J Comp Physiol A (2006) 192: 761–768
DOI 10.1007/s00359-006-0112-y

ORIGINAL PAPER

M. O. Schäfer · V. Dietemann · C. W. W. Pirk
P. Neumann · R. M. Crewe · H. R. Hepburn
J. Tautz · K. Crailsheim

Individual versus social pathway to honeybee worker reproduction (*Apis mellifera*): pollen or jelly as protein source for oogenesis?

Received: 16 October 2005 / Revised: 31 January 2006 / Accepted: 5 February 2006 / Published online: 1 March 2006

Abstract Honeybee workers, *Apis mellifera*, can reproduce in queenless colonies. The production of queen-like pheromones may be associated with their reproductive activity and induce nestmates to respond by feeding them. Such frequent trophallaxis could supply their protein needs for oogenesis, constituting a social pathway to worker reproduction. However, some individuals can develop ovaries without producing queen pheromones. The consumption of protein-rich pollen could be an alternative solitary pathway for them to satisfy this dietary requirement. In order to investigate the way in which workers obtain proteins for oogenesis, we created orphaned worker groups and determined ovarian and pheromonal development in relation to pollen consumption of selected workers. Individuals that did not consume pollen had significantly more developed ovaries and produced significantly more queen mandibular pheromone than workers that fed directly on pollen. Our results suggest that workers producing queen-like secretions are fed trophallactically. However, reproductive workers that lacked queen pheromones had consumed little or no pollen, suggesting that they also obtained trophallaxis. Although pollen consumption might contribute to sustaining oogenesis, it does not appear to be sufficient. Trophallaxis as a means of obtaining proteins seems to be necessary to attain reproductive status in queenless honeybee colonies.

Keywords Honeybees · Worker reproduction · Pheromonal dominance · Trophallaxis · Pollen consumption

M. O. Schäfer (✉)
Chemisches und Veterinäruntersuchungsamt Freiburg,
Am Moosweiher 2, 79108, Freiburg, Germany
E-mail: mos.mellifera@gmx.de

V. Dietemann · C. W. W. Pirk · R. M. Crewe
Department of Zoology and Entomology,
University of Pretoria, 0002, Pretoria, South Africa

P. Neumann
Institut für Zoologie, Martin-Luther-Universität Halle-Wittenberg,
Hoher Weg 4, 06099, Halle (Saale), Germany

H. R. Hepburn · P. Neumann
Department of Zoology and Entomology, Rhodes University,
6140, Grahamstown, South Africa

J. Tautz
Bee-group, Biozentrum der Universität Würzburg,
Am Hubland, 97074, Würzburg, Germany

K. Crailsheim
Institut für Zoologie, Universität Graz,
Universitätsplatz 2, 8010, Graz, Austria

Introduction

One of the major characteristics of social Hymenoptera is the division of reproductive labour between two female castes: the queens and the workers. Generally, one or only a few queens lay eggs while workers supply food and maintain the nest. However, in most species the workers are capable of reproducing when the queen is removed (Bourke 1988; Choe 1988; Hoover et al. 2003). Independent of caste, individuals that reproduce require proteins to sustain oogenesis (Wheeler 1996). In the honeybee, *Apis mellifera*, pollen is the main source of protein (Grogan and Hunt 1979) and workers metabolise it to produce royal jelly (Crailsheim et al. 1992). This jelly is fed to the queen by trophallaxis (the exchange of liquid food or glandular secretions among nestmates, Wilson 1971) and this fulfils all of her nutritional requirements, including proteins for oogenesis (Allen 1955, 1960; Haydak 1970; Rutz and Lüscher 1974; Crailsheim 1991; Free et al. 1992).

Queen honeybees produce specific pheromones to attract a retinue of workers that care for their needs. The main source of these pheromones is the mandibular gland and the main compound produced by queens is (E)-9-keto-2-decenoic acid (9-ODA, Barbier and Lederer 1960; Slessor et al. 1988). The major compound produced in workers' mandibular glands is 10-hydroxy-(E)2-decenoic acid (10-HDA, Callow et al. 1959; Pankiw

et al. 1996), and is also a component of royal jelly (Boch et al. 1979). However, workers can switch to the production of 9-ODA as they start reproducing (Crewe and Velthuis 1980; Pankiw et al. 1996). These workers are then treated like queens and function like them: they attract a retinue of attendants (Sakagami 1958; Velthuis 1970), inhibit ovarian development (Velthuis et al. 1990) and prevent changes in the mandibular bouquet of their nestmates (Hemmling et al. 1979).

