This is an electronic appendix to the Biology Letter by Wenseleers & Ratnieks 2004 Tragedy of the commons in *Melipona* bees. *Proc. R. Soc. Lond.* B (Suppl.) **271**, S310–S312. (DOI 10.1098/rsbl.2003.0159.)

ELECTRONIC APPENDIX A

Description of the data set

To test the effect of kinship on queen production we collated all available data on male parentage and female caste ratio in *Melipona*. A total of 32 studies and 4 reviews across 13 species were found. However, we only used studies where male parentage and caste ratio were known for the same population, and were measured under natural conditions. This left 12 studies on four species: *Melipona beecheii* (3 studies, all from Yucatan, Mexico)*, M. favosa* (Trinidad)*, M. quadrifasciata* (various sites, Brazil) and *M. subnitida* (Rio Grande do Norte, Brazil) (Tables 1, 2).

Worker reproduction, as inferred from either genetic or observational studies, ranges from high to low. In *M. favosa*, 95% of males are workers' sons, in *M. subnitida* and *M. quadrifasciata* the workers and queen share in male production, and in *M. beecheii* all of the males are queen's sons (Table 1). These differences are statistically significant (Table 1). In species where worker reproduction occurs, R_m>0.25, leading to the prediction that fewer excess queens should be produced. In all four species colonies are headed by a single singly-mated queen (Kerr et al. 1962; da Silva et al. 1972; Kerr 1975; Contel & Kerr 1976; Peters et al. 1999; Paxton et al. 2001), so that R_f=0.75. (Double mating by queens was erroneously reported for *M. beecheii* due to the accidental inclusion of workers that drifted between colonies, Paxton et al. 1999).

Table 2 sumarizes caste ratios in the same four species. Caste ratios, too, vary considerably. *M. beecheii* produces most queens (16%), *M. subnitida* and *M. quadrifasciata* produce an intermediate amount (9%) and *M. favosa* produces the least queens (5%). The differences between species with high, intermediate and low queen production are highly significant (Table 2). To determine whether queen production correlated with male production by workers we used the gamma statistic (Siegel & Castellan 1988) to test predicted rankings. *M. favosa* and *M. beecheii* were coded as having high and low predicted and observed queen production (the two studies on *Melipona beecheii,* Darchen & Delage-Darchen 1975 and Moo-Valle et al. 2001, were averaged into one data point). The predicted and observed ranks of *M. subnitida* and *M. quadrifasciata* were intermediate and considered to be tied, as both had nonsignificantly different caste ratios and levels of worker reproduction (Tables 1, 2).

All data were considered phylogenetically independent. This can be defended given that both the level of worker reproduction and caste ratios are evolutionary labile. For example, cytochrome oxidase I sequencing has shown that *M. favosa* and *M. beecheii* are phylogenetically very closely related (J.C. Nieh, unpublished data), yet they are at two extremes in terms of both caste ratio and male parentage. Conversely, *M. subnitida* and *M. quadrifasciata* produce similar caste ratios and have similar levels of worker reproduction, but are not closely related phylogenetically, since they belong to two different subgenera, *Melipona* sensu strictu and *Michmelia* Moure, 1975 (Moure 1992).

References

Chinh, T. X., Grob, G. B. J., Meeuwsen, F. J. A. J. & Sommeijer, M. J. 2003 Patterns of male production in the stingless bee *Melipona favosa* (Apidae, Meliponini). *Apidologie* 34, 161-170.

Contel, E. P. B. & Kerr, W. E. 1976 Origin of males in *Melipona subnitida* estimated from data of an isozymic polymorphic system. *Genetica* 46, 271-279.

da Silva, D. L. N. 1977 Estudos bionomicos em colônias mistas de Meliponinae. *Boletim de Zoologia, São Paulo* 2, 7-106.

da Silva, D. L. N., Zucchi, R. & Kerr, W. E. 1972 Biological and behavioural aspects of the reproduction in some species of *Melipona* (Hymenoptera, Apidae, Meliponinae). *Animal Behaviour* 20, 123-132.

Darchen, R. & Delage-Darchen, B. 1975 Contribution à l'étude d'une abeille du Mexique *Melipona beecheii* B. (Hymenoptère : Apide). *Apidologie* 6, 295-339.

Kerr, W. E. 1950 Genetic determination of castes in the genus *Melipona*. *Genetics* 35, 143-152.

Kerr, W. E. 1975 Evolution of population structure in bees. *Genetics* 79, 73-84.

Kerr, W. E., Zucchi, R., Nakadaira, J. T. & Butolo, J. E. 1962 Reproduction in the social bees (Hymenoptera: Apidae). *Journal of the New York Entomological Society* 70, 265-276.

Koedam, D., Contrera, F. A. L. & Imperatriz-Fonseca, V. L. 1999 Clustered male production by workers in the stingless bee *Melipona subnitida* Ducke (Apidae, Meliponinae). *Insectes Sociaux* 46, 387-391.

McCullagh, P. & Nelder, J. A. 1989 *Generalized Linear Models*. London: Chapman and Hall.

Moo-Valle, H., Quezada-Euan, J. J. G. & Wenseleers, T. 2001 The effect of food reserves on the production of sexual offspring in the stingless bee *Melipona beecheii* (Apidae, Meliponini). *Insectes Sociaux* 48, 398-403.

