GENETICS

Levels of Polyandry and Intracolonial Genetic Relationships in *Apis dorsata* (Hymenoptera: Apidae)

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ABSTRACT Pupae and adults of 4 *Apis dorsata* F. colonies from northern Thailand were collected in liquid nitrogen. DNA was extracted, and microsatellite genotypes at 3 loci determined for 42–194 workers per colony. From these data, the queen genotype was inferred, and the number of males with which each queen mated deduced. These *A. dorsata* queens mated with a mean of 26.75 ± 5.42 (SEM) drones (range, 13–39). The mean within-colony genetic relatedness was 0.29 ± 0.009. All colonies were monogynous. Possible reasons for the very high level of polyandry and the great variance are discussed. A null allele was detected for 1 microsatellite locus in 1 queen progeny.

KEY WORDS polyandry, microsatellite, multiple mating, relatedness, null allele

EVOLUTION OF EUSOCIALITY in the Hymenoptera was probably facilitated by the high relatedness among workers which is a consequence of haplodiploidy and single once-mated queens (Hamilton 1964, Pamilo 1991b). However, in many eusocial species, multiple mating (polyandry) or multiple queens (polygyny) occurs, which causes a reduction in average genetic relatedness among worker nest mates to levels slightly higher than that of half-siblings (Page and Metcalf 1982, Keller and Reeve 1995). Eusociality can be maintained despite multiple mating because the benefits to workers of remaining in a social way of life outweigh the costs. However, the almost universal occurrence of polyandry or polygyny in ants and honey bees is of great interest (Page and Metcalf 1982, Keller and Reeve 1994). Cole (1983) suggested that multiple mating may have evolved because queens of large long-lived colonies require a large reservoir of sperm. However, this hypothesis seems implausible, because there seems no reason why males could not evolve to produce more semen (Crozier and Page 1985). Indeed, at least in *Apis mellifera*, males produce approximately the same amount of semen as is typically found in the spermatheca of mated queens, but queens still mate many times (Koeniger and Koeniger 1990, 1991).

Keller and Reeve (1994) argued that the evolutionary shift from monandry and monogyny to polyandry or polygyny was predicated by selective advantages for an increase in intracolonial genetic variance. After an extensive survey of the literature, they convincingly demonstrated that nearly all ant species are either polygynous or polyandrous, but rarely both. They then argued that because polyandry has a cost (for example from increased risk of predation or sexually transmitted disease), the nearly universal presence of polyandry in monandrous species gives strong support for the genetic variance hypothesis. (However, more critical examination of their data might suggest that the apparently significant association between polyandry and monandry might only be the result of phylogenetic inertia).

There are 4 hypotheses as to why intracolonial genetic variance confers selective advantages on queens, colonies, and individuals (Crozier and Page 1985, Keller and Reeve 1994). (1) Genetic variance allows an increased expression of caste polymorphism (Crozier and Page 1985). (2) Genetic variance increases the range of environments the colony can tolerate (Crozier and Brückner 1981; Oldroyd et al. 1992a, b). (3) Polyandry reduces the variance in the production of diploid males among colonies (Page 1980, Ratnieks 1990). (4) Genetic variance increases colonial resistance to parasites and pathogens (Sherman et al. 1988; Shykoff and Schmid-Hempel 1991a, b).

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Table 1. PCR conditions and primer sequences for 3 microsatellite loci used to determine pedigrees in *A. dorsata*

Locus	Primers	Annealing temp, °C	$MgCl_2$, mM	No. cycles
A14	5'GTGTCGCAATCGACGTAACC 5'GTCGATTACCGATCGTGACG	58	1.5	30
A88	5'CGAATTAACCGATTTGTCG 5'GATCGCAATTATTGAAGGAG	50	1.5	30
B124	5'GCAACAGGTCGGGTTAGAG 5'CAGGATAGGGTAGGTAAGCAG	55	1.5	30

Primer sequences are from Estoup et al. (1993, 1994) and Oldroyd et al. (1995).