Workers of the Cape honeybee, *A. m. capensis*, are particularly prone to reproduce. They have a short latency to produce queen-like pheromones (Crewe and Velthuis 1980) and to start reproducing after queen loss (3 days, Anderson 1963; 6.5 days, Ruttner and Hesse 1981). Another distinct characteristic of *A. m. capensis* workers is the production of diploid offspring by thelythokous parthenogenesis (Onions 1909; Moritz and Haberl 1994; Baudry et al. 2004). This mode of reproduction generates a high level of reproductive competition among *A. m. capensis* workers (Greeff 1996; Moritz et al. 1996), promoting a clear differentiation between reproductively dominant and subordinate workers. In dominant workers, ovarian development correlates with the production of 9-ODA (Hepburn 1992; Simon et al. 2005) and Moritz and Hillesheim (1985) showed that the workers producing these queen-like pheromones received trophallaxis more frequently. In orphaned colonies, workers that do not produce queen-like pheromones may nevertheless have developed ovaries (Crewe and Velthuis 1980; Hepburn 1994). These laying workers represent a particularly interesting category as they manage to produce eggs despite their reduced probability of gaining proteins via trophallaxis. These individuals could possibly obtain the proteins necessary for ovarian development from pollen consumption (Bitondi and Simões 1996; Cremonez et al. 1998). Thus, honeybee workers have two possible means of obtaining proteins to sustain oogenesis: they can solicit and obtain jelly (i.e. use a social pathway to obtain proteins), which is more likely if they produce queen-like pheromones, or they can feed on pollen stores directly (i.e. use a solitary pathway).

We exploited the proneness of *A. m. capensis* workers to reproduce in order to determine the way in which they obtained proteins for oogenesis. The basis of this study is not dependant on the ploidy of the oocytes produced. The differentiation of trophallactically dominant and subordinate *A. m. capensis* workers (Greeff 1996; Hillesheim et al. 1989) was enhanced by rearing them in mixed groups with *A. m. scutellata* workers. Indeed, *A. m. capensis* workers easily dominate *A. m. scutellata* workers pheromonally, trophallactically and reproductively in terms of ovary development (Crewe and Velthuis 1980; Velthuis et al. 1990; Hepburn and Allsopp 1994; Neumann and Hepburn 2002; Wossler 2002) and the latter can therefore be used as food providers. We hypothesise that the production of queen-like mandibular pheromones enables individuals to obtain high quality food to sustain oogenesis. Therefore, we expect those *A. m. capensis* workers producing queen-like secretions to obtain proteins for oogenesis primarily via the social pathway. In contrast, those workers without queen-like pheromones are expected to have obtained proteins mainly via the solitary pathway, by feeding on pollen directly.

Materials and methods

Sampling and experimental design

Four *A. m. capensis* colonies from Heidelberg (Western Cape Province, South Africa) and four queenright colonies of *A. m. scutellata*, obtained from Pretoria (Gauteng Province, South Africa) were used for the experiments. All the colonies were queenright, unrelated and equally strong with approximately 20,000 individuals. The colonies represent authentic samples of the natural wild populations because honeybee breeding is not practised in these regions (Hepburn et al. 2004).

Frames with capped worker brood were taken from each colony and placed in an incubator until adult emergence. Groups ($n = 48$) of workers were confined within hoarding cages for the duration of the experiment. The hoarding cages were supplied with water (25 ml), a mixture of honey and icing sugar (1:4; 45 g), a piece of empty comb (6×7 cm) and pollen (1.5 g). The cages (15×11×8 cm) were kept in a dark room at 31°C with a relative humidity of 55%. Each group consisted of 50 *A. m. scutellata* and 10 freshly emerged *A. m. capensis* workers (< 20 h old). A 5:1 ratio was chosen to ensure the presence of sufficient *A. m. scutellata* workers to feed the *A. m. capensis* workers. To avoid comparing individuals belonging to different subspecies, we focussed on the *A. m. capensis* workers for our analyses. The *A. m. capensis* workers were paint-marked on the thorax so that they could be easily distinguished from *A. m. scutellata* workers. After 9 days, the experiment was terminated and the hoarding cages refrigerated to calm down the workers. All living *A. m. capensis* workers were decapitated and their heads placed in 200 µl dichloromethane in order to extract mandibular gland secretions. Extractions lasted for at least 24 h after which gas-chromatographic (GC) analyses were performed (Simon et al. 2001). These workers' abdomens were stored at −20°C until they were dissected to determine their degree of ovarian development and to record their pollen consumption.

Ovarian development and pollen consumption

The abdomens of *A. m. capensis* workers were dissected and ovarian development was categorised according to Velthuis (1970): undeveloped, without vitellus or visible oocytes; intermediate, corresponding to the early stages in which the developing oocytes are bean-shaped; and developed, with chorionated oocytes (Fig. 1a–c).

