

1 TITLE: Queen-controlled sex ratios and worker-reproduction in the
2 bumble bee *Bombus hypnorum*, as revealed by microsatellites

3
4 AUTHORS: MJF Brown^{1,2}, R Schmid-Hempel² & P Schmid-Hempel²

5
6 ¹Department of Zoology, Trinity College Dublin, Dublin 2, Ireland

7 ²Ecology & Evolution, Experimental Ecology, ETH-Zürich, ETH Zentrum NW, Zürich,
8 CH-8092, Switzerland

9
10 DATE OF RECEIPT:

11
12 KEYWORDS: reproductive conflict, social insects, *Bombus*, worker reproduction,
13 microsatellites, sex ratio

14
15 ADDRESS FOR CORRESPONDENCE: MJF Brown
16 Department of Zoology
17 Trinity College Dublin
18 Dublin 2, Ireland
19 Fax: +353 (0)1 677 8094
20 Email: mabrown@tcd.ie

21
22 RUNNING TITLE: Queen-worker conflict in bumble bees

23 **Abstract**

24

25 Social insect colonies provide model systems for the examination of conflicts among
26 parties with different genetic interests. As such, they have provided the best tests of
27 inclusive fitness theory. However, much remains unknown about in which party's favour
28 such conflicts are resolved, partly due to the only recent advent of the molecular tools
29 needed to examine the outcome of these conflicts. Two key conflicts in social insect
30 colonies are over control of the reproductive sex ratio and the production of male
31 offspring. Most studies have examined only one of these conflicts, but in reality they
32 occur in tandem and may influence each other. Using microsatellite analyses, we
33 examined the outcome of conflict over sex ratios and male production in the bumble bee,
34 *Bombus hypnorum*. We determined the genotypes of mother queens, their mates and
35 males for each of ten colonies. In contrast to other reports of mating frequency in this
36 species, all of the queens were singly-mated. The population sex ratio was consistent with
37 queen control, suggesting that queens are winning this conflict. In contrast, workers
38 produced over 20% of all males in queen-right colonies, suggesting that they are more
39 effective in competing over male-production. Combining our results with previous work,
40 we suggest that worker-reproduction is a labile trait that may well impose only small
41 costs on queen fitness.

42 **Introduction**

43

44 At first sight, colonies of social insects appear to be cooperative units *par excellence*.

45 However, their internal genetic structure (haplodiploidy and its consequent asymmetric
46 relationships) makes them an arena for the playing out of numerous genetic conflicts.

47 This attribute has made social insects focal organisms in testing the predictions of the
48 genetic theory of conflict and cooperation (for a recent review see (Sundström &
49 Boomsma 2001)). Most such tests have focussed on two particular conflicts – over sex
50 ratio and male production (Sundström & Boomsma 2001).

51

52 In haplo-diploid social insects, workers and new queens develop from fertilised, diploid
53 eggs, while males develop from unfertilised haploid eggs. Consequently, assuming single
54 mating, mother queens are related to their male and female sexual offspring equally ($r =$
55 0.5), while workers, who rear the sexual offspring, are more highly related to new sister
56 queens ($r = 0.75$) than to their brothers ($r = 0.25$). Given this, Trivers & Hare (1976)
57 predicted that under worker control the reproductive sex ratio of colonies should be 0.75
58 (calculated as $F/(F+M)$) whilst under queen control it should be 0.5 . Recent reviews
59 (Bourke & Franks 1995; Queller & Strassmann 1998) suggest that sex allocation patterns
60 often match the predictions from inclusive fitness theory given worker control, although
61 exceptions (see Sundström & Boomsma (2001)) indicate that queen control or factors
62 other than relatedness play a role in determining sex allocation patterns.

63

64 As well as conflict over relative investment into male and female reproductives, workers
65 and queens also disagree over who should produce the males. In general, workers should
66 always prefer that their own sons ($r = 0.5$) should be reared over those of their mother
67 queen ($r = 0.25$). Furthermore, in singly-mated colonies, workers prefer both their own
68 sons and their nephews ($r = 0.375$) over their brothers. In contrast, in multiply-mated
69 colonies, workers prefer brothers over nephews, resulting in the evolution of worker-
70 policing behaviour (Ratnieks 1988). Evidence in support of these predictions comes from
71 both honey bees and wasps (Foster & Ratnieks 2000; Foster & Ratnieks 2001; Ratnieks
72 1988). However, recent work has also shown that worker reproduction is either absent in
73 many singly-mated species, despite the predictions of relatedness (Foster *et al.* 2000;
74 Walin *et al.* 1998), or present at low or variable levels (Tóth *et al.* 2002a; Tóth *et al.*
75 2002b). In such cases, the costs of worker reproduction may well overwhelm the benefits
76 derived through relatedness (Ratnieks & Reeve 1992).