Testing these alternative hypotheses requires data across a range of species. The genus *Apis* is an ideal one for comparative studies because the 6 species have very different ecological ranges and life history patterns (Ruttner 1988). Most of what is known about the numbers of matings in most species has been obtained from sperm counts, which can be unreliable (Koeniger et al. 1990, Oldroyd et al. 1995). However, recent work using reliable genetic markers has shown that in honey bees, *Apis mellifera* L., queens mate 7–20 times, with a mean effective number of matings of 12.4 ± 2.5 (±SEM) and a mean average genetic relatedness of 0.30 ± 0.009 (Estoup et al. 1994). *Apis florea* (Fabricius, 1787) mate at least 5–14 times with a mean effective number of matings of 5.6 ± 1.04, and a mean average genetic relatedness of workers of 0.35 ± 0.018 (Oldroyd et al. 1995).

Apis dorsata (Fabricius, 1793) is the largest species in its genus, and is found throughout tropical Asia (Ruttner 1988). Colonies are very large, with 40,000 individuals in an average nest (Seeley et al. 1982). Colonies are either found singly or in aggregations of up to 60 colonies (Koeniger and Koeniger 1980, Seeley et al. 1982). By comparing the number of spermatozoa in the spermathecae of 2 *A. dorsata* queens with that found in the seminal vesicles of 5 drones, Koeniger et al. (1990) demonstrated that *A. dorsata* queens mate with at least 2 drones. However, they also suggested that *A. dorsata* queens may mate many more times than this if, as in *A. mellifera*, queens expel most of the semen of each male, assimilating only a small portion of each males ejaculate into the spermatheca.

We report on the number of matings in *A. dorsata* using microsatellites as genetic markers to infer maternity and paternity in worker progeny (Choudary et al. 1993; Estoup et al. 1993, 1994; Evans 1993; Hamaguchi et al. 1993; Queller et al. 1993). We also compute the "effective" number of matings (Chevalet and Cornuet 1982, Oldroyd and Moran 1983), or the "effective promiscuity" (Starr 1984) which takes into account the number and proportion of paternities represented in worker offspring.

Materials and Methods

Combs containing *A. dorsata* pupae were bought in street markets in northern Thailand where they are sold as food. Two combs were bought at the market in the town of Lampang and 1 in the city of Chiang Mai. We could not ascertain from where the combs were harvested, but believe they were cut locally because the pupae were still alive. Combs were definitely from separate colonies because they were purchased on different days from different vendors (this was later confirmed genetically). A 4th sample of adult bees was obtained from a colony nesting on a building in Chiang Mai. All samples were frozen in liquid nitrogen for transport to the laboratory, where they were stored at −70°C. Use of brood rather than adults eliminates the possibility of bees drifting among colonies contributing to the results. The colony we collected from was isolated, and drifting was unlikely.

The DNA was extracted from individual bees (Crozier et al. 1991) and resuspended in 50 μl of TE. Samples were prepared for polymerase chain reaction (PCR) using 3 sets of primers (A14, A88, and B124), which are known to amplify microsatellite sequences in *A. mellifera* (Estoup et al. 1994, Oldroyd et al. 1995). For each primer pair, 1 primer was radio-actively endlabeled. In a total reaction volume of 10 μl, the γ-phosphate from ^{33}P-dATP (Dupont, Boston, MA) was transferred to the 5'-terminus primer-2, using T4 polynucleotide kinase (Promega, Madison, WI). The reaction contained 70 mM Tris-HCl, 10 mM $MgCl_2$, 2 μM primer, 5 μl ^{33}P-dATP, and 4 units of polynucleotide kinase. The reaction was incubated for 30 min at 37°C and stopped by heating to 90°C for 2 min.

