

Measuring the cost of worker reproduction in honeybees: work tempo in an ‘anarchic’ line

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Abstract – Worker reproduction is extremely rare in queen-right honey bee (*Apis mellifera*) colonies, despite the fact that workers are capable of laying eggs and can potentially increase their direct fitness by doing so. Factors that tip selective forces in favour of functional worker sterility may be related to colony-level costs of worker reproduction. We examined one possible cost by comparing work rates of ‘anarchist’ (a selected line showing high rates of worker reproduction) and wild-type honey bees fostered into anarchic and wild-type host colonies. We observed a lower work rate among anarchist workers compared to wild-type workers. The difference was small but significant and likely contributes to the reduced viability of anarchic colonies. This colony-level cost of anarchistic behaviour counterbalances the increased personal fitness of anarchist bees and partly explains the extreme rarity of anarchic honey bee colonies.

Apis mellifera / anarchy / worker reproduction / policing / worker sterility

1. INTRODUCTION

In honey bee (*Apis mellifera* L.) colonies the queen is usually the sole female reproductive and her thousands of daughters (the workers) are functionally sterile. Should a colony lose its queen workers are able to change their reproductive status. In queenless colonies some workers undergo ovary activation and worker-laid eggs are observed in large numbers (Ruttner and Hesse, 1981; Page and Erickson, 1988). A consequence of the haplodiploid sex determination system of the honey bee is that eggs laid by the unmated workers always develop into males (drones).

Even though workers are physiologically capable of reproducing, worker derived drones are extremely rare in queenright *A. mellifera* colonies. Only one worker in 10 000 has full-sized eggs in her ovaries, showing that very few workers are capable of laying eggs (Ratnieks, 1993; Visscher, 1996). These few workers can

contribute about 7% of the total male eggs in a colony (Visscher, 1996), but most worker-laid drone eggs are eaten by other workers (a phenomenon known as worker policing, Ratnieks and Visscher, 1989). Consequently very few worker-laid eggs develop, so that only about 0.1% of a colony’s males are workers’ sons (Visscher, 1989; Ratnieks 1993; Visscher, 1996).

Evolutionary theory predicts that if a honey bee worker could circumvent the mechanisms that usually prevent worker reproduction she would benefit from vastly increased personal fitness (Hamilton, 1972). Such ‘cheating’ by workers does occur, and in some, very rare, colonies worker reproduction is observed at high frequency (Oldroyd et al., 1994). In 1995 Oldroyd identified a queenright colony of bees in which there was extensive worker reproduction (Montague and Oldroyd, 1998). We have line bred this colony to produce a strain of ‘anarchistic’ (AN) bees in which workers

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Table I. Categories and descriptions of worker behaviour.

Behaviour	Description
Cell cleaning	The focal worker was observed placing her head in a cell or picking at cell edges with her mandibles.
Queen and brood care	The worker attended the queen or placed her head in a cell containing an egg or larvae. Capping of larva cells was also included in this behaviour as were antennating and mandibulating (Visscher and Dukas, 1995) brood cell caps.
Food storage	The worker was observed placing her head in a cell containing nectar or pollen. This category also included trophallaxis.
Nest maintenance	The worker was observed building cells (picking at wax with mandibles). This category also included wood smoothing (worker moves mouthparts back and forwards over wooden parts of the frame) and hive ventilation, via fanning.
Social interactions	The worker was observed in antennal contact with another bee or in an act of grooming with another bee. Grooming was classified as one worker picking at another with her mandibles or rubbing her proboscis on another worker.
Inactive	The worker was motionless or walking but performed none of the above tasks within the interval that it was followed.

reproduce at high frequency even in the presence of the queen (Oldroyd et al., 1999; Oldroyd and Osborne, 1999). Furthermore, the vast majority of the male offspring of these colonies are worker's sons (Montague and Oldroyd, 1998), indicating that workers not only activate their ovaries, but that levels of policing are lower in AN colonies than in wild-type (WT) colonies (Oldroyd and Ratnieks, 2000).

AN colonies are extremely rare as evidenced by the few the authors have seen despite extensive searches. While it is impossible to say what proportion of natural colonies is anarchistic, it is probably between one per several hundred to several thousand (Barron et al., 2001).

