboriosa*. Specifically, he reports that this species constructs a large single comb in an open space in high cliffs at altitudes ranging from 1200 to 3600 m, makes a seasonal migration depending on the availability of nectar and pollen resources, and tends to colonize one site in a reproductive season. However, the comparative levels of polyandry in giant honeybee colonies are unknown within the genus *Apis*.

In the present study, we used microsatellite DNA analysis to estimate queen mating frequency and the genetic relatedness among workers of two colonies of the Himalayan giant honeybee *A. laboriosa*.

In June of 1999, two colonies of *A. laboriosa* were collected by local honey hunters of Rai tribe in Sadhi Village, Nepal. These colonies had nested in the same cliff site. We were unable to obtain the queen of either colony, but we collected from 45 to 53 worker pupae from each, and these were stored at 99% ethanol until DNA extraction. We chose to sample pupae in order to avoid the problem of drifted individuals which may contaminate the result. Microsatellite DNA analysis was conducted using 4 primers designed by Estoup et al. (1994), A14, A88, A107 and B124. The levels of polyandry were calculated according to Oldroyd et al. (1996). Genotype frequency was observed at the selected four microsatellite loci with 4, 5, 3 and 5 alleles at loci A14, A88, A107 and B124, respectively. The results are given in Table I. The queen of Colony 1 was found to have mated with 23 drones and the queen of Colony 2 with 21. The number of effective matings revealed 22.12 in the Colony 1, and 17.01 in Colony 2. The genetic relatedness of nestmate workers were 0.272 in Colony 1 and 0.278 in Colony 2. Our study demonstrates that queens of two *A. laboriosa* colonies mate 20 times, a similar level of polyandry to that found in *A. dorsata* (Moritz et al., 1995; Oldroyd et al., 1996). However, the level of polyandry of *A. mellifera carnica* queens ranged from 6 to 24 patrilines within species (Neumann et al., 1999; Tarpy and Nielsen, 2002). It is possible that the mating frequency of *A. laboriosa* queens has an equally large range, but more colonies need to be analyzed to estimate more precisely the levels of polyandry in *A. laboriosa*.

Note scientifique sur les niveaux de polyandrie de deux reines de l'abeille géante de l'Himalaya, *Apis laboriosa*.

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Table I. Genotypes of queens and paternal drones in 2 colonies of *A. laboriasa*. Q1 and Q2 indicate the queen genotypes, and from P1 to P23 indicate the paternal genotypes.

	Microsatellite loci				Number of samples	Microsatellite loci				Number of samples
	A14	A88	A107	B124		A14	A88	A107	B124	
Colony No. 1						Colony No. 2				
Q1	216	128	214	202		220	132	214	202	
Q2	222	132	222	212		224	138	222	206	
P1	216/222	128	214/222	121	3	220/224	138	214/222	208	1
P2	216	128/132	214/222	202/206	2	216	130	210	200	1
P3	216/222	128/132	214	212	1	222	134	214	200	1
P4	216	128	214/222	200	4	222	134	214/222	202	2
P5	220	128	214/222	200	2	224	130	214	200	3
P6	220	138	210	200	2	220/224	134	210	202	4
P7	224	134	214	212	1	216	130	210	202	2
P8	216	138	214	200	2	222	130	210	206	3
P9	216	138	222	208	3	222	130	214/222	200	2
P10	220	130	222	208	2	224	128	222	200	2
P11	222	138	214/222	212	2	220	130	214/222	206	2
P12	224	138	214/222	212	2	222	130	214	208	4
P13	220	128	214/222	208	3	220	130	214	200	4
P14	224	138	214/222	206	1	222	130	210	202	3
P15	220	138	214/222	202	2	220/224	128	210	202	1
P16	216/222	128/132	214/222	202	3	220	134	214	200	1
P17	224	130	214/222	202/206	1	216	134	210	200	2
P18	220	138	214/222	206	2	220/224	128	214	208	1
P19	216/222	134	210	200	2	224	130	210	208	3
P20	216/222	138	210	200	4	224	128	214/222	200	2
P21	216	128/132	214/222	208	2	216	138	210	200	1
P22	224	138	214/222	3						
P23	216	130	210	202	4					
				Total	53				Total	45

Wissenschaftliche Notiz über Anzahl der Paarungen von zwei Königinnen der Riesenhonigbiene des Himalaya *Apis laboriosa*.

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