One-microliter aliquots of 1/10 dilution sample DNA were amplified using primers (1 endlabeled) and PCR temperature profiles specified in Table 1. PCR reactions were performed in a total volume of 10 μl containing 0.167 mM of each dNTP, 1 μg BSA, 0.4 μM unlabeled primer, 0.02 μM labeled primer, 1 × Promega reaction buffer, $MgCl_2$ and 0.4 units of Promega Taq polymerase. PCR products were run on standard 6% polyacrylamide sequencing gels with M13 control DNA sequencing reactions run on the same gel as size standards. Microsatellite alleles were scored as fragment lengths in base pairs. Primer sequences are given in Table 1.

Where possible, the queen genotype was determined for each locus in each colony. When an allele was present in every worker, the queen was

considered homozygous for that allele. When every worker carried 1 of 2 alleles, the queen was assumed heterozygous for those 2 alleles (Estoup et al. 1994). Paternal alleles of each worker were deduced by subtraction (Estoup et al. 1994). That is, the paternal allele of each worker is that allele not carried by the queen. Where a queen is heterozygous at a particular locus *A*, paternity at that locus is uncertain for all workers with the same genotype as the queen. That is, for these workers, one cannot tell if a particular allele is paternal or maternal in origin. Our approach to these workers was as follows: Homozygous workers of genotype A_1A_1 and A_2A_2 were unambiguously of different subfamilies *X* and *Y* and were allocated to their appropriate paternity groups accordingly. The ambigous heterozygous workers, A_1A_2, were then allocated to the 2 alternative paternity classes based on the proportion of the 2 homozygous classes. That is, if the number of A_1A_1 workers was x and A_2A_2 workers y, then the z A_1A_2 workers would be allocate to subfamily *X* in the proportion

$$z\left(\frac{x}{x+y}\right)$$ and *Y* in the proportion $$z\left(\frac{y}{x+y}\right).$$

The average coefficient of relatedness, *G*, weighted according to the relative proportions of each subfamily in our samples, was computed from:

$$G = \sum_{i=1}^{k} \{([0.75p_i] + [0.25(1 - p_i)])p_i\} \quad (1)$$

(Laidlaw and Page 1984), where p_i = the relative frequency of the i^{th} subfamily and k is the number of subfamilies. The effective number of matings (m) was computed from:

$$m = 1/\sum_{i=1}^{k} p_i^2 \quad (2)$$

(Starr 1984).

Results

While scoring the gels we became aware of a null allele carried by the queen of colony 3 for microsatellite A14. That is, we assumed that the sequence corresponding to one of the primers designed to amplify this microsatellite contained a mutation, which prevented amplification. The null allele was manifested by 6 different homozygous worker genotypes in this colony for locus A14. In the absence of a null allele, only 2 kinds of homozygous workers are possible at any 1 locus, if colonies contain only a single laying queen. Our initial hypothesis therefore, was that colony 3 had at least 3 queens. However, this possibility was excluded, and the presence of a null allele assumed because locus B124 reveals only 1 kind of queen allele whereas locus A88 showed only 2 different kinds of queen allele. A more complex distribution of alleles correlated with locus A14 would be expected if the colony was indeed polygynous. Heterozygous individuals in this colony all carried a common 206 bp allele for locus A14. Support for the possibility of polygyny would require the assumption that all 3–5 queens carried the 206 bp allele, which seems extremely unlikely. Fig. 1 gives the most parsimonious pedigree for colony 3 under the assumption of a null allele. There was no evidence of polygyny in any of the 3 other colonies.

In colony 1, the queen mated with at least 30 drones. We observed 13–39 (mean = 26.75, 95% CI = 9.49–44.00) patrilines in the 4 colonies studied (Tables 2 and 3). Mean average relatedness ($G \pm SE\mu$) for the 5 colonies was 0.28 (95% CI = 0.26–0.32) and the mean effective number of matings (m) was 11.36 (95% CI = −1.23–41.05) (Table 3).