77

78 Obviously, conflicts over sex ratio and male production occur concurrently, and their
79 results can feed back on each other. (Bourke 1997) detailed how predicted sex ratios vary
80 depending upon levels of worker reproduction. In essence, in queen-right populations, if
81 the queens control sex ratios, then workers can only increase their inclusive fitness
82 through male production and such worker reproduction has no effect on the predicted
83 population sex ratio because the queen will compensate in her own favour (Bourke 1997),
84 Table 3). In contrast, under worker control of the sex ratio, workers still gain through
85 male-production, but such reproduction in turn drives the preferred population sex ratio
86 to become relatively (although never absolutely) more male-biased. In contrast, worker

87 reproduction in populations with queenless colonies always drives preferred sex ratios, be
88 they under queen or worker control, towards a relatively (and sometimes absolutely)
89 male-biased level. Whether workers gain more from attempting to control sex ratios,
90 male production, or both, remains unknown and must depend not only on relatedness
91 factors but also on both the costs of such conflict in terms of the absolute biomass of
92 reproductives produced and the relative power of queens and workers over the two
93 outcomes (Bourke & Ratnieks 1999). Evidence for worker controlled sex ratios in the
94 absence of worker reproduction exist (reviewed in Sundström & Boomsma (2001)), but
95 we know of no studies that examined the control of sex ratios in populations with worker
96 reproduction.

97

98 Bumble bees provide a good model system with which to address this question. Worker
99 reproduction is known and common in bumble bees, although the absolute level of
100 worker reproduction is generally unclear (Bourke 1988a; Bourke 1997). As annual
101 species, the absolute sex ratios of bumble bee colonies and populations can be measured
102 within a year, thus avoiding the potential problem of changes from year-to-year. Finally,
103 the molecular tools with which to examine the origin of males exist (Estoup *et al.* 1995;
104 Estoup *et al.* 1993). Here, we investigate which parties control sex ratio and male
105 production in the bumble bee *Bombus hypnorum*.

106 **Methods**

107

108 Colonies of *B. hypnorum* were raised under laboratory conditions (red light, 30°C, 60% r.
109 h., *ad libitum* pollen and sugar water) from queens caught in May 1999 in Uppsala,
110 Sweden, and Åland, Finland. We placed queens in rearing boxes (acrylic glass, 12.5 x 7.5
111 x 5.5 cm) and checked them every two days for brood-rearing behaviour. After the first
112 workers hatched we transferred colonies to observation hives (Pomeroy & Plowright
113 1980) that were attached to a feeding box. We recorded, for each colony, the date of the
114 first eggs, the eclosion date for all workers, males and new queens, and the death date for
115 the queen and colony. In cases where queen death date could not be unambiguously
116 recorded, e.g., when the queen was no longer seen in a colony but her dead body was not
117 found, death date was estimated in the following way. First, we determined the
118 emergence date of the last definitively queen-produced bee (in all cases this bee was a
119 young queen). Queen death date was calculated as this date minus 23 days (the average
120 development time from egg to emergence for *B. hypnorum* queens (Röseler & Röseler
121 1974)). This is a conservative estimate, as the queen may still have been alive and
122 interacting with her workers after this date, but just not producing successful eggs.
123 Colony death date was either when all brood had hatched, or approximately 25 days after
124 the observed queen death, at which point most, if not all queen-laid eggs would have
125 hatched out (Röseler & Röseler 1974). This period post-queen death is also similar to the
126 average length of time bumble bee colonies live for in the field after queen death (Brown
127 unpublished data). All workers, males and queens were marked individually with
128 Opalith® tags to facilitate later analyses. To determine the biomass of males and queens,

129 we dried 20 individuals of each sex from each colony (where colonies did not produce 20
130 males or queens, we weighed as many as possible of each sex) at 60°C for four days, and
131 then weighed them individually to the nearest 100th of a milligram.

132

133 To determine the mating frequency of foundress queens and the degree of male-
134 production by workers, we used 6 highly variable microsatellite markers (Table 1) to
135 genotype the mother queen and 8-10 workers per colony. For each colony, one
136 informative locus (i.e., a locus with a paternal allele that was different and not found in
137 the mother queen) was used to genotype all the males. Note that only adult animals were
138 genotyped in this study – we did not assess the genotypes of eggs, larvae or pupae. On
139 average, half of the males produced by workers will carry a maternal allele, and thus the
140 number of worker-produced males was estimated as twice the number of worker-derived
141 males (as confirmed by the presence or absence of the paternal allele); binomial variances
142 and associated 95% confidence limits for these estimates were calculated using the
143 binomial distribution.

