

Four QTLs that Influence Worker Sterility in the Honey Bee (*Apis mellifera*)

Peter R Oxley^{*}, Graham J Thompson^{*†}, Benjamin P Oldroyd^{*}

*Behaviour and Genetics of Social Insects Laboratory

School of Biological Sciences A12,

University of Sydney

Sydney, NSW 2006

Australia

†Present address: Department of Biology

The University of Western Ontario

London, Ontario, N6A 5B7

Canada

Running head: QTL mapping of ovary activation

Key words: worker sterility, altruism, eusocial, interval mapping, microsatellite DNA

Corresponding author: Peter Oxley

School of Biological Sciences

Macleay Building A12

University of Sydney, 2006

Australia

(ph) +61 2 9351 3642

(fax) +61 2 9351 4771

Peter.Oxley@usyd.edu.au

ABSTRACT

The all-female worker caste of the honey bee (*Apis mellifera*) is effectively barren in that workers refrain from laying eggs in the presence of a fecund queen. The mechanism by which workers switch off their ovaries in queenright colonies is pheromonally cued, but there is genetically-based variation among individuals: some workers have high thresholds for ovary activation, while for others the response threshold is lower. Genetic variation for threshold response by workers to ovary-suppressing cues is most evident in ‘anarchist’ colonies in which mutant patriline have a proportion of workers that activate their ovaries and lay eggs, despite the presence of a queen. In this study we use a selected anarchist line to create a backcross queenright colony that segregated for high and low levels of ovary activation. We used 191 informative microsatellite loci, covering all 16 linkage groups to identify QTLs for ovary activation and test the hypothesis that anarchy is recessively inherited. We reject this hypothesis, but identify four QTLs that together explain approximately 25% of the phenotypic variance for ovary activation in our mapping population. They provide the first molecular evidence for the existence of quantitative loci that influence selfish cheating behavior in a social animal.

In societies where individuals act to benefit other members of the society at cost to their own direct fitness, there is a selective advantage for individuals that ‘cheat’, reaping the benefits of group living while avoiding the cost of contributing personally. Where genes influence cooperation among individuals, single mutations at key loci may permit selfish behavior to arise that advantages the carrier, but reduces the fitness of the group. Thus, identifying mutations for cheating behavior provides the opportunity to characterize the genetic architecture of cooperation – a key goal of sociogenomics (ROBINSON *et al.* 2005). However, while cheating phenotypes have been identified and studied in some microorganisms (ENNIS *et al.* 2000; FOSTER *et al.* 2004; HAIG 1996; QUELLER *et al.* 2003; VELICER *et al.* 2000), no equivalent cheater mutants have been characterized in higher eukaryotes (GILBERT *et al.* 2007).

Reproductive division of labor, in which members of the worker caste have reduced reproductive capacity or are even sterile, is one of the defining features of eusocial insects (WILSON 1971). The honey bee (*Apis mellifera*), typically lives in colonies comprised of a single fecund female (the queen), tens of thousands of female workers and several hundred male drones. The resident queen is the mother of all drones and workers. The drones arise from unfertilized eggs and are thus haploid. Queens and workers, by contrast, arise from fertilized eggs and are diploid (WINSTON 1987).

In wildtype honey bee colonies, workers do not typically activate their ovaries when a queen is present. Pheromones play an important role in signaling to workers that queen and brood are present (JAY 1970; JAY and NELSON 1973; WINSTON and SLESSOR 1998). After a queen’s death the workers no longer receive this signal, and up to 30% will activate their ovaries and produce eggs (BARRON and OLDROYD 2001). Furthermore, there is genetic variation in the propensity for individual workers to activate their ovaries following queen death, or when workers otherwise

fail to receive the signal that maintains their effective sterility (MARTIN *et al.* 2004; ROBINSON *et al.* 1990).

Oldroyd *et al.* (1994) discovered a naturally occurring ‘anarchistic’ colony, in which all males sampled were offspring of workers instead of the queen. Microsatellite DNA analysis of workers from this colony revealed the queen had mated with at least 12 males, producing 12 subfamilies of workers. Furthermore, microsatellite analysis showed that 49 of the 50 males examined were offspring of workers from a single subfamily – the grandsons of just one of the males involved in the mating. Subsequently, two other naturally-occurring anarchist colonies were identified; one in Australia (MONTAGUE and OLDROYD 1998) and the other in the United Kingdom (CHÂLINE *et al.* 2002). In these colonies also, the males were offspring of just one or a small number of patriline.