Discussion

These results demonstrate that *A. dorsata* queens mate with at least 13 drones and very often many more. In colony 1, where many individuals were sampled, we found 30 patrilines. Because of the high number of alleles at loci A14 and A88, and the high sample size, it seems likely that nearly all the patrilines present were detected. However, some large groupings (for example, drones 16–18 of colony 1) probably contained >1 patriline. Colony 2 revealed only 13 patrilines, which may be an underestimate because of relatively low sample size, whereas colony 4 had at least 39 patrilines. These results indicate a high level of variance in the number of matings in *A. dorsata*, and a level of polyandry unprecedented in social insects, and an order of magnitude higher than that suggested from sperm counts (Koeniger et al. 1990). They confirm results found independently by Moritz et al. (1995) in a population of *A. dorsata* sampled in Borneo.

Why should *A. dorsata* queens mate so often, when in doing so there must be some increased risk of predation or sexually transmitted disease? Additional matings beyond 6 do not substantially alter within-colony genetic relatedness (Hamilton 1964, Oldroyd and Moran 1983, Crozier and Page 1985). Therefore, despite the extremely high levels of polyandry that are apparently present in *A. dorsata*, levels of within-colony genetic relatedness and the effective numbers of matings are very similar to those found in *A. mellifera* (Estoup et al. 1994) and *A. florea* (Oldroyd et al. 1995).

Arguments for the evolution of polyandry based on the genetic load imposed by the sex locus predict that mating by at least 6 drones, but no additional benefit from a significantly larger number of drones (Shaskolsky 1976, Page 1980, Ratnieks 1990). Thus, the large number of mates observed in *A. dorsata* cannot be explained adequately in this way.

Arguments relating to worker/queen conflict over sex allocation may predict a large number of