Paxton, R. J., Ruhnke, H., Shah, M., Bego, L. R., Quezada-Euan, J. J. G. & Ratnieks, F. L. W. 2001 Social evolution in stingless bees: are the workers or is the queen in control of male production? In *II Seminario Mexicano sobre abejas sin aguijón*, pp. 104-107. Universidad Autónoma de Yucatán, Mérida, Yucatán, México.

Paxton, R. J., Weissschuh, N., Engels, W., Hartfelder, K. & Quezada-Euan, J. J. G. 1999 Not only single mating in stingless bees. *Naturwissenschaften* 86, 143-146.

Peters, J. M., Queller, D. C., Imperatriz-Fonseca, V. L., Roubik, D. W. & Strassmann, J. E. 1999 Mate number, kin selection and social conflicts in stingless bees and honeybees. *Proceedings of the Royal Society of London Series B-Biological Sciences* 266, 379-384.

Siegel, S. & Castellan, N. J. 1988 *Nonparametric statistics for the behavioral sciences*. New York: McGraw-Hill.

Sommeijer, M. J., Chinh, T. X. & Meeuwsen, F. J. A. J. 1999 Behavioural data on the production of males by workers in the stingless bee *Melipona favosa* (Apidae, Meliponinae). *Insectes Sociaux* 46, 92-93.

Sommeijer, M. J., de Bruijn, L. L. M., Meeuwsen, F. J. A. J. & Martens, E. P. 2003 Natural patterns of caste and sex allocation in the stingless bees *Melipona favosa* and *M. trinitatis* related to worker behaviour. *Insectes Sociaux* 50, 38-44.

Tóth, E., Strassmann, J. E., Nogueira-Neto, P., Imperatriz-Fonseca, V. L. & Queller, D. C. 2002 Male production in stingless bees: variable outcomes of queen-worker conflict. *Molecular Ecology* 11, 2661-2667.

Table 1. Data on male parentage in 4 Melipona *species with sample sizes and 95% confidence limits.* Levels of worker reproduction are significantly different ($p < 0.05$) when the 95% confidence intervals do not overlap; significant differences are indicated by distinct letters in column 8. The difference between *M. beecheii* and *M. favosa* is significantly different at the $p<10^{-17}$ level (*GLZ*).

Species	Type of study (# of loci)	# Colonies	# Males	% males workers' sons	Overall estimate	95% confidence limits[a]	Significant differences	References
M. beecheii	genetic (6)	13	108	0%	0%	0-3%	a	Paxton et al. 2001
M. subnitida	genetic (1)[b]	13	224	33%	36%	8-70%	b	Contel & Kerr 1976
	observational	1	23	65%				Koedam et al. 1999
M. quadrifasciata	genetic (4)	2	47	64%	51%	48-53%	b	Tóth et al. 2002
	observational	2	1,291[c]	50%				da Silva 1977
M. favosa	observational	4	604	95%	95%	92-96%	c	Chinh et al. 2003; Sommeijer et al. 1999
total		35	2,297					

[a]Calculated by fitting a generalized linear (*GLZ*) model (McCullagh & Nelder 1989) with binomial error structure on the assigned parentage of the males ('COLONY' was nested within 'SPECIES'; for the genetic studies the maximum likelihood estimate of the proportion of workers' sons (Tóth et al. 2002) was used as the expected value). [b]Male parentage was inferred from typing at just one diagnostic locus, which causes some uncertainty on the parentage assignments. To account for this, we calculated means and 95% confidence limits for this species on Arcsine-root transformed proportions of worker-produced males (the given figures are backtranformed to the original scale). [c]Two colonies were given equal weight by subsampling to N=518 for both colonies.

Table 2. Caste ratio data in the same 4 Melipona *species with sample sizes and 95% confidence limits.* Fewer females selfishly become queens in *M. favosa*, where nearly all males are worker's sons, than in *M. subnitida* and *M. quadrifasciata*, where only 35-51% are workers' sons (Table 1). *M. subnitida* and *M. subnitida* in turn produce fewer queens than in *M. beecheii* where all males are queen's sons (Table 1). These differences are very highly significant (column 5, *GLZ*, $p<10^{-17}$). The negative relationship between queen overproduction and the level of worker reproduction is also significant overall ($\gamma = 1$, $Z=2.04$, $p=0.04$). This supports the prediction that there should be less exploitation within groups when the cost is borne by close relatives (nephews, $r = 0.75$, rather than brothers, $r = 0.25$).

Species	# Colonies	# Individuals	# Months	Caste ratio (% of queens)	95% confidence limits[a]	Significant differences	References
M. beecheii	13	10,638	12	15.8%	15.1-16.5%	a	Darchen & Delage-Darchen 1975; Moo-Valle et al. 2001
M. subnitida	4	2,383	2	7.1%	6.0-8.1%	b	Koedam et al. 1999
M. quadrifasciata	9	2,806	11	8.5%	7.4-9.5%	b	Kerr 1950
M. favosa	78	13,514	10	5.1%	4.7-5.5%	c	Sommeijer et al. 2003
total	104	29,341					

[a]Calculated from a fitted *GLZ* model (McCullagh & Nelder 1989) with binomial error structure and 'SPECIES', 'COLONY' and 'SEASON' (month of the year) as hierarchically nested factors.