Recurrent selection for worker reproduction in a line derived from the colony identified by Montague and Oldroyd (1998) established a line in which up to 40% of 12 day old workers have eggs in their ovarioles despite the presence of a fecund queen (BARRON *et al.* 2001; THOMPSON *et al.* 2006). The significant association between worker ovary activation and patriline in naturally occurring anarchistic colonies, and the strong response to selection for reproductive behavior by workers, indicate a strong genetic basis for the anarchy phenotype (BARRON *et al.* 2001). Because anarchist workers not only show higher rates of ovary activation than wildtype workers in queenless colonies, but also display significant levels of activation in queenright colonies, we hypothesized that ovary activation is under the control of a single genetic regulatory pathway that suppresses ovary activation when appropriate environmental (pheromonal) cues are present. In anarchist workers, we speculated that a putative single mutation (*An*) in this pathway has resulted in a partial loss of response to the normal ovary-suppressing signal emitted from

queen and brood (BARRON *et al.* 2001), allowing some workers to selfishly activate their ovaries in the presence of the queen.

Comparison of anarchist workers over the 10 years of selection for this character reveals an increasing frequency of individuals showing ovary activation; from roughly 9% (OLDROYD and OSBORNE 1999) to nearly 40% (THOMPSON *et al.* 2006). This directional response to selection on the mutant line may be explained by the presence of additional loci of small to medium effect that influence the propensity of workers to activate their ovaries, in this case when the pathway is already mutant and no longer fully suppressing ovary activation, i.e., when it is showing the anarchist phenotype.

The genes that regulate worker ovary activation in honey bees and other social insects are key to the evolution of cooperation among workers, enabling this helper caste to subsume their individual reproduction into the reproductive output of the whole colony. Such ‘genes for altruism’ have been frequently postulated (e.g. DAWKINS 1989; HAMILTON 1972) but molecular-genetic evidence for their existence has to date proved elusive. The phenomenon of the anarchistic phenotype in honey bees provides one opportunity for the discovery of genes for altruism: anarchists are likely mutant along any pathway that normally promotes cooperation among workers. If so, then finding this mutation could reveal how cheater mutants arise from within reproductively altruistic systems, and how altruism (in honey bees) works at a molecular level. Though anarchic colonies are rare, their existence is consistent with the expectation for cheaters to occasionally arise out of the persistent reproductive conflict between each worker’s direct fitness potential and the fitness of the worker collective (RATNIEKS 1988).

Oldroyd and Osborne (1999) performed a crossing experiment in which wild type queens were inseminated with either anarchist worker-laid males or a mix of both anarchist worker-laid

males and wildtype queen-laid males. These crosses produced a range of reproductive phenotypes among worker offspring (OLDROYD and OSBORNE 1999). In some colonies only workers fathered by anarchist males had activated ovaries, while in others there was no correlation between paternity and ovary activation. This inconsistent pattern suggests a strong G x E interaction for the anarchy phenotype, and further suggested that the postulated anarchy locus controlling worker reproduction in the anarchist line is recessive to the wild type allele.

Here we report an attempt to confirm the hypothesis that anarchy is recessive and to locate the postulated *An* locus, using QTL mapping on an anarchist backcross. All microsatellite markers and the linkage map were obtained from the honeybee genome project (SOLIGNAC *et al.* 2007).

MATERIALS AND METHODS

Genetic line and crosses: The anarchist line has been maintained for c.a. 10 generations from a single colony identified from the field in 1995 (MONTAGUE and OLDROYD 1998). Selection for anarchy in each generation has led to an average annual increase of 2% in the frequency of ovary activation in colonies (BARRON and OLDROYD 2001; HOOVER *et al.* 2005a; OLDROYD and OSBORNE 1999; OLDROYD *et al.* 2001; THOMPSON *et al.* 2006).

For our mapping population we used a backcross design (Figure 1), consisting of a hybrid queen (the daughter of an Anarchist queen and wildtype drone) artificially inseminated by a single worker-laid drone. Assuming a recessive mode of inheritance, offspring from this backcross will segregate into two phenotypic classes: anarchist (ovaries activated) and wildtype (ovaries not activated), depending on the maternal allele inherited.