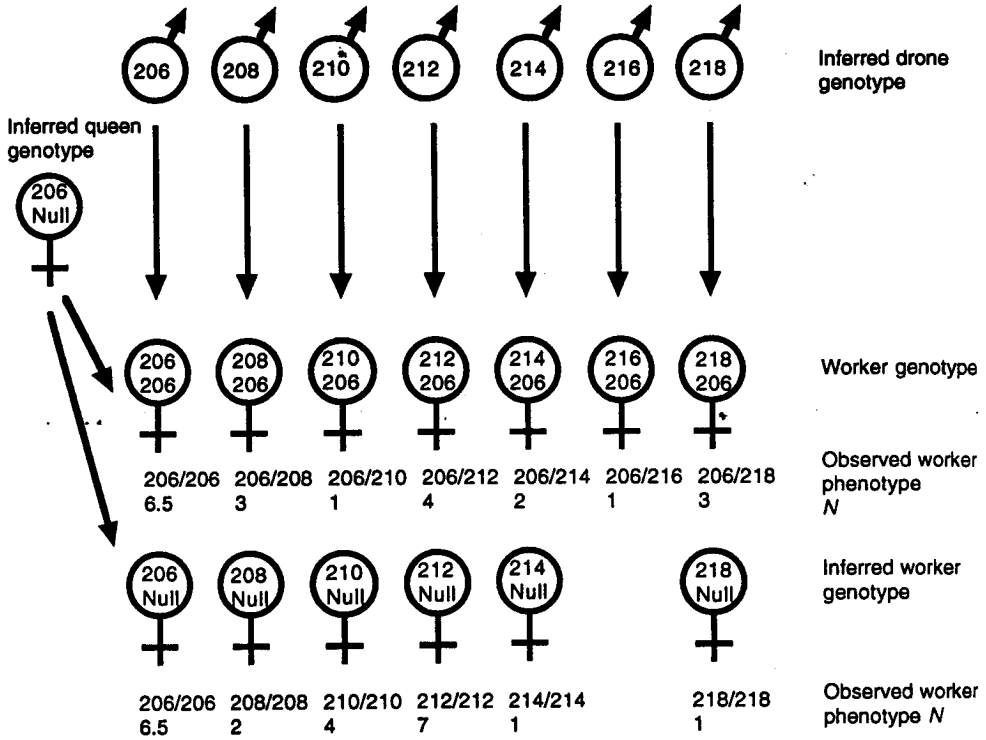

Fig. 1. Parsimonious pedigree of inferred from worker phenotypes for microsatellite A14 (Estoup et al. 1994) in colony 3. Note that each male symbol represents several drones that were distinguished by other microsatellite loci. The 13 workers with 206/206 phenotype were equally allocated to the 206/null and 206/206 genotypes.

matings may evolve under quite specific circumstances (Queller 1993) that may exist in honey bees. Because of asymmetries in relatedness of workers and queens to brood, there are potential conflicts between queens and workers in their preferred sex ratios for their colony's sexuals. Briefly, in monandrous colonies, workers prefer a more female biased sex ratio (because they are more closely related to female offspring than male offspring), whereas queens prefer a 50:50 sex ratio (Trivers and Hare 1976, Moritz 1985). Multiple mating reduces the asymmetries of relatedness between queens and workers, and may be 1 reason for the evolution of polyandry (Trivers and Hare 1976, Moritz 1985). Queller's (1993) argument extends that of Moritz (1985) and Pamilo (1991a). He suggests that if, selection increases the number of times queens mate, workers should respond to this increase by allowing the production of more males. In a population with many males per colony, queens will again be selected to increase male production, and they can induce workers to do this by increasing the number of times they mate. This evolutionary arms race will only continue while there is variance in levels of polyandry and male production among colonies. If all queens mate with the same number of males, and all colonies produce the same numbers of males, this equilibrium number of matings and sex ratio will be stable (Queller 1993).

In honey bees, variance in male production among colonies results in part from seasonal and environmental factors (Seeley 1985). Migration may mix populations with different optimal sex ratios. Thus, optimal levels of polyandry for queens and workers may constantly change. The very high numbers of matings observed in *A. dorsata*, and the great variance in number of mates that we now have observed within each of the 3 species of *Apis* now studied, may suggest that the conditions for the evolutionary arms race proposed by Queller exist in the genus, and that such races are indeed occurring. The weakness of this argument is that it seems very unlikely that workers can accurately detect the number of times their queen mated and make appropriate adjustments to the production of males. It is plausible that they could distinguish between monandry and low levels of polyandry, but once the levels of polyandry are relatively high (again ≈6 males) it is doubtful that workers could

Table 2. Genotypes (microsatellite length in base pairs) of queens and paternal drones in 4 colonies of *A. dorsata*

	Microsatellite locus			Observed no. workers
	B124	A88	A14	
		Colony 1		
Queen allele 1	218	138	206	
Queen allele 2	220	142	208	
Drone 1	218	138	214	1
Drone 2	218	140	212	5
Drone 3	218	140	206/208	1
Drone 4	218	142	208	9
Drone 5	218	144	210	2
Drone 6	218	144	212	18
Drone 7	218	144	214	6
Drone 8	218	148	208	4
Drone 9	218	142/138	210	13
Drone 10	218	142/138	212	2
Drone 11	218	142/138	214	5
Drone 12	220	135	214	7
Drone 13	220	138	214	2
Drone 14	220	140	210	3
Drone 15	220	140	212	1
Drone 16	220	144	210	13
Drone 17	220	144	212	33
Drone 18	220	144	214	21
Drone 19	220	144	206/208	8
Drone 20	220	142/138	206	11
Drone 21	220	142/138	212	2
Drone 22	220	142/138	214	2
Drone 23	224	142	?	2
Drone 24	218/220	133	210	3
Drone 25	218/220	135	212	1
Drone 26	218/220	138	210	2
Drone 27	218/220	138	212	6
Drone 28	218/220	138	206/208	3
Drone 29	218/220	146	210	5
Drone 30	218/220	148	210	3
			Total	194
		Colony 2		
Queen allele 1	218	140	206	
Queen allele 2	220	140	206	
Drone 1	218	136	206	3
Drone 2	218	136	214	2
Drone 3	218	138	214	2
Drone 4	218	144	206	2
Drone 5	220	138	210	4
Drone 6	220	138	214	12
Drone 7	220	140	210	3
Drone 8	220	142	208	5
Drone 9	220	148	208	1
Drone 10	218/220	133	208	5
Drone 11	218/220	142	206	1
Drone 12	228	138	208	1
Drone 13	230	138	214	3
			Total	44
		Colony 3		
Queen allele 1	218	138	206	
Queen allele 2	218	142	null	
Drone 1	218	136	212	2
Drone 2	218	138	206	3
Drone 3	218	138	210	1
Drone 4	218	142	206	1
Drone 5	218	142	212	1
Drone 6	218	144	208	1
Drone 7	218	144	210	2
Drone 8	218	144	212	1
Drone 9	218	144	216	1
Drone 10	218	144	218	3
Drone 11	218	146	208	3
Drone 12	218	150	214	1