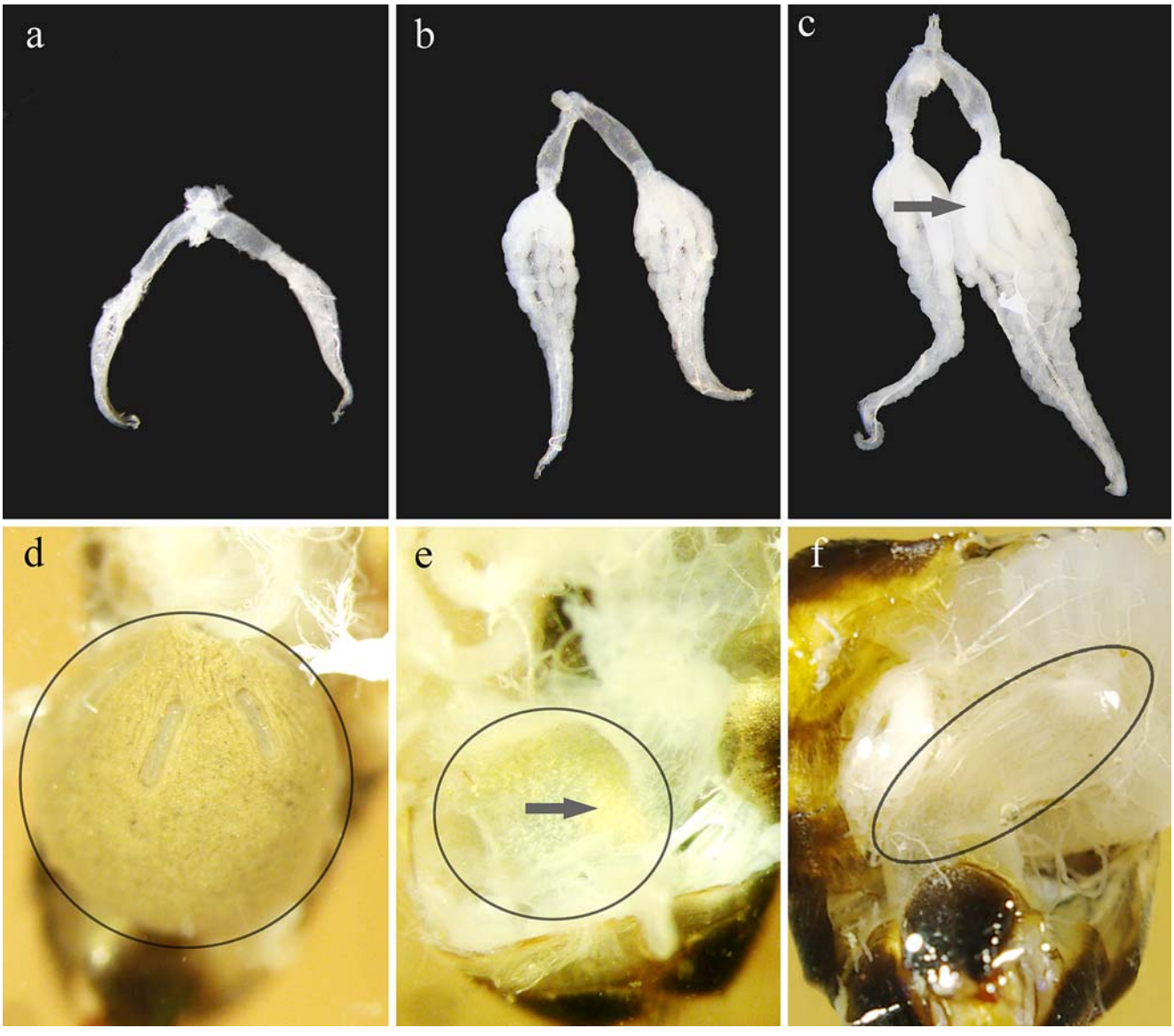

Fig. 1 Dissected abdomens of the tested *A. m. capensis* workers showing different classes of ovarian development (**a** undeveloped; **b** intermediate; **c** developed with chorionated oocytes marked with an *arrow*) and categories of pollen in the rectum (rectums are *encircled*; **d** large amount of pollen; **e** small amount of pollen (marked with an *arrow*); **f** no pollen

Digestion of the food ingested by honeybees takes place in the midgut (Happ 1984; Moritz and Crailsheim 1987). Undigested pollen grain walls accumulate in the rectum because workers do not defecate before their first orientation flight (Buttel-Reepen 1900; Sakagami 1953; Vollbehr 1975). We therefore assessed pollen consumption from the quantity of pollen found in the rectum of the workers. These quantities were classified in three categories: no pollen, small amount of pollen and large amount of pollen (Fig. 1d–f). Workers were considered as having consumed large amounts of pollen when their rectum was fully distended by pollen (Fig. 1d). Jelly, which consists primarily of nutrients in liquid form, is likely to be metabolised completely, leaving no visible signs of its digestion in the gut. Thus, we assumed that workers with developed ovaries had been fed via trophallaxis if they had no pollen in the rectum. Trophallaxis itself was not monitored, as there is no way of determining whether carbohydrates or proteins are exchanged between workers when it occurs. For this reason, observations of trophallaxis events would not necessarily indicate an exchange of proteins for oogenesis.