Biological material: We introduced the focal queen (individual F₁:2 in Figure 1) into a standard Langstroth hive containing a colony comprising eight frames of honey and queen-laid

brood. When the host colony's progeny were eventually replaced by progeny from our focal queen (after two months) we removed a single comb of emerging brood and incubated it overnight (34.5°). The following day we paint-marked (Posca Poster Pens, Mitsubishi Pencil Co.) approximately 1,000 newly emerged workers and re-introduced them into their colony of origin. After 14 days, 595 marked workers were recaptured and snap frozen on dry-ice. We then dissected individual worker abdomens and scored their ovaries according to the degree of development (after DADE 1977). Specifically, we scored ovaries on an arbitrary five-point scale; 0 indicated that ovarioles (the finger-like projections at the distal end of each ovary) were thin and lacking any sign of activation, and 4 indicating that at least one ovary had a mature (oval shaped) ovum. Scores within this range reflect intermediate stages of ovary activation. For the purposes of this study and to allow comparisons with previous work, workers with a score of 2-4 were considered to have 'activated' ovaries.

From the total number of individuals scored from our backcross population ($n = 595$) we selectively genotyped the 96 individuals with the lowest numerical score (0) and 96 individuals with the highest numerical scores (2-4). This subset of individuals represents the maximum contrast in ovary activation phenotypes. DNA was extracted from these 192 individuals by grinding one hind leg on dry ice, followed by addition of 0.5 mL Chelex solution (5%) and boiling for 15 Minutes (WALSH *et al.* 1991). After centrifugation (13 000 g, 10 mins), we added proteinase K (1 μ g) to the DNA-containing supernatant and incubated it at 37° for 3 hours, then 80° for 15 minutes to deactivate the enzyme. The solution was diluted 1:1 in sterile H₂O and stored at 4° prior to use in PCR.

We genotyped all 192 individuals at 417 microsatellite loci (SOLIGNAC *et al.* 2007), distributed across all 16 linkage groups. For 'touchdown' PCR genotyping (whereby T_a

decreased from 58° to 52° in single degree increments, with 5 cycles per degree), we used fluorescently labeled tags (Pet, Fam, Ned and Vic; Applied Biosystems) at a final concentration of 0.25µM (PET/FAM/NED/VIC-CCTGGCGACTCCTGGAG), with 0.0165 µM tagged reverse primers (CCTGGCGACTCCTGGAG-RevPrimer) and 0.25 µM forward primer. We electrophoresed amplified products using a 3130xl Analyzer (Applied Biosystems), and subsequently visualized and acquired individual genotypes at each locus using the associated software (Genemapper Software 3.7, Applied Biosystems).

Genetic linkage map: We assembled markers into linkage groups using the positions and inter-marker distances published in version AmelMap3 of the honey bee genome assembly (SOLIGNAC *et al.* 2007). We assessed the likelihood of marker order for each of our linkage groups using the Map-Manager QTX (MANLY *et al.* 2001) ‘ripple’ function. The marker order of the most likely map deviated from the published map (SOLIGNAC *et al.* 2007) at only 11 loci, and map choice did not change the results of the interval mapping analysis (below).

Statistical analysis: In order to determine the association of any QTLs to individual markers we used a regression analysis of marker genotype on ovary score, as implemented in the computer program Map-Manager QTX (MANLY *et al.* 2001). This initial approach is map-independent and was primarily to search for evidence of QTLs outside the region screened using interval mapping. To minimize type-1 errors associated with testing over multiple loci, we adjusted the associated *P*-values to maintain a False Discovery Rate (FDR) of 5% (Q Value = 0.05 BENJAMINI and HOCHBERG 1995; VERHOEVEN *et al.* 2005).

Subsequently, Map-Manager QTX was used to perform interval mapping, using the Kosambi mapping function. We identified QTLs for each linkage group by first calculating significance thresholds using permutation (CHURCHILL and DOERGE 1994) to determine the *P*-value for the

highest LOD score in each linkage group. These *P*-values (Table 1) were then ranked and assessed for significance at a 5% FDR across all linkage groups.

RESULTS

Worker ovary activation scores spanned the full range, from 0 to 4 (Table 2). A total of 119 workers (20 %) were classified as having active ovaries.