Table 2. Continued

	Microsatellite locus			Observed no. workers
	B124	A88	A14	
Drone 13	218	142/138	206	2
Drone 14	218	142/138	208	1
Drone 15	218	142/138	212	3
Drone 16	220	133	210	2
Drone 17	220	135	206	1
Drone 18	220	135	218	1
Drone 19	220	138	206	1
Drone 20	220	140	206	1
Drone 21	220	144	212	3
Drone 22	220	144	214	2
Drone 23	220	150	206	3
Drone 24	220	142/138	206	1
Drone 25	224	140	212	1
			Total	42
		Colony 4		
Queen allele 1	177	97	167	
Queen allele 2	183	99	171	
Drone 1	177	92	165	1
Drone 2	177	92	169	1
Drone 3	177	97	165	2
Drone 4	177	101	165	2
Drone 5	177	101	167/171	10
Drone 6	177	101	173	5
Drone 7	177	103	167/171	1
Drone 8	177	105	169	1
Drone 9	177	107	167/171	1
Drone 10	177	113	173	1
Drone 11	183	92	165	1
Drone 12	183	97	165	2
Drone 13	183	101	167/171	1
Drone 14	183	103	173	1
Drone 15	177/183	92	169	2
Drone 16	177/183	94	169	1
Drone 17	177/183	94	167/171	1
Drone 18	177/183	97	169	1
Drone 19	177/183	97	173	1
Drone 20	177/183	101	169	1
Drone 21	177/183	107	167/171	3
Drone 22	177/183	97/99	171	1
Drone 23	179	92	167/171	1
Drone 24	179	94	165	1
Drone 25	179	95	169	1
Drone 26	179	97/99	165	3
Drone 27	179	99	171	1
Drone 28	179	101	171	1
Drone 29	179	103	165	1
Drone 30	179	103	173	1
Drone 31	179	103	167/171	2
Drone 32	179	105	165	1
Drone 33	179	105	169	1
Drone 34	179	107	171	1
Drone 35	179	111	167/171	1
Drone 36	179	117	167/171	2
Drone 37	187	97	165	3
Drone 38	187	99	173	1
Drone 39	192	97/99	165	1
			Total	64

/ Indicates 2 alternative genotypes where paternal and maternal alleles cannot be distinguished. ? Indicates genotype not determined.

accurately detect mate number and make appropriate adjustments (Queller 1993). We therefore reject this hypothesis (as does Queller) to explain the evolution of extreme polyandry beyond 6–10 mates.