GC analyses

For each extract, the dichloromethane was evaporated to dryness under a stream of nitrogen and the residue was re-dissolved in 20 µl internal standard (0.38 mg octanoic acid and tetradecane in 0.25 ml dichloromethane) and 20 µl bis-trimethylsilyl-trifluoroacet-amid

was added (Simon et al. 2001). One microlitre of this solution was injected into a gas chromatograph (Hewlett Packard 5890) using the analytical conditions described in Simon et al. (2001). To assess how 'queen-like' a mandibular gland bouquet was, we calculated the ratios of amounts of 9-ODA/(9-ODA + 10-HDA) (Moritz et al. 2000, 2004). If the ratio is close to one, this indicates a queen-like blend, whereas a ratio close to zero indicates a worker-like blend. Based on published data (Crewe and Velthuis 1980; Crewe 1988; Velthuis et al. 1990), we established the range of 9-ODA/(9-ODA + 10-HDA) ratios in queens and workers of *A. m. capensis* and defined three groups of pheromonal status: worker-like from 0 to 0.5, intermediate from 0.5 to 0.9 and queen-like from 0.9 to 1.

Data analyses

Kruskal-Wallis ANOVAs and post hoc tests (multiple comparisons of mean ranks) with the pollen consumption category as the grouping variable were performed to evaluate differences in ovarian development and pheromonal status between the three pollen consumption categories. A Spearman rank correlation was calculated to investigate the relationship between pollen consumption and ovarian development. Log-linear models allow testing for significant interactions between factors. Thus, we used a log-linear model to test the predictions that mandibular pheromones can influence diet and that diet, in turn, can influence ovarian status. We predicted that if both pollen consumption and trophallaxis sustain oogenesis, the interaction between diet and ovarian development should not contribute to the model. We included three factors in this model: pheromonal status, pollen consumption and ovarian development. All tests were performed using Statistica© (Statsoft Inc., Tulsa, OK, USA).

Results

All the categories of orphaned *A. m. capensis* workers described by Hepburn (1994) were found in our experiment. There were 98 normal workers (worker-like pheromones; undeveloped ovaries) representing 35.4 % of all *A. m. capensis* workers. Only one laying worker (worker-like pheromones; developed ovaries) was found (0.4%), while there were 84 (30.3%) false queens (queen-like pheromones; with undeveloped ovaries or intermediate ovarian development) and 24 (8.7%) surrogate queens (queen-like pheromones; developed ovaries). In addition, there were five workers (1.8%) with worker-like pheromonal blends that had intermediate ovarian development. These individuals do not fit the definition of laying workers, but it is likely that they would have eventually become laying workers. The remaining 65 *A. m. capensis* workers (23.4%) were intermediate between these categories. Overall, 39% ($n = 108$ out of 277) of the tested individuals had a queen-like, 23.5% ($n = 65$) had an intermediate and 37.5% ($n = 104$) a worker-like mandibular gland ratio.

The workers that consumed different amounts of pollen showed significant differences in relation to their pheromonal bouquets (Kruskal-Wallis test: H (2, $n = 277$) = 69.99; $P < 0.0001$). Workers with a large amount of pollen in their rectum had a significantly more worker-like bouquet than workers that had no pollen or a small amount of pollen ($P < 0.0001$). Workers that had no pollen in their rectum showed pheromonal bouquets similar to those individuals that consumed a small amount of pollen ($P = 0.89$). Despite significant differences in the pheromonal bouquets between individuals that consumed different amounts of pollen, 50% (54 out of 108) of the individuals that produced queen-like pheromonal bouquets had pollen (small and large amounts) in their rectum. This was also the case for 78.5% (51 out of 65) of the individuals with intermediate pheromonal blends.

Overall, 73.6% of the tested individuals had undeveloped ovaries, 15.2% had intermediate ovarian development and 11.2% had fully developed ovaries. There were significant differences in ovarian development among the tested workers grouped according to pollen consumption (Kruskal-Wallis ANOVA: H (2, $n = 294$) = 89.60; $P < 0.0001$). Workers that consumed no pollen ($n = 87$) had significantly higher ovarian development than workers that consumed only a small amount of pollen ($n = 62$; $P < 0.0001$) or a large amount of pollen ($n = 145$; $P < 0.0001$; Fig. 2). In turn, workers that consumed a small amount of pollen had significantly higher ovarian development than workers that consumed a large amount of pollen ($P < 0.05$; Fig. 2). Pollen consumption and the ovarian development of Cape honeybee workers were significantly negatively correlated (Spearman rank order correlation: $n = 294$; $R = -0.54$; $P < 0.0001$) despite the occurrence of five individuals out of 31 (16.1%) that had developed ovaries and had consumed pollen (small or large amounts). Figure 2 summarises the results about pheromonal status, pollen consumption and ovarian development in the tested workers. With increasing ovarian development individuals with queen-like mandibular blends increased in frequency, whereas those that consumed a large amount of pollen decreased in frequency.