From 417 markers, 191 showed allelic variation between the two parents (Individuals P:1 and P:3 in Figure 1). These informative markers produced a near-saturated linkage map, whereby 95.7% of the genome was within 20 cM of at least one marker. A full 93.1% of the genome was delimited by markers less than 50 cM apart. All 16 honey bee chromosomes were represented in our map (not shown), with the average distance between markers being 22.4 cM with a standard deviation of 11.83 cM.

Interval mapping revealed four significant QTLs, one each on linkage groups 15, 1, 7 and 13 (in order of greatest effect, Figure 2). We label these QTLs *OvA1*, *OvA2*, *OvA3* and *OvA4*, respectively, and we estimate they account for 8%, 6%, 6% and 5% of the total phenotypic variation (Table 3).

DISCUSSION

This study has not found evidence to support the hypothesis that anarchy is recessively inherited, but has identified four quantitative trait loci that influence ovary activation rates in queenright honey bee workers. These are the first QTLs for cheating to be mapped in a eusocial insect. We reject our initial model of inheritance based on the number, direction and magnitude of QTL effects identified in our map. This is because, under the assumption of a recessive mutation (BARRON *et al.* 2001; OLDROYD and OSBORNE 1999), we expect to find a single QTL of large effect. However, we found multiple QTLs which together account for only 25% of total

phenotypic variation. Furthermore, the wild type allele from one QTL increased ovary activation (*OvA1*; Table 3), rather than decreasing, as was expected. Given the high saturation of our linkage map (95.7% of the genome lies within 20 cM of at least one marker), it is unlikely that a single recessive mutation accounting for the majority of phenotypic variation would remain undetected following our open-ended screen. We therefore suggest that the *An* locus exhibits a more complex mode of inheritance and propose a new hypothesis, that the anarchy allele is dominant to its wild type counterpart. This hypothesis remains consistent with the conditional expression of the anarchy phenotype following an anarchist x wildtype cross (OLDROYD and OSBORNE 1999) provided we allow for incomplete penetrance. Analysis of ovary activation in the reciprocal backcross to that used in this study would likely prove useful in understanding the mode of inheritance of this trait, and is the subject of current research in our laboratory.

Under a dominant model, all workers in our backcross population would be either *An/An* or *An/an* (cf Figure 1), and would therefore be genetically predisposed to ovary activation. However, we observed only 20% of workers with active ovaries, indicating a low phenotypic penetrance for anarchy. The QTLs identified in the present study are therefore likely to be secondary modifiers of the dominant but incompletely penetrating anarchy phenotype: these loci influence the likelihood that an anarchist worker already predisposed at the major locus will activate her ovaries.

The role of our QTLs in wildtype workers is not yet known and cannot be assessed from the current study. However, we suggest a role similar to that in anarchists in that it influences a workers' propensity to activate her ovaries in the absence of pheromonal suppression. In anarchists, this absence of suppression results from the as-yet-unmapped *An* mutation or loss of queen, whereas in wildtypes it results solely from loss of the queen. In our mapping population,

the allele for high ovary activation was inherited from the anarchist line in three of the four QTLs (*OvA2-4*), as would be expected given the continuous selection for anarchistic alleles since 1998.

Candidate Genes

The strongest QTL identified, *OvA1*, explains 8% of variance in ovary activation scores and contains 86 candidate genes within its 95% confidence interval (Figure 2A). Based on a functional annotation from GO terms (<http://www.geneontology.org/>), we highlight four genes of particular interest.

***OvA1* dopamine receptor type D2:** The neurotransmitter dopamine has been found to mediate a wide range of honey bee behaviors, including hygienic (SPIVAK *et al.* 2003) and foraging (BARRON *et al.* 2007; BARRON *et al.* 2002; SCHULZ *et al.* 2002) behavior in workers. Dombroski *et al.* (2003) showed that when queenless workers are fed a diet supplemented with dopamine the workers show increased levels of ovary activation. Thus genes associated with dopamine activity may be important to ovary activation. *OvA1* encompasses a dopamine receptor type D2 gene (*Dop2*). This gene may therefore be associated with a change in responsiveness to dopamine in workers already cued to activate their ovaries.