The final plausible hypothesis is that extreme polyandry has evolved to maximize genetic variance within colonies and thereby increase the range of conditions that a colony can tolerate by increasing the total behavioral plasticity of workers within colonies (Calderone and Page 1988, 1991; Robinson and Page 1988; Calderone et al. 1989; Kolmes et al. 1989; Page et al. 1989; Oldroyd et al. 1992a, 1993, 1994; Robinson 1992; Fewell and

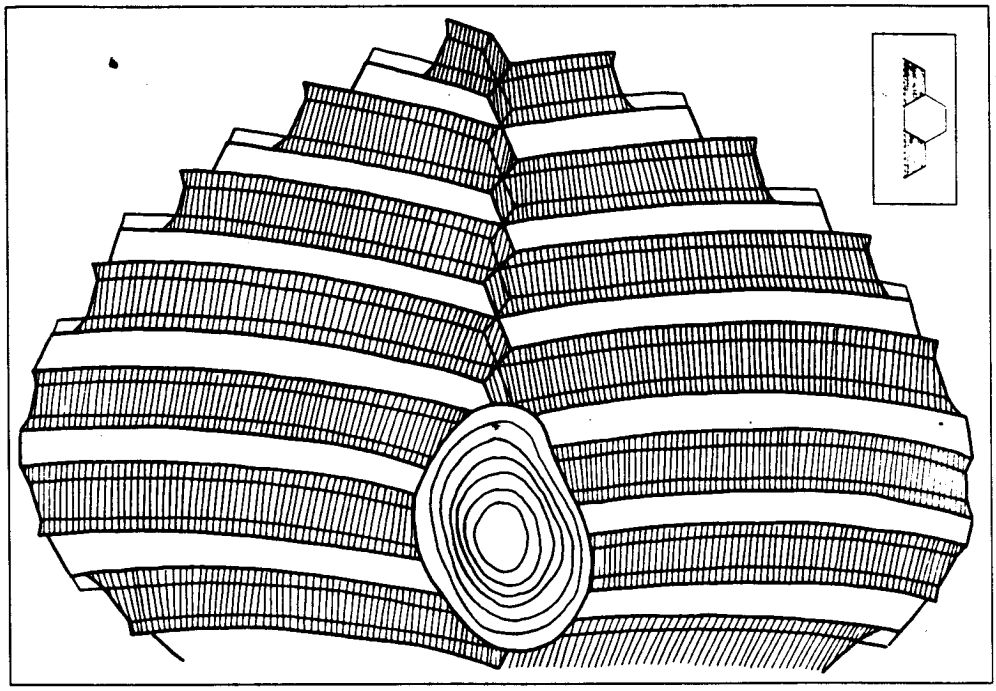

FIG. 7. Diagram of a cross-section view of the honey storage area or crown of a nest of *Apis andreniformis* showing the internal relationships of the honey storage cells above the supporting branch. The central concentric circles represent the supporting branch. The insert at the top right provides an interpretation of open and closed cells. The diagram shows a clear mid-rib structure.

very long and extend to the supporting branch. Above this area, cells coming from opposite sides have their base at the sides of cells coming from the other side. Cells coming from the top of the crown have this same pattern, however the use of an adjacent sidewall as a base is more extreme with some cells open to the top surface having their base well away from the base of the supporting cell.

This contrasts with the crown of an *A. andreniformis* nest. This crown has a characteristic crest appearance when viewed from the outside surface. Each cell has a regular hexagonal shape. Cells are arranged in layers with each layer offset by the width of half a cell, much like roofing tiles. A slight curvature of cells in combination with cells having different lengths (and being present or not being present) provides the adjustments that produce tapering and rounding (figs. 5 and 6). Each layer of cells distal from the supporting branch is narrower than the previous layer with the opening of the cell longer at the bottom than at the top. Hence, the arrangement of cells is quite regular. A cross section shows a clear mid-rib structure where the bases of opposing cells come together in the same way as cells in the brood nest area (fig. 7).