The log-linear analysis showed that the two-way interaction between diet and ovarian status significantly contributes to the model (marginal association $\chi^2 = 83.2$, $DF = 4$, $P < 0.01$). The same is true for the two-way interaction between pheromonal status and pollen consumption (marginal association $\chi^2 = 69.9$, $DF = 4$, $P < 0.01$) and for the interaction between pheromonal and ovarian status (marginal association $\chi^2 = 59.7$, $DF = 4$, $P < 0.01$). All two-ways must be considered in our model (Maximum Likelihood $\chi^2 = 13.51$, $DF = 8$, $P = 0.10$), as the removal of one of the two-way interactions always resulted in a statistically significant deviation from the observations (pollen consumption × ovarian development, partial association $\chi^2 = 46.27$,

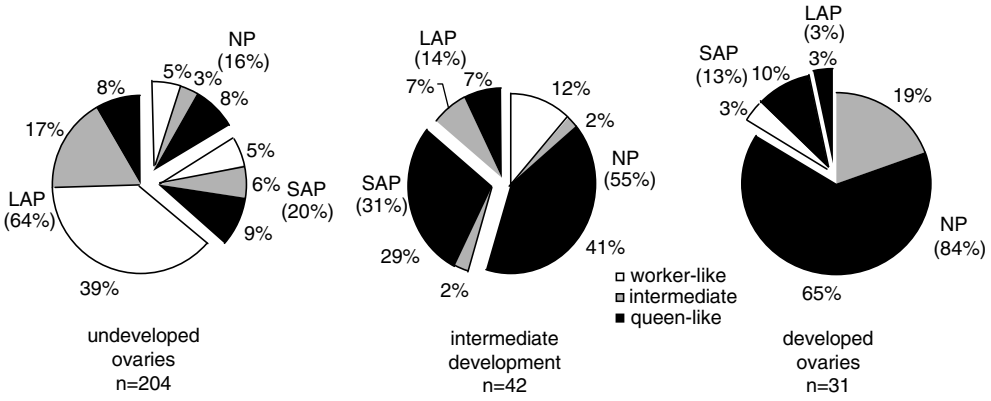

Fig. 2 Pheromonal status and diet of *A. m. capensis* workers with different ovarian development stages. Pollen quantity found in the workers' rectum is indicated clockwise as follows: *NP* no pollen visible; *SAP* small amount of pollen; *LAP* large amount of pollen

$DF = 4$, $P < 0.01$; ovarian development × pheromonal status, partial association $\chi^2 = 22.74$, $DF = 4$, $P < 0.01$; pollen consumption × pheromonal status partial association $\chi^2 = 32.92$, $DF = 4$, $P < 0.01$). This demonstrates that the interaction between the three factors monitored explains the observed data.

Discussion

The results clearly show an inverse relationship between pollen consumption by *A. m. capensis* workers and both their pheromonal bouquet and their ovarian development. Workers that consumed a large amount of pollen had mostly undeveloped ovaries, whereas workers with no pollen in their rectum had significantly more highly developed ovaries. This indicates that pheromonal status could influence diet and that diet could affect ovarian development. Thus, our results support the idea that the positive correlation between ovarian development and queen-like mandibular gland ratio results from trophallactic dominance favoured by a queen-like pheromonal bouquet. Our data show that orphaned workers use the social rather than the solitary pathway to obtain proteins for oogenesis. Indeed, in the absence of any other protein source than jelly obtained by trophallaxis or pollen, the large number of individuals with developed ovaries and rectums without traces of pollen must have obtained their proteins via the social pathway. The presence of pollen in the rectum of workers that produced queen-like pheromones and showed ovarian development could result from their consumption of pollen before they started producing queen-like pheromones. The occasional presence of pollen does not invalidate the link between queen-like pheromone production and diet that favours oogenesis. Although proteins extracted from pollen could contribute to oogenesis, the high frequency of workers with developed ovaries, but without pollen in their rectum indicates that pollen consumption is not required.

Pollen consumption varies with age and function of honeybee workers. Typically, nurse bees are the main pollen consumers and have a higher proteolytic activity than other workers (Moritz and Crailsheim 1987; Crailsheim et al. 1992; Loidl and Crailsheim 2001). They extract proteins from pollen and metabolise it to produce jelly in their hypopharyngeal and mandibular glands (Ribbands 1953; Callow et al. 1959; Boch et al. 1979). This jelly is then fed to larvae as well as adults (both workers and queens). In our experimental groups, workers were of the same age cohort and there was no brood to rear and no need for foraging. It is thus likely that the division of labour in these orphaned worker groups was primarily based on competition for reproduction. We thus assumed that the observed digestive status was linked to reproductive status. We found correlative evidence that subordinate workers feed on pollen to produce the food given to the dominant individuals that start to reproduce, supporting the work of Moritz and Hillesheim (1985). Via trophallaxis, subordinates can increase their indirect fitness by feeding dominant workers in a way that is similar to that of workers in queenright colonies when they feed the queen who can then invest all her energy in egg laying.

What is the physiological advantage for reproductively dominant individuals to get fed and not to feed on pollen directly? Although pollen and royal jelly may have the same protein content (e.g. Howe et al. 1985; Roulston et al. 2000), Lin and Winston (1998) showed that the protein-rich royal jelly promotes ovarian development better than pollen. This is probably because the exine shell of pollen grains makes digestion difficult (Stanley and Linskens 1974) so that the liberation of proteins from these grains is a time-consuming process (Moritz and Crailsheim 1987). The length of time from ingestion until digested pollen grains are found in the rectum can range from a few hours to more than 1 day (Barker and Lehner 1972; Klungness and Peng 1984a; Peng et al. 1986; Crailsheim 1990). In addition, only a certain proportion of the pollen grains are digested (Klungness and Peng 1984b; Crailsheim et al. 1992), whereas jelly is likely to be rapidly and completely digested. Feeding directly on pollen is thus likely to be more costly both in terms of time and energy than being fed pre-processed royal jelly.