144

145 Molecular methods were as follows. All material was kept at -80°C. Total DNA was
146 extracted from one leg, which was put into a 1.5 ml Eppendorf tube, immersed in liquid
147 nitrogen and pulverized with fitting grinders. 500 - 750 µl of 10 % Chelex® (Bio-Rad
148 Laboratories) was added. This suspension was incubated at 95°C for 15 minutes,
149 vortexed and spun down for two minutes at 13,000 rpm. PCR-amplification was carried
150 out in a volume of 10 µl containing 2 µl of DNA template, 0.5 µl of forward and reverse
151 primers, 1.2 mM of MgCl₂, 0.1 mM of dATP, dCTP, dGTP and dTTP each, and 0.425

152 units of Taq Polymerase. Buffer and distilled water were added to make up the volume.
153 0.8 μl of $\alpha\text{-P}^{33}\text{-dATP}$ was added for radioactive PCR's (used for the determination of
154 queen mating frequency). Non-radioactive PCR's were used for the determination of male
155 parentage. Cycling conditions varied only in the annealing temperature and were as
156 follows: an initial denaturing step of 3 minutes at 94°C followed by 35 cycles of 30
157 seconds at 92°C , 30 seconds at the optimal annealing temperatures (48°C - 58°C ; see
158 Table 1) and elongation at 72°C for 30 seconds. The elongation step of the last cycle was
159 extended to 10 minutes. Radioactive PCR products were electrophoresed on 6%
160 denaturing sequencing gels. A DNA-size marker (SequaMark®, Research Genetics Inc.)
161 was run along with our samples to determine the length of the alleles. Non-radioactive
162 products were separated on Spreadex® gels (Elchrom Scientific, Switzerland) and
163 electrophoresed at a constant temperature of 55°C . DNA size-markers (M3 Marker,
164 Elchrom Scientific, Switzerland; 10 bp DNA Ladder, Gibco BRL®) were used to
165 determine the length of the DNA fragments. Bands were made visible by staining the gels
166 with SYBR® Gold (Molecular Probes, Eugene, Oregon USA).

167

168 All summary data are shown as mean \pm standard deviation, unless otherwise noted. Sex
169 ratios are based on biomass, rather than numbers (Bourke 1997), and are presented as the
170 proportion of investment into female biomass (i.e., $F/(F + M)$); with 95% confidence
171 limits calculated as shown in Box 5.1, pp. 160-161 of Bourke & Franks (1995). The
172 expected sex ratios under queen and worker control were calculated according to Bourke
173 (1997), Table 3. All statistics were done using SPSS 10 for the Macintosh.

174 **Results**

175

176 Given that the queens and colonies described below showed no significant differences in
177 any of their genetic or life-history characteristics, and that the generally large spatial scale
178 of bumble bee population structure (e.g., *B. terrestris* (Estoup *et al.* 1996); *B. lucorum*
179 (Mikkola 1984); *B. pascuorum* (Widmer & Schmid-Hempel 1999)) we treat all the
180 colonies in this study as members of a single population.

181

182 Of 32 foundress queens, 15 laid eggs, 11 hatched out workers, but only 10 made a
183 complete colony (as judged by the production of >1 worker and sexuals). Colony size
184 (i.e., the number of workers produced) ranged from 3 to 86 (23.5 ± 24.87 ; Table 2). All
185 10 colonies produced males (from 4 – 282, 162.4 ± 88.79 ; Table 2), but only 8 colonies
186 produced new queens (0 – 224, 58.1 ± 77.03 ; Table 2). There were significant and
187 positive correlations between colony size and number of males produced ($N = 10$, $r =$
188 0.6748 , $P = 0.032$), number of queens produced ($N = 9$, $r = 0.9390$, $P < 0.001$), and total
189 number of sexuals produced (males + queens) ($N = 10$, $r = 0.9058$, $P < 0.001$).

190

191 Microsatellite analyses showed that the queens that produced colonies were all most
192 likely singly-mated. Heterozygosity values for the 6 microsatellites used ranged from
193 0.27 to 0.8 (number of alleles = 6.3 ± 1.97 , range = 3-8; heterozygosity = 0.65 ± 0.158).
194 Using Boomsma & Ratnieks (1996) to analyse the resolving power of these markers, we
195 calculated a non-detection error of 0.0012, suggesting that a second father is not detected
196 only 0.12% of the time.