DISCUSSION

This study reinforces the concern that previous literature concerning *A. florea* might derive from work done with *A. andreniformis* before it was recognized as a separate species (Rinderer *et al.*, 1995). The figures of *A. florea* nests by Ruttner (1988) indicate the presence of a mid-rib, a characteristic of the nests of *A. andreniformis*, not *A. florea*. More correct drawings are provided by Ruttner (1992). However, Ruttner's (1992) figures are only accurate in correctly indicating a mid-rib for the nest of *A. andreniformis* and the lack of a mid-rib in for the nest of *A. florea*. The descriptions and figures presented here provide an accurate representation of the arrangements of cells and their physical relationships to one another in the nests of both species.

Although the specific measurements of *A. florea* nests are generally larger than those of *A. andreniformis* nests, the overall impression of the nests is similar and only measurements would permit the identification of a nest using size characteristics. However, the external appearance of the crowns of the two nests are clearly distinct and can be used to quickly identify them. From the examination of cross-sections it is clear that the external crown differences derive from the presence of a honey storage area mid-rib in nests of *A. andreniformis*

and the lack of a honey storage mid-rib in nests of *A. florea*.

The use of a mid-rib above the supporting branch may influence the size of the branch chosen to support the nest by *A. andreniformis*. However, the variance in branch diameter is large, and the difference in branch sizes chosen by the two species is marginal. If a true difference exists, it is small and may arise from slight differences in habitat selection .

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REFERENCES

The numbers given at the end of references denote entries in *Apicultural Abstracts*.

FABRICIUS, J C (1787) *Mantissa Insectorum*. Vol I Proft. Hafniae.

GUZMAN, DE L I; FORBES, M; CERVANCIA, C; RINDERER, T E; SOMERA, S JR (1992) *Apis andreniformis* Smith in Palawan, Philippines. *Journal of Apicultural Research* 32(2): 111. 93/827

OTIS, G W (1990) Diversity of *Apis* in Southeast Asia. *Proceedings of 11th International Congress of International Union for the Study of Social Insects*; pp 104–105.

RINDERER, T E; OLDROYD, B P; WONGSIRI, S; SYLVESTER, H A; GUZMAN, DE L I; POTICHOT, S; SHEPPARD, W S; BUCHMANN S L (1993) Time of drone flight in four honey bee species in southeastern Thailand. *Journal of Apicultural Research* 32(1): 27–33. 93/113

RINDERER, T E; OLDROYD, B P; WONGSIRI, S; SYLVESTER, H A; GUZMAN, DE L I; STELZER, J A; RIGGIO, R M (1995) A morphological comparison of the dwarf honey bees of southeastern Thailand and Palawan, Philippines. *Apidologie* 26: 387–394.

RUTTNER, F (1988) *Biogeography and taxonomy of honeybees*. Springer-Verlag; Berlin, Germany; 284 pp. 88/1155

RUTTNER, F (1992) *Naturgeschichte der honigbienen*. Ehrenwirth Verlag; Munich, Germany; 360 pp. 93/1188

SMITH, F (1858) Catalogue of the Hymenopterous insects collected at Sarawak, Borneo; Mount Ophir, Malacca; and at Singapore, by A R Wallace. *Journal of the Proceedings of the Linnean Society London* 2: 42–130.

WONGSIRI, S; LIMBIPICHAI, K; TANGKANASING, P; MARDAN, M; RINDERER, T; SYLVESTER, H A; KOENIGER, G; OTIS, G (1990) Evidence of reproductive isolation confirms that *Apis andreniformis* (Smith, 1858) is a separate species from sympatric *Apis florea* (Fabricius, 1787). *Apidologie* 21(1): 47–52. 90/1074

WU, Y-R; KUANG, B (1986) [A study of the genus Micrapis (Apidae).] *Zoological Research* 7(2): 99–102 (in Chinese). 88/59

WU, Y-R; KUANG, B (1987) Two species of small honeybee — a study of the genus *Micrapis*. (A translation of *Zoological Research* 7: 99–102). *Bee World* 68(3): 153–155. 88/60