We found all the categories of workers described by Hepburn (1994) in our experimental groups of *A. m. capensis*. In the field, there is a majority of laying workers and few false and surrogate queens (Hepburn 1994). In queenless colonies of European subspecies, the number of individuals with queen-like mandibular gland secretion is even smaller (Sakagami 1958). In contrast, we obtained more surrogate (queen-like pheromones; developed ovaries) and false queens (queen-like pheromones; undeveloped ovaries) than laying workers. This could be due to the use of mixed groups in which *A. m. capensis* workers can easily dominate *A. m. scutellata* workers reproductively. The high ratio of subordinate to dominant individuals in our experimental design might have resulted in reduced competition among *A. m. capensis* workers and in a larger proportion of them attaining reproductive status.

Production of queen-like pheromones and reproductive activity covary in *A. m. capensis* (Hepburn 1992). However, some workers that do not produce queen-like pheromones show ovarian development (laying workers) and some workers that produce queen-like secretions do not reproduce (false queens). To explain these cases is of broad interest for our understanding of the pheromonal regulation of reproduction and of reproductive conflicts in social insects. If the production of queen-like pheromones without reproduction in false queens can be due to a delay in ovarian maturation, the cases where workers reproduce without secreting the semiochemicals associated with reproductive activity are more difficult to explain. It is not known what determines the uncoupling of pheromones and reproductive status. Workers that for some reason are not able to produce queen-like pheromones could nevertheless develop their ovaries through the consumption of pollen. However, the laying worker and the few putative laying workers we found in this study consumed little or no pollen, indicating that they were also fed some jelly. Consumption of pollen alone therefore does not seem to sustain oogenesis in queenless colonies of *A. m. capensis* and feeding on jelly via the social pathway seems to be required or preferred. This also indicates that the production of queen pheromones might not be a prerequisite for obtaining jelly in sufficient quantity for ovarian development. An alternative explanation for this unexpected association between worker-like secretions and reproduction is that these individuals behave in a cryptic manner by avoiding the production of queen-like pheromones while reproducing. In a social context where the production of these pheromones by workers can be detrimental because it can provide cues for their recognition and their attacks by nestmates (Visscher and Dukas 1995), their secretion as a means of soliciting trophallaxis might have been selected against.

Despite the unnaturally low proportion of laying workers in our experiment, we believe that our results accurately reflect the physiological mechanisms that determine oogenesis in individual workers. If our conclusion regarding the diet of laying workers is undermined by the low sample size obtained, the effect of pheromone production on the diet of false and surrogate queens is well supported. Our results suggest that orphaned *A. m. capensis* workers that engage in reproduction satisfy their protein needs mainly through pheromone-induced trophallaxis. Manipulation of signal production and diet could establish the causal link between these factors and ovarian development. However, the mode of action of queen pheromones is not understood sufficiently yet and their application on workers in order to determine their effect on diet is likely to trigger attacks on them by nestmates (Pettis et al. 1998). Another possibility could be radioactive labelling of proteins in order to follow their transfer between individuals.

Although *A. m. capensis* appears unique among honeybee subspecies due to its mode of worker reproduction by thelytokous parthenogenesis, our conclusions can be generalised to other subspecies of honeybees. We used *A. m. capensis* for their propensity to reproduce, but the dietary requirements to sustain oogenesis are likely to be the same in other subspecies that simply need a longer time to activate their ovaries. The interactions between nestmates described in this study show the complexity of social regulation of reproductive division of labour that is present in honeybee colonies. These interactions affect the behaviour, pheromonal and ovarian status of workers and they constitute examples of socio-physiological mechanisms (e.g. Robinson 1999). Investigating these processes has deepened our understanding of the behavioural plasticity of colony members and of the complex colonial organisation of social insects.

Acknowledgments Appreciation is addressed to Jürgen Liebig for support in the beginning of this study and to Thomas Mürrle for his technical assistance. We thank two anonymous referees for their constructive comments. Financial support was granted by a DAAD fellowship (MS), an Emmy Noether fellowship of the DFG (PN), the Volkswagen Foundation, the National Research Foundation of South Africa and the University of Pretoria (CP, VD and RC). The experiments comply with the ''Principles of animal care'', publication No. 86–23, revised 1985 of the National Institute of Health, and with the current laws of South Africa where the experiments were performed.