197

198 We successfully genotyped 1,304 adult males (80% of all males produced). Using
199 informative microsatellite loci (which varied among colonies), we detected worker-
200 produced males in nine of the 10 colonies (Table 2). The expected proportion of worker-
201 produced males in these nine colonies ranged from 6.5 (95% C.I. of 3.8 – 13.9) to 100 %
202 (17.1 - 100). At the population level, 19.6 % (19.6 – 19.7) of males were worker-
203 produced.

204

205 In all of the colonies where workers reproduced, the eggs that developed into the first
206 worker-produced males must have been laid before the death of the mother queen, and
207 while she herself was still laying successful male eggs (Table 3). The proportion of the
208 period of worker male-production that overlapped queen male-production ranged from 18
209 - 100% (N = 8, $82.0 \pm 30.03\%$, see Table 3). Worker male-production started, on average,
210 27.6 ± 21.09 days after the beginning of male production (regardless of male origin) in a
211 colony (Table 3).

212

213 There were no significant correlations between the proportion of worker-produced males
214 in a colony and colony size, colony life, queen life, or the proportion of the colony cycle
215 for which the queen was alive (Spearman's rank correlation: all N = 10, $r = -0.4073$, -
216 0.2867 , -0.2893 , -0.0976 , respectively, all $P > 0.24$). In addition, the proportion of males
217 produced by workers in a colony was unrelated to the sex ratio of queen-derived
218 reproductives (Spearman's rank correlation: Queen sex ratio, N = 9, $r = 0.2167$, $P =$
219 0.576).

220

221 Colony sex ratios ranged from 0 – 0.7. As colony size increased, the sex ratio became
222 significantly less male biased (Spearman's rank correlations, $N = 10$, $r = 0.939$, $P <$
223 0.001 ; Figure 1). The population sex ratio was 0.52 (0.369 - 0.679) indicating queen
224 control, since the expected sex ratio under queen control with worker reproduction is 0.5
225 (Bourke 1997). In contrast, the expected sex ratio under worker control is 0.71, with
226 upper and lower confidence limits of 0.693 and 0.721 (based on the confidence limits for
227 the expected number of worker-produced males, see Methods). Both the expected
228 worker-control sex ratio and its upper confidence limits are significantly more female-
229 biased than the 95% confidence limits for the measured sex ratio (Figure 1).

230

231 There was no trade-off between the quality (body mass) and quantity (number) of sexuals
232 produced by a colony. There was no significant correlation between number of males
233 produced and male size (Spearman's rank correlation: $N = 10$, $r = 0.2485$, $P = 0.489$). In
234 contrast, there was a significant and positive correlation between the number of new
235 queens produced by a colony and their size (Spearman's rank correlation: $N = 10$, $r =$
236 0.8571 , $P = 0.007$). When controlling for colony size (the number of queens produced
237 increases with colony size, see above) this significant correlation disappeared (partial
238 correlation coefficient: $r = 0.5227$, $P = 0.229$). Finally, there was a significant positive
239 correlation between colony size and queen mass (Spearman's rank correlation: $N = 8$, $r =$
240 0.802 , $P = 0.017$), but not for male body mass (Spearman's rank correlation: $N = 10$, $r =$
241 0.468 , $P = 0.172$). This means that larger colonies produce both more and heavier queens,
242 but only more males of the same mass.

243 **Discussion**

244

245 Workers in social insect colonies are in conflict with their mother queen over both sex
246 ratio, i.e., how many sexual males and females are produced, and parentage of the males
247 produced. From our data it appears that, while workers in *Bombus hypnorum* colonies
248 have at least some control over male parentage, the population sex ratio remains under
249 queen control.

250

251 While worker reproduction is widespread in the social insects (Bourke 1988b), significant
252 levels of worker-produced males from queen-right colonies are only known from a few
253 taxa (Meliponine bees (Tóth *et al.* 2002a; Tóth *et al.* 2002b), *Bombus* spp., reviewed in
254 Bourke (1988b); *Dolichovespula saxonica* (Foster & Ratnieks 2000)). However, to our
255 knowledge, sex ratios have only been assessed in one of these taxa. Bourke (1997)
256 analysed sex ratio and worker reproduction data from Owen & Plowright's (1982) study
257 of *Bombus melanopygus*. Owen & Plowright (1982) found that 20% of males produced in
258 queenright colonies were worker-produced, surprisingly similar to the results we report
259 for *Bombus hypnorum* (in total, including production in queenless colonies, 39% of *B.*
260 *melanopygus* males were worker-produced). However, in stark contrast to our results,
261 (Bourke 1997) showed that the strongly male-biased sex ratio of 0.27 of *B. melanopygus*
262 was not consistent with either queen (expected sex ratio of 0.47) or worker (expected sex
263 ratio of 0.69) control. We thus believe that our study is the first to demonstrate a sex-
264 ration consistent with queen-control despite high levels of worker reproduction.