If anarchistic behaviour leads to increased personal fitness, why is it so rare? A possibility is that there are counterbalancing fitness costs associated with anarchy. In this paper we explored a potential cost by examining the relative levels of activity in cohorts of WT and AN workers cross fostered into WT and AN colonies. If AN bees worked less than WT bees there would be selection against anarchy at the colony level to counterbalance the increased personal fitness of the anarchists.

2. MATERIALS AND METHODS

One AN colony (ie from the line of bees selected for worker reproduction by Oldroyd & Osborne,

1999) and one WT colony were each established in two-frame observation hives. Each colony contained approximately 2000 bees and a laying queen. The colonies were matched as closely as possible for population, brood area and food resources. These observation hive colonies served as host colonies for our experiments.

Each host colony received 250 WT and 250 AN day-old workers, who were the subjects of the behavioural observations. These focal bees were individually marked using a combination of numbered marking disks (Opalithplättchen, Graz) on the thorax and coloured paints (Posca Poster Pens, Mitsubishi Pencil Co.) on the abdomen. The paint dot on the abdomen allowed identification of marked workers even if their head and thorax was inserted into a cell of the comb. Focal bees were taken from different source colonies than the host colonies. This experiment was duplicated. The first replicate was established on October 15, 2000 and the second on November 10, 2000.

Each colony was observed for two hours daily (replicate 1: October 16-30, 2000, replicate 2 November 11-26, 2000). The order in which the two colonies of each pair were observed was assigned randomly so as to avoid any temporal effects on behaviour. Observations on each colony were conducted by randomly sampling one of 360 2.5-cm² squares marked on the windows of the observation hives. The behaviour of any marked bee observed within the focal square was recorded. Focal bees were observed for one minute and if, during that time, they engaged in any of the tasks defined in Table I they were classified as 'active'. When the same marked bee was observed twice, both

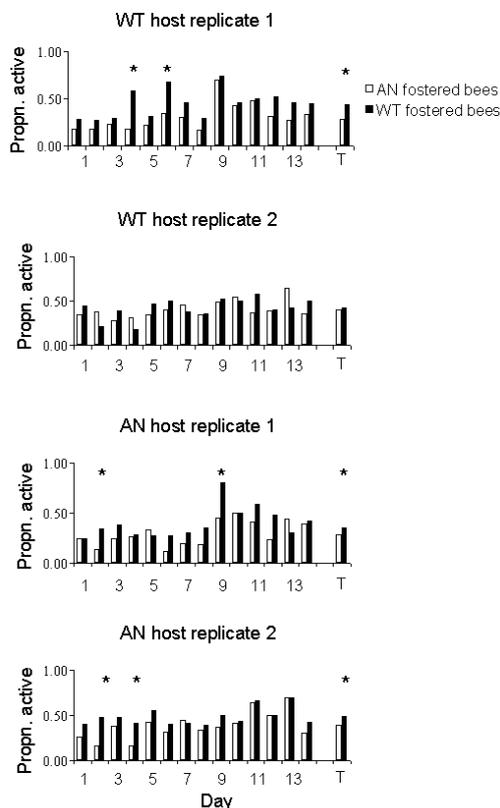


Figure 1. Proportion of AN and WT focal bees actively engaged in work in two AN and two WT host colonies. Asterisks indicate that a significantly higher proportion of WT bees than AN bees were actively engaged in a task on that day or overall (in clusters marked T). Significance was determined by *G*-tests of 2-way contingency tables of genotype (AN and WT) by activity (active and non active). A mean of 28.5 (sd = 10.6) AN and 29.2 (sd = 7.6) WT bees were observed during each day.

observations were used in the analysis, but this was a rare occurrence. Observers were unaware of the genotypes of the marked bees.

The analysis compared the numbers of anarchist and wild-type focal bees that were recorded as active and inactive in each of the two host colonies. We chose not to analyse each task separately because differences between the two genotypes could reflect differences in rates of age polyethism (the relationship between behaviour and age, Lindauer, 1961; Calderone and Page, 1992) rather than overall activity.

3. RESULTS

Our analysis compared the numbers of bees scored as active and inactive in the AN and WT colonies (Fig. 1). Each day was analysed separately as a 2×2 contingency table and the distribution of active and inactive bees in WT and AN colonies was compared with a *G*-test.