References

Allen MD (1955) Observations of honeybees attending their queen. Brit J Anim Behav 3:66–69

Allen MD (1960) The honeybee queen and her attendants. Anim Behav 8:201–208

Anderson RH (1963) The laying worker in the Cape honeybee *Apis mellifera capensis*. J Apic Res 2:85–92

Barbier M, Lederer E (1960) Structure chimique de la 'substance royale' de la reine d'abeille (*Apis mellifica*). C R Acad Sci (Paris) 250:4467–4469

Barker RJ, Lehner Y (1972) A look at honey bee gut functions. Am Bee J 112:336–338

Baudry E, Kryger P, Allsopp M, Koeniger N, Vautrin D, Mougel F, Cornuet JM, Solignac M (2004) Whole-genome scan in thelytokous-laying workers of the Cape honeybee (*Apis mellifera capensis*): central fusion, reduced recombination rates and centromere mapping using half-tetrad analysis. Genetics 167:243–252

Bitondi MMG, Simões ZLP (1996) The relationship between level of pollen in the diet and juvenile hormone titres in africanized *Apis mellifera* workers. J Apic Res 35:27–36
Boch R, Shearer DA, Shuel RW (1979) Octanoic and other volatile acids in the mandibular gland of the honeybee and in royal jelly. J Apic Res 18:250–253
Bourke AFG (1988) Worker reproduction in the higher eusocial Hymenoptera. Q Rev Biol 63:291–311
Buttel-Reepen H v (1900) Sind die Bienen Reflexmaschinen? Leipzig
Callow RK, Johnston NC, Simpson J (1959) 10-hydroxy-delta 2-decenoic acid in the honeybee (*Apis mellifera*). Experientia 15:421–422
Choe J (1988) Worker reproduction and social evolution in ants (Hymenoptera: Formicidae). In: Trager JC (ed) Advances in myrmecology. Brill EJ, Leiden, pp 163–187
Crailsheim K (1990) The protein balance of the honey bee worker. Apidologie 21:417–429
Crailsheim K (1991) Interadult feeding of jelly in honeybee (*Apis mellifera* L.) colonies. J Comp Physiol B 161:55–60
Crailsheim K, Schneider LHW, Hrassnigg N, Bühlmann G, Brosch U, Gmeinbauer R, Schöffmann B (1992) Pollen consumption and utilization in worker honey bees (*Apis mellifera carnica*): dependence on individual age and function. J Insect Physiol 38:409–419
Cremonez TM, De Jong D, Bitondi MMG (1998) Quantification of hemolymph proteins as a fast method for testing protein diets of honey bees (Hymenoptera: Apidae). J Econ Entomol 91:1284–1289
Crewe RM (1988) Natural history of honey-bee mandibular gland secretions: development of analytical techniques and the emergence of complexity. In: Needham GR, Page RE, Delfinado-Baker M, Bowman CE (eds) Africanized honeybees and bee mites. Ellis Horwood Limited, England, pp 149–158
Crewe RM, Velthuis HHW (1980) False queens: a consequence of mandibular gland signals in worker honeybees. Naturwissenschaften 67:467–469
Free JB, Ferguson AW, Simpkins JR (1992) The behaviour of queen honeybees and their attendants. Physiol Entomol 17:43–55
Greeff JM (1996) Thelytokous versus arrhenotokous worker reproduction in the Cape honeybee and other eusocial Hymenoptera. Hereditas 124:99–103
Grogan DE, Hunt JH (1979) Pollen proteases: their potential role in insect digestion. Insect Biochem 9:309–313
Happ GM (1984) Major life systems. In: Evans HE (eds) Insect biology. Addison-Wesley publishing, Reading, MA, pp 70–92
Haydak MH (1970) Honey bee nutrition. Ann Rev Entomol 15:143–156
Hemmling C, Koeniger N, Ruttner F (1979) Quantitative Bestimmung der 9-Oxodecensäure im Lebenszyklus der Kapbiene (*Apis mellifera capensis* Escholtz). Apidologie 10:227–240
Hepburn HR (1992) Pheromonal and ovarian development covary in Cape Honeybees, *Apis mellifera capensis*. Naturwissenschaften 79:523–524
Hepburn HR (1994) Reproductive cycling and hierarchical competition in Cape honeybees, *Apis mellifera capensis* Esch. Apidologie 25:38–48
Hepburn HR, Allsopp MH (1994) Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*. S Afr J Sci 90:247–249
Hepburn HR, Neumann P, Radloff SE (2004) Genetic variation in natural honeybee populations, *Apis mellifera capensis* Esch. Naturwissenschaften 91:447–450
Hillesheim E, Koeniger N, Moritz RFA (1989) Colony performance in honeybees (*Apis mellifera capensis*) depends on proportion of subordinate and dominant workers. Behav Ecol Sociobiol 24:291–296
Hoover SER, Keeling CI, Winston ML, Slessor KN (2003) The effect of queen pheromones on worker honey bee ovary development. Naturwissenschaften 90:477–480
Howe SR, Dimick PS, Benton AW (1985) Composition of freshly harvested and commercial royal jelly. J Apic Res 24:52–61
Klungness LM, Peng YS (1984a) A histochemical study of pollen digestion in the alimentary canal of honeybees (*Apis mellifera* L). J Insect Physiol 30:511–521
Klungness LM, Peng YS (1984b) Scanning electron microscope observation of pollen food bolus in the alimentary canal of honeybees. Can J Zool 62:1316–1319
Lin H, Winston ML (1998) The role of nutrition and temperature in the ovarian development of the worker honey bee (*Apis mellifera*). Can Entomol 130:883–891
Loidl A, Crailsheim K (2001) Free fatty acids from pollen and triolein in the honeybee (*Apis mellifera carnica* Pollmann) midgut. J Comp Physiol B 171:313–319
Moritz B, Crailsheim K (1987) Physiology of protein digestion in the midgut of the honeybee (*Apis mellifera* L.). J Insect Physiol 33:923–931
Moritz RFA, Hillesheim E (1985) Inheritance of dominance in honeybees (*Apis mellifera capensis*). Behav Ecol Sociobiol 17:87–89
Moritz RFA, Haberl M (1994) Lack of meiotic recombination in thelytokous parthenogenesis of laying workers of *Apis mellifera capensis* (the Cape honeybee). Heredity 73:98–102
Moritz RFA, Kryger P, Allsopp M (1996) Competition for royalty in bees. Nature 384:522
Moritz RFA, Simon UE, Crewe RM (2000) Pheromonal contest between honeybee workers. Naturwissenschaften 87:395–397
Moritz RFA, Lattorff HMG, Crewe RM (2004) Honeybee workers (*Apis mellifera capensis*) compete for producing queen-like pheromone signals. Biol Lett Proc R Soc Lond (Suppl) 271:98–100
Neumann P, Hepburn HR (2002) Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.). Apidologie 33:165–192
Onions GW (1909) Bees: workers and queens from eggs laid by workers. Agric J Cape Good Hope 35:527
Pankiw T, Winston ML, Plettner E, Slessor KN (1996) Mandibular gland pheromone components of European and Africanized honey bee queens (*Apis mellifera* L.). J Chem Ecol 22:605–615
Peng YS, Nasr ME, Marston JM (1986) Release of alfalfa, *Medicago sativa*, pollen cytoplasm in the gut of the honey bee, *Apis mellifera* (Hymenoptera: Apidae). Ann Entomol Soc Am 79:804–807
Pettis JS, Westcott LC, Winston ML (1998) Balling behaviour in the honey bee in response to exogenous queen mandibular gland pheromone. J Apic Res 37:125–131
Ribbands CR (1953) The behaviour and social life of honeybees. Bee Research Association, London
Robinson GE (1999) Integrative animal behaviour and sociogenomics. TREE 14:202–205
Roulston T, Cane JH, Buchmann SL (2000) What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? Ecol Monogr 70:617–643
Ruttner F, Hesse B (1981) Rassenspezifische Unterschiede in Ovarienentwicklung und Eiablage von weisellosen Arbeiterinnen der Honigbiene *Apis mellifera* L. Apidologie 12:159–183
Rutz W, Lüscher M (1974) The occurrence of vitellogenin in workers and queens of *Apis mellifera* and its transmission to the queen. J Insect Physiol 20:897–909
Simon UE, Moritz RFA, Crewe RM (2001) The ontogenetic pattern of mandibular gland components in queenless worker bees (*Apis mellifera capensis* Esch.). J Insect Physiol 47:735–738
Simon U, Moritz RFA, Crewe RM (2005) Reproductive dominance among honeybee workers in experimental groups of *Apis mellifera capensis*. Apidologie 36:413–419
Sakagami SF (1953) Untersuchungen über die Arbeitsteilung in einem Zwergvolk der Honigbiene. Beiträge zur Biologie des Bienenvolkes. Jap J Zool 11:117–185
Sakagami SF (1958) The false queen: fourth adjustive response in dequeened honeybee colonies. Behaviour 13:280–296