265

266 Population sex ratios consistent with queen control *per se* are not uncommon in bumble
267 bees (six of 11 cases reviewed by Bourke (1997), despite their more publicised highly
268 male-biased sex ratios (Beekman & Van Stratum 1998; Bourke 1997). In contrast to
269 earlier suggestions that sex ratios in bumble bees are strongly affected by laboratory
270 conditions (Müller *et al.* 1992), Bourke (1997) found no consistent effect of laboratory
271 vs. field conditions on population sex ratios. In addition, our sex ratio results (both
272 population sex ratio and increasing female bias with colony size) are consistent with
273 those from a population of captive but free-foraging *B. hypnorum* (Paxton *et al.* 2001).
274 Thus, we have no reason to believe that our sex ratio results are an artefact of laboratory
275 rearing conditions.

276

277 The ability of worker bumble bees to reproduce is well-known (Bourke 1988b), but with
278 the general absence of genetic studies, actual levels of worker reproduction in queenright
279 colonies remain controversial. In *B. terrestris*, perhaps the most well-studied bumble bee
280 species, results are conflicting. Duchateau & Velthuis (1988) found no evidence for
281 worker reproduction in their laboratory populations, while van Honk *et al.* (1981) stated
282 that workers produced up to 82% of adult males in their laboratory populations. Thus,
283 considerable variation appears to exist among populations in this species for worker
284 reproduction. In another species, *B. melanopygus*, Owen & Plowright (1982) used a
285 phenotypic marker and found high levels of worker reproduction in a laboratory
286 population (see above). Finally, using microsatellites, Paxton *et al.* (2001) found no
287 evidence for worker reproduction in free-flying (but captive) colonies of *B. hypnorum* in
288 Scandinavia.

289

290 In general, theory predicts successful worker reproduction in bumble bees (Ratnieks
291 1988; but see Ratnieks & Reeve (1992)), and our results are in line with this general
292 expectation. In our colonies, we observed no queen-worker aggression, as was also found
293 in a previous behavioural study (Ayasse *et al.* 1995), and worker-produced males hatched
294 in synchrony with queen-produced males. Furthermore, we found no evidence for a
295 positive correlation between the production of queens and worker egg-laying (Product
296 moment correlation, $N = 7$, $r = -0.6462$, $P = 0.117$, Power = 0.7673), as was predicted by
297 Bourke & Ratnieks (2001) for bumble bee species where queen caste is determined solely
298 by larval food intake. Given this congruence of our results with theoretical expectations,
299 and the known variability across populations of bumble bees in levels of worker-
300 reproduction, we have no reason to believe that worker reproduction in our colonies was
301 either abnormal or due to laboratory conditions. Rather, we suspect, from the evidence
302 cited above, that at the colony and population levels worker reproduction is a labile trait
303 that may depend upon variation in resource availability, colony demography and
304 phenology. Further studies of both laboratory and field colonies under various resource
305 regimes are clearly needed to determine accurately when, how and why worker
306 reproduction occurs in queen-right bumble bee colonies.

307

308 Calculating relative inclusive fitness gains and losses for queens and workers in our
309 population may shed light on who is winning the outcome of this combined sex-
310 ratio/male production conflict. As our base case, we take a sex ratio of 0.52 (as found in
311 this study), no worker reproduction and the assumption of no costs to conflict over sex

312 ratios or male production. With worker reproduction and assuming that all worker-
313 produced males were nephews of an individual worker (the most conservative case), the
314 mean inclusive fitness of workers across our population was only 2% higher because of
315 worker reproduction. The least conservative case, calculating fitness for a single worker
316 responsible for producing all 20.2% of the males in a colony, yields a fitness margin of
317 5%. The queen's inclusive fitness would decline by 5% in both cases. In contrast, if
318 workers left male production to the colony queen but instead controlled the sex ratio, as
319 seen in other social insect species (e.g., Sundström (1994)), they would see an increase in
320 fitness of 23%.

321

322 The queens in our population all appeared to be singly mated, although *B. hypnorum* is a
323 facultatively polyandrous species (Estoup *et al.* 1995; Paxton *et al.* 2001; Schmid-
324 Hempel & Schmid-Hempel 2000). Prior studies have suggested both population (Estoup
325 *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000) and temporal (Paxton *et al.* 2001)
326 variation in mating frequencies. Given the current interest in mating frequencies in social
327 insects (reviewed in Strassmann (2001) and Sundström & Boomsma (2001)), a large
328 scale study of mating frequencies in this species might be particularly illuminating.