As Figure 1 shows we frequently observed more activity among WT bees than AN bees in both the WT and AN host colonies, but this was rarely significant when analysing each day separately. Considering the conservative nature of the *G*-test and that the sample sizes of each of our daily comparisons was quite low (mean sample size < 30 bees per colony) this test would have insufficient statistical power to detect a subtle difference between the two bee genotypes.

To increase the power of our statistical analysis we determined an overall estimate of the significance of the difference in activity between the two genotypes of focal bees by summing the *G* test statistics for each of the 14 days of observation for each colony and obtaining the associated *P* value from tables at 13 degrees of freedom. This is a valid statistical method for pooling data (Robinson et al., 1994) and greatly improved the power of our test. Results from the daily comparisons and overall comparisons are summarised in Table II. These data show a small but detectable decrease in activity in AN workers compared to WT in both AN and WT host colonies.

4. DISCUSSION

Slightly fewer AN workers were observed actively engaged in tasks compared to WT bees in both AN and WT host colonies. The reduced work rate by AN workers is a small but significant component of the colony-level fitness costs of anarchistic behaviour.

Despite recent additions of new genetic material, our anarchistic line is probably more inbred than typical wild-type bees. Inbreeding may be associated with reductions in activity levels in workers, and potentially, the effects we observed could be due to inbreeding effects alone. However, the main effect of inbreeding in honey bees is reduced brood viability at the

Table II. Summary of comparisons of activity in WT and AN workers fostered to WT and AN host colonies. All colonies were observed for 14 days.

Host colony	No. days lower proportion of AN workers active than WT	No. days significantly lower proportion of AN active than WT	Overall comparison of activity. G test, df = 13
WT trial 1	14	2	$P < 0.001$
WT trial 2	9	0	$P > 0.05$
AN trial 1	10	2	$P = 0.02$
AN trial 2	11	2	$P = 0.007$

sex locus (Page and Marks, 1982), and this effect was unimportant in these experiments on a fixed number of adult workers.

Reduced work rates in reproductively active worker honey bees have been observed in other contexts. When a WT honeybee colony is both queenless and broodless, many workers activate their ovaries and begin reproducing. Several of these workers may develop into 'false queens' (Sakagami, 1958). These individuals have queen-like mandibular pheromone secretions (Plettner et al., 1993), attract a retinue and adopt many of the behavioural characteristics of queens (Sakagami, 1958). They perform none of the workers' usual tasks. The AN bees differ from false queens in that AN workers have never been observed with a retinue, and egg-laying AN workers have a normal worker behavioural repertoire (Oldroyd et al., 1999), but are slightly less active than WT workers.

A relationship between worker reproductive status and worker activity is also observed in the cape honey bee, *A. m. capensis* of South Africa. Hillesheim et al. (1989) classified workers of the cape honey bee, into two behavioural morphs. Dominant workers performed very little work, had more developed ovaries and produced a more queen-like pheromonal blend compared to 'subordinate' workers (Hillesheim et al., 1989). When queenless, dominant workers were more likely to activate their ovaries and lay eggs than subordinate workers, but colonies comprised entirely of subordinate workers out-performed colonies comprised entirely of dominant workers for important fitness correlates like brood rearing, comb building and food hoarding. Mixed colonies were intermediate between exclusively dominant and exclusively subordinate colonies (Hillesheim et al., 1989).

In the cape honey bee the trade-off between individual fitness (dominant workers are more likely to reproduce directly via egg laying Moritz and Hillesheim, 1989; Moritz et al., 1996) and colony-level fitness (colonies of subordinate workers are presumably more likely to produce swarms and queen-produced males) is clear. Similarly while AN *A. mellifera* bees are more likely to reproduce than WT bees, AN colonies are far less successful. The difference in work rates between AN and WT workers is likely part of this trade off, but the difference is so slight it is unlikely to be the whole story. Further fitness costs associated with the rearing of large numbers of drones in worker cells (Barron et al., 2001), which imposes a heavy demand on the colonies resources and further reduces the numbers of workers present in these colonies, probably constitute other important colony level fitness costs of anarchistic behaviour.