Slessor KN, Kaminski LA, Ki GGS, Borden JH, Winston ML (1988) Semiochemical basis of the retinue response to queen honey bees. Nature 332:354–356

Stanley RG, Linskens HF (1974) Pollen: biology, biochemistry, management. Springer, Berlin Heidelberg New York

Velthuis HHW (1970) Ovarian development in *Apis mellifera* worker bees. Entomol Exp Appl 13:377–394

Velthuis HHW, Ruttner F, Crewe RM (1990) Differentiation in reproductive physiology and behaviour during the development of laying worker honeybees. In: Engels W (eds) Social Insects. Springer, Berlin Heidelberg New York, pp 231–243

Visscher PK, Dukas R (1995) Honey bees recognise development of nestmates' ovaries. Anim Behav 49:542–544

Vollbehr J (1975) Zur Orientierung junger Honigbienen bei ihrem 1. Orientierungsflug. Zool Jb Physiol 79:33–69

Wheeler DE (1996) The role of nourishment in oogenesis. Ann Rev Entomol 41:403–431

Wilson EO (1971) The insect societies. Harvard University Press, Cambridge

Wossler TC (2002) Pheromone mimicry by *Apis mellifera capensis* social parasites leads to reproductive anarchy in host *Apis mellifera scutellata* colonies. Apidologie 33:139–163