329

330 In general, our remaining results reinforce the findings of Paxton *et al.* (2001). Colony
331 sizes (in number of workers) were similar in both studies (their study 28 ± 3.5 workers,
332 our study 24 ± 7.9 workers, $t = 0.63$, $df = 22$, $P = 0.535$). Similarly, the production of the
333 most expensive sexual class, young queens, was not significantly different between the
334 two studies (their study 28 ± 13.3 queens, our study 58 ± 24.4 queens, $t = 1.21$, $df = 9.38$,

335 $P = 0.258$). However, colonies in our population produced significantly more males than
336 did those of Paxton *et al.* (2001) (their study 14 ± 4.2 males, our study 150 ± 32.1 males,
337 $t = 4.22$, $df = 9.31$, $P = 0.002$). In addition, we found no evidence that colonies trade-off
338 the number and the quality of sexuals produced. 10 of 10 colonies and eight of 10
339 colonies produced males and gynes, respectively, in the current study, while 12 of 14
340 colonies studied by Paxton *et al.* (2001) produced both sexes. In both studies there was a
341 positive relationship between the number of new queens a colony produced and the
342 number of males produced, indicating the absence of split sex ratios in *B. hypnorum*, in
343 contrast to *B. terrestris* (Beekman & Van Stratum 1998; Duchateau & Velthuis 1988).
344 Sexual productivity increased with colony size in both studies, and in the current study
345 we found that this relationship persisted for both males and new queens when considered
346 independently. Overall, therefore, the productivity characteristics of *B. hypnorum*
347 colonies seem to differ little between laboratory and free-foraging conditions.

348

349 In conclusion, our results show that the outcome of genetic conflicts in social insect
350 colonies can oppose each other – in this case, workers clearly win control over a
351 meaningful proportion of male production, whilst queens control the sex ratio. However,
352 given the low payoff for workers of direct reproduction vs. sex ratio manipulation, these
353 results suggest that queens may only be sacrificing a small amount of fitness, in contrast
354 to their gain from controlling the sex ratio.

355 **Acknowledgements**

356

357 This work was financially supported by grants of the Swiss NSF (# 3100-66733.01) and
358 the EU's IHP - program (HPRN-CT-2000-00052) to PSH. We would like to thank
359 Andrew Bourke for comments on an earlier version of this manuscript. Thanks to Nicole
360 Duvoisin and Roland Loosli for technical assistance.

361 **References**

362

363 Ayasse M, Marlovits T, Tengo J, Taghizadeh T, Francke W (1995) Are there pheromonal
364 dominance signals in the bumblebee *Bombus hypnorum* L (Hymenoptera,
365 Apidae)? *Apidologie* **26**, 163-180.

366 Beekman M, Van Stratum P (1998) Bumblebee sex ratios: Why do bumblebees produce
367 so many males? *Proceedings of the Royal Society of London Series B* **265**, 1535-
368 1543.

369 Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. *Philosophical*
370 *Transactions of the Royal Society of London B Biological Sciences* **351**, 947-975.

371 Bourke AFG (1988a) Dominance orders, worker reproduction, and queen-worker conflict
372 in the slave-making ant *Harpagoxenus sublaevis*. *Behavioural Ecology and*
373 *Sociobiology* **23**, 323-333.

374 Bourke AFG (1988b) Worker reproduction in the higher eusocial hymenoptera.
375 *Quarterly Review of Biology* **63**, 291-311.

376 Bourke AFG (1997) Sex ratios in bumble bees. *Philosophical Transactions of the Royal*
377 *Society of London B* **352**, 1921-1933.

378 Bourke AFG, Franks NR (1995) *Social Evolution in Ants* Princeton University Press,
379 Princeton.

380 Bourke AFG, Ratnieks FLW (1999) Kin conflict over caste determination in social
381 Hymenoptera. *Behavioral Ecology and Sociobiology* **46**, 287-297.

382 Bourke AFG, Ratnieks FLW (2001) Kin-selected conflict in the bumble-bee *Bombus*
383 *terrestris* (Hymenoptera: Apidae). *Proceedings of the Royal Society of London, B*
384 **268**, 347-355.

385 Duchateau MJ, Velthuis HHW (1988) Development and reproductive strategies in
386 *Bombus terrestris* colonies. *Behaviour* **107**, 186-207.

387 Estoup A, Scholl A, Pouvreau A, Solignac M (1995) Monoandry and polyandry in
388 bumble bees (Hymenoptera: Bombinae) as evidenced by highly variable
389 microsatellites. *Molecular Ecology* **4**, 89-93.