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Résumé – Mesure du coût de la reproduction des ouvrières chez l'Abeille domestique : le rythme de travail chez une lignée « anarchique ». La reproduction des ouvrières est un fait extrêmement rare dans les colonies d'abeilles domestiques (*Apis mellifera* L.) qui possèdent une reine, bien que les ouvrières soient capables de pondre des œufs. Habituellement les ouvrières s'imposent mutuellement leur stérilité en mangeant les œufs des autres ouvrières, mais il existe de rares colonies « anarchiques » dans lesquelles une large proportion de mâles proviennent d'œufs pondus par les ouvrières. L'accroissement évident de la valeur adaptative (fitness)

individuelle pour les ouvrières d'une part et l'extrême rareté des colonies anarchiques d'autre part laissent penser que le comportement anarchique doit être lié à des inconvénients (coûts) importants. L'un des moyens pour que la reproduction des ouvrières impose un coût de la fitness au niveau de la colonie serait que les ouvrières qui se reproduisent apportent une contribution moindre à la colonie que les ouvrières normales. Nous avons comparé les niveaux d'activité d'ouvrières de type sauvage (WT), ou normales, et d'ouvrières anarchiques (AN) pour mieux comprendre les forces d'évolution qui maintiennent la rareté de la reproduction des ouvrières.

Les ouvrières AN ont été obtenues à partir d'une lignée sélectionnée pour la reproduction des ouvrières. Des ouvrières AN et WT ont été marquées à 1 j et introduites dans des colonies hôtes AN ou WT installées dans des ruches d'observation. Les colonies ont été observées tous les jours et le comportement des abeilles rencontrées dans l'un des carrés de 2,5 cm² a été classé selon les catégories du tableau I. Si au bout d'une minute l'abeille observée n'avait accompli aucune des tâches répertoriées, elle était classée comme inactive. Les ouvrières AN ont été légèrement moins actives que les WT, aussi bien dans les colonies AN que dans les WT (Fig. 1). Nous en concluons que l'intensité plus faible du travail chez les ouvrières AN est un élément petit mais significatif des coûts de la fitness au niveau de la colonie.

***Apis mellifera* / anarchie / reproduction des ouvrières / stérilité des ouvrières / régulation**

Zusammenfassung – Messung der Kosten für die Reproduktion von Arbeiterinnen in der Honigbiene: das Tempo bei Arbeitsabläufen in „anarchistischen“ Linien. Die Reproduktion von Arbeiterinnen ist in weiselrichtigen Bienenvölkern extrem selten, obwohl Arbeiterinnen Eier legen können. Normalerweise zwingen sich die Arbeiterinnen gegenseitig zur funktionellen Sterilität, indem sie die von anderen Arbeiterinnen gelegten Eier auffressen. Aber in selten vorkommenden „anarchistischen“ Völkern stammt ein großer Anteil der adulten Drohen von Arbeiterinnen ab. Diese offensichtliche Steigerung der individuellen Fitness für die Arbeiterinnen, bei gleichzeitigem extrem seltenem Auftreten von „anarchistischen“ Völkern lässt vermuten, dass das anarchistische Verhalten mit erheblichen Nachteilen verbunden sein muss. Ein möglicher der Nachteil bei der Reproduktion von Arbeiterinnen wäre, wenn die sich vermehrenden Arbeiterinnen weniger an den Aufgaben der normalen Arbeiterinnen beteiligen. Wir verglichen den Aktivitätsgrad von normalen und anarchischen Arbeiterinnen, um die Evolutionswirkung besser zu verstehen, die die Seltenheit der Reproduktion von Arbeiterinnen aufrecht erhalten. Anarchistische Arbeiterinnen der Honigbienen wurden von einer Linie genommen, die auf Reproduktion

von Arbeiterinnen gezüchtet worden war. Markierte, einen Tag alte anarchische und Wildtyp - Arbeiterinnen wurden in anarchische und Wildtyp - Wirtsvölker in Beobachtungstöcke gesetzt. Die Völker wurden täglich beobachtet und das Verhalten der betreffenden Bienen klassifiziert, wie in Tabelle I angegeben. Wenn nach einer Minute der Beobachtung die betreffende Biene keine der in Tabelle I beschriebenen Aufgaben durchgeführt hatte, wurde sie als „inaktiv“ eingeordnet. Anarchische Arbeiterinnen waren geringfügig weniger aktiv als normale Bienen, sowohl in anarchischen als auch in normalen Völkern (Abb. 1). Wir schließen daraus, dass eine geringere Arbeitsintensität der anarchischen Arbeiterinnen eine kleine aber bedeutende Komponente der „Fitness Kosten“ auf dem Volksniveau ist.