390 Estoup A, Solignac M, Cornuet JM, Goudet J, Scholl A (1996) Genetic differentiation of
391 continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in
392 Europe. *Molecular Ecology* **5**, 19-31.

393 Estoup A, Solignac M, Harry M, Cornuet J-M (1993) Characterization of (GT)_n and
394 (CT)_n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*.
395 *Nucleic Acids Research* **21**, 1427-1431.

396 Foster KR, Ratnieks FLW (2000) Facultative worker policing in a wasp. *Nature* **407**,
397 692-693.

398 Foster KR, Ratnieks FLW (2001) Convergent evolution of worker policing by egg eating
399 in the honeybee and common wasp. *Proceedings of the Royal Society of London*,
400 *B* **268**, 169-174.

401 Foster KR, Ratnieks FLW, Raybould AF (2000) Do hornets have zombie workers?
402 *Molecular Ecology* **9**, 735-742.

403 Mikkola K (1984) Migration of wasp and bumble bee queens across the Gulf of Finland
404 (Hymenoptera: Vespidae and Apidae). *Notulae Entomologica* **64**, 125-128.

405 Müller CB, Shykoff JA, Sutcliffe GH (1992) Life history patterns and opportunities for
406 queen-worker conflict in bumblebees (Hymenoptera: Apidae). *Oikos* **65**, 242-248.

407 Owen RE, Plowright RC (1982) Worker-queen conflict and male parentage in bumble
408 bees. *Behavioral Ecology and Sociobiology* **11**, 91-99.

409 Paxton RJ, Thorén PA, Estoup A, Tengö J (2001) Queen-worker conflict over male
410 production and sex ratio in a facultatively polyandrous bumble bee, *Bombus*
411 *hypnorum*: the consequences of nest usurpation. *Molecular Ecology* **10**, 2489-
412 2498.

413 Pomeroy N, Plowright RC (1980) Maintenance of bumble bee colonies in observation
414 hives (Hymenoptera: Apidae). *Canadian Entomologist* **111**, 289-294.

415 Queller DC, Strassmann JE (1998) Kin selection and social insects. *Bioscience* **48**, 165-
416 175.

417 Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial
418 Hymenoptera. *American Naturalist* **132**, 217-236.

419 Ratnieks FLW, Reeve HK (1992) Conflict in single-queen hymenopteran societies - the
420 structure of conflict and processes that reduce conflict in advanced eusocial
421 species. *Journal of Theoretical Biology* **158**, 33-65.

422 Röseler P-F, Röseler I (1974) Morphological and physiological differentiation of the
423 castes in the bumblebee species *Bombus hypnorum* (L.) and *Bombus terrestris*
424 (L.). *Zoologisches Jahrbuch Allgemeine Zoologische Physiologie Tiere:*
425 *Physiologie* **78**, 175-198.

426 Schmid-Hempel R, Schmid-Hempel P (2000) Female mating frequencies in *Bombus* spp.
427 from Central Europe. *Insectes Sociaux* **47**, 36-41.

428 Strassmann J (2001) The rarity of multiple mating by females in the social Hymenoptera.
429 *Insectes Sociaux* **48**, 1-13.

430 Sundström L (1994) Sex ratio bias, relatedness asymmetry and queen mating frequency
431 in ants. *Nature* **367**, 266-268.

432 Sundström L, Boomsma JJ (2001) Conflicts and alliances in insect families. *Heredity* **86**,
433 515-521.

434 Tóth E, Queller DC, Imperatriz-Fonseca VL, Strassmann JE (2002a) Genetic and
435 behavioral conflict over male production between workers and queens in the
436 stingless bee *Paratrigona subnuda*. *Behavioral Ecology and Sociobiology* **53**, 1–
437 8, DOI 10.1007/s00265-00002-00543-00266.

438 Tóth E, Strassmann JE, Nogueira-Neto P, Imperatriz-Fonseca VL, Queller DC (2002b)
439 Male production in stingless bees: variable outcomes of queen–worker conflict.
440 *Molecular Ecology* **11**, 2661–2667.

441 Trivers RL, Hare H (1976) Haplodiploidy and the evolution of the social insects. *Science*
442 **191**, 249-263.

443 van Honk CGJ, Röseler P-F, Velthuis HHW, Hoogeveen JC (1981) Factors influencing
444 the egg laying of workers in a captive *Bombus terrestris* colony. *Behavioral*
445 *Ecology and Sociobiology* **9**, 9-14.

446 Walin L, Sundstrom L, Seppa P, Rosengren R (1998) Worker reproduction in ants: A
447 genetic analysis. *Heredity* **81**, 604-612.