***Apis mellifera* / Anarchie / Reproduktion von Arbeiterinnen / „Policing“ / Sterilität von Arbeiterinnen**

REFERENCES

- Barron A.B., Oldroyd B.P., Ratnieks F.L.W. (2001) Worker reproduction in honey bees (*Apis*) and the anarchic syndrome, *Behav. Ecol. Sociobiol.* 50, 199–208.
- Calderone N.E., Page R.E. (1992) Effects of interactions among genotypically diverse nestmates on task specialization by foraging honey bees (*Apis mellifera*), *Behav. Ecol. Sociobiol.* 30, 219–226.
- Hamilton W.D. (1972) Altruism and related phenomena in social insects, *Annu. Rev. Ecol. Syst.* 3, 193–232.
- Hillesheim E., Koeniger N., Moritz R.F.A. (1989) Colony performance in honeybees (*Apis mellifera capensis* Esch) depends on the proportion of subordinate and dominant workers, *Behav. Ecol. Sociobiol.* 24, 291–296.
- Lindauer M. (1961) *Communication Among Social Bees*, Harvard University Press, Cambridge Massachusetts.
- Montague C.E., Oldroyd B.P. (1998) The evolution of worker sterility in honey bees: an investigation into a behavioural mutant causing failure of worker policing, *Evolution* 52, 1408–1415.
- Moritz R.F.A., Hillesheim E. (1989) Inheritance of dominance in honeybees (*Apis mellifera capensis* Esch), *Behav. Ecol. Sociobiol.* 17, 87–89.
- Moritz R.F.A., Kryger P., Allsopp M.H. (1996) Competition for royalty in bees, *Nature* 384, 31.
- Oldroyd B.P., Halling L., Rinderer T.E. (1999) Development and behaviour of anarchistic honey bees, *Proc. R. Soc. London B* 266, 1875–1878.
- Oldroyd B.P., Osborne K.E. (1999) The evolution of worker sterility in honeybees: the genetic basis of

- failure of worker policing, *Proc. R. Soc. London B* 266, 1335–1339.
- Oldroyd B.P., Ratnieks F.L.W. (2000) Anarchistic honey bee workers evade worker policing by laying eggs that have low removal rates, *Behav. Ecol. Sociobiol.* 47, 268–273.
- Oldroyd B.P., Smolenski A.J., Cornuet J.-M., Crozler R.H. (1994) Anarchy in the beehive, *Nature* 371, 749.
- Page R.E., Erickson E.H. (1988) Reproduction by worker honey bees (*Apis mellifera* L.), *Behav. Ecol. Sociobiol.* 23, 117–126.
- Page R.E., Marks R.W. (1982) The population genetics of sex determination in honey bees: random mating in closed populations, *Heredity* 48, 263–270.
- Plettner E., Slessor K.N., Winston M.L., Robinson G.E., Page R.E. (1993) Mandibular gland components and ovarian development as measures of caste differentiation in the honey bee (*Apis mellifera* L.), *J. Insect Physiol.* 39, 235–240.
- Ratnieks F.L.W. (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies, *Behav. Ecol. Sociobiol.* 32, 191–198.
- Ratnieks F.L.W., Visscher P.K. (1989) Worker policing in the honeybee, *Nature* 342, 796–797.
- Robinson G.E., Page R.E., Arensen N. (1994) Genotypic differences in brood rearing in honey bee colonies: context-specific? *Behav. Ecol. Sociobiol.* 34, 125–137.
- Ruttner F., Hesse B. (1981) Rassenspezifische Unterschiede in Ovaentwicklung und Eiblage von weisellosen Arbeiterinnen der Honigbiene *Apis mellifera* L., *Apidologie* 12, 159–183.
- Sakagami S.F. (1958) The false queen: fourth adjustive response in dequeen honey bee colonies, *Behaviour* 13, 280–296.
- Visscher P.K. (1989) A quantitative study of worker reproduction in honey bee colonies, *Behav. Ecol. Sociobiol.* 25, 247–254.
- Visscher P.K. (1996) Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing, *Behav. Ecol. Sociobiol.* 39, 237–244.
- Visscher P.K., Dukas R. (1995) Honey bees recognise development of nestmates' ovaries, *Anim. Behav.* 49, 542–544.