448 Widmer A, Schmid-Hempel P (1999) The population genetic structure of a large
449 temperate pollinator species, *Bombus pascuorum* (Scopoli) (Hymenoptera:
450 Apidae). *Molecular Ecology* **8**, 387-398.

451 **Author Information Box**

452

453 This work was conducted in Paul Schmid-Hempel's Ecology and Evolution group at
454 ETH-Zürich. Paul and Regula Schmid-Hempel are conducting long-term studies on the
455 ecology and evolution of social insects and their parasites. Mark Brown is now at Trinity
456 College Dublin, where his group is investigating host-parasite interactions and
457 conservation ecology and genetics in bumble bees.

458 **Figure legends**

459

460 Figure 1

461

462 Sex ratio (percentage female biomass) becomes less male-biased as colony size increases.

463 Each data point represents one colony. The solid line represents the population sex ratio,

464 whilst the three dotted lines represent the sex ratio (and confidence limits) expected under

465 worker control (see text for calculation).

466 Table 1. List of microsatellite loci used in the analysis of worker reproduction and female
 467 mating frequency. The third column shows the annealing temperature used for PCR. For
 468 further details, see Estoup *et al.* (1995) & Estoup *et al.* (1996).
 469

Loci	Primer Sequence	T ^m (°C)
B10	5'-GTGTAAC TTTCTCTCGACAG-3'	52
	5'-GGGAGATGGATATAGATGAG-3'	
B11	5'-GCAACGAAACTCGAAATCG-3'	52
	5'-GTTTCATCCAAGTTTCATCCG-3'	
B96	5'-GGGAGAGAAAGACCAAG-3'	48
	5'-GATCGTAATGACTCGATATG-3'	
B121	5'-GAACATGTGGAACGACGG-3'	48
	5'-GAACAATCGATATGTCACCG-3'	
B131	5'-GATCGCCTATCTTCTCGG-3'	54
	5'-GAGGCGCTGTCGAGCTC-3'	
B132	5'-GAAATTCGTGCGGAGGG-3'	58
	5'-CAGAGAACTACCTAGTGCTACGC-3'	

470

471

Table 2. Productivity data for the 10 *B. hypnorum* colonies (ordered by increasing colony size). Columns give the numbers of each caste, the biomass sex ratio (proportion females) of each colony, the mean biomass of the two sexual castes, the percentage of males produced by workers, and the upper and lower 95% confidence limits calculated using the binomial distribution, see text).

Colony	Workers	Males	Queens	Sex ratio	Male mass (mg)	Queen mass (mg)	% of males produced by workers*	Lower 95% CL	Upper 95% CL
20	3	4	0	0.00	66.75	-	100.0	17.1	100
7	6	125	0	0.00	49.64	-	14.4	10.1	21.1
15	12	173	8	0.11	53.73	148.30	33.8	28.4	39.6
24	13	108	7	0.10	57.60	97.90	6.5	3.8	13.9
28	14	184	69	0.44	68.29	143.21	39.2	34.3	45.7
26	15	104	17	0.25	62.97	131.57	20.0	15.5	29.1
11	20	115	23	0.35	63.09	171.46	0.0	0.0	14.5
12	20	257	71	0.46	62.33	192.68	12.5	9.8	16.5
25	46	282	162	0.64	66.36	208.28	13.7	11.8	18.2
37	86	272	224	0.71	70.67	205.90	25.0	21.7	30.2

*As the percentage was calculated only from those males that were successfully genotyped (80% of all males), the percentage values do not necessarily yield integer values when multiplied by the total male production of a given colony.

Table 3. Temporal patterns in colony development. Day 0 is the day on which the queen was placed under rearing conditions. Production periods are the days between which members of each caste hatched out from their pupal cases. For calculation of uncertain queen death dates (marked with '?'), see text.

Colony	1st egg	Period of worker production	Period of male production	Period of worker-male production	Period of queen production	Queen died	Colony died
7	4	22 - 42	53 - 98	53 - 85	-	73	98
11	4	19 - 61	50 - 117	-	34 - 117	94?	117
12	4	19 - 47	49 - 105	86 - 105	45 - 105	82?	105
15	4	25 - 72	44 - 105	88 - 117	57 - 88	72	117
20	4	25 - 80	-	77 - 80	-	98	98
24	4	19 - 50	34 - 98	98	37 - 41	98	98
25	4	25 - 98	47 - 95	56 - 105	61 - 105	82?	105
26	4	19 - 44	48 - 105	83 - 105	36 - 75	93	105
28	17	26 - 102	52 - 74	68 - 102	46 - 102	79?	102
37	17	21 - 90	57 - 90	73 - 90	42 - 90	82	90