Odorant receptors: Ovary activation is in part mediated by pheromones emitted from mature larvae that signal workers to refrain from ovary activation. Brood pheromones are less effective at inhibiting ovary activation in the anarchist line than in wildtypes (BARRON and OLDROYD 2001; HOOVER *et al.* 2005a; OLDROYD and OSBORNE 1999). It is therefore possible that Odorant Binding Protein 9 (*Obp9*) or the gene Similar to Putative Odorant Receptor 13a (LOC72693) are involved in the detection of brood pheromones, and thereby play a role in the regulation of ovary activation. Similarly, the gene Neuronal Nicotinic Acetylcholine Apis α 7-2

Subunit (GB17254) is expressed in brain regions associated with olfactory learning, as well as being expressed in the antennal lobes of workers (JONES *et al.* 2006; THANY *et al.* 2005). It may also be associated with response to brood signals.

Differentially expressed loci: Thompson *et al.* (2006) used a cDNA microarray of honey bee brain ESTs to identify loci that are differentially expressed between 4-day-old wildtype and anarchist workers. Of approximately 5,500 genes screened, three were significantly differentially expressed. These loci are candidates for the regulation of worker sterility (THOMPSON *et al.* 2006), but are not associated with any of our *OvA*-series QTLs. However, among the secondary candidates reported from that study, three (GB17541, GB13621 and GB14785) are located within the genomic regions of *OvA1*, *OvA2* and *OvA3*, respectively. An additional two secondary candidates (BI511564 and BI509796) are associated with *OvA2* and *OvA4*, respectively. These five genes, first identified from the microarray screen, are therefore of interest in the context of the current study.

The identification of QTLs and corresponding candidate genes for ‘modifiers of ovary activation’ are a step toward uncovering molecular pathways influencing the expression of cheating behaviour in animal societies. Our use of the FDR is appropriate because it controls both type I and II errors, substantially increasing the power to detect QTLs without compromising the family-wise error rate (BENJAMINI and HOCHBERG 1995). Because this method requires all tests to be statistically independent (BENJAMINI and HOCHBERG 1995; CHEN and STOREY 2006) we have applied it to the uncorrected *P*-values associated with the single highest LOD score on each linkage group ($N = 16$), rather than the *P*-value for each (non-independent) mapping interval within groups. The dependency of intervals within linkage groups were taken into account by using permutation (CHURCHILL and DOERGE 1994).

Genotype by Environment Interactions

Quantification of QTL effects can be complicated by the presence of genotype x environment interactions, which have been suggested in previous studies of anarchy (BARRON and OLDROYD 2001; OLDROYD *et al.* 1999). Worker ovary activation has been shown to be more inhibited by the presence of wildtype brood than by anarchist brood (OLDROYD *et al.* 2001). The backcross workers in our experiment were exposed to signals from both homozygous and heterozygous anarchist pupae, potentially resulting in an inhibitory signal from the brood that was stronger than a pure anarchist colony, but weaker than from wildtype brood. Therefore, the small percentage of workers with ‘activated’ ovaries observed in our backcross compared to studies of pure anarchist colonies (20% vs. 38%; e.g THOMPSON *et al.* 2006) could be explained in part by the segregation of ovary activation loci (i.e., *OvA*) combined with an increased inhibitory signal from the brood.

It should be noted that the level of ovary activation observed is still three orders of magnitude greater than that expected in wild type colonies (RATNIEKS 1993). Furthermore, by genotyping individuals with the most extreme ovary activation scores, the frequency of workers with active ovaries in our mapping population was magnified to 50%.

Further Considerations

An alternative genetic model to dominance is one in which many small loci contribute to the phenotype additively. However, as the largest significant additive effect we identified inherited from the anarchist line was a mere 6%, it would require each of the original anarchist colonies (CHÂLINE *et al.* 2002; MONTAGUE and OLDROYD 1998; OLDROYD *et al.* 1994) to have inherited very large numbers of loci to produce the large differences in ovary activation rates between wild

type workers and anarchists. It is therefore more probable that there exist a small number of QTLs, of large dominant effect.

The four QTLs identified in our study explain approximately 25% of the phenotypic variance in our backcross. If anarchy is dominant, then further testing would be necessary to determine if there are more QTLs that contribute to variation in anarchists' ovary activation, or whether the rest is due to G x E interactions.

Conclusions: This study represents the first QTL mapping project in honey bees to utilize the complete microsatellite-based linkage map and gives further corroboration to the positioning of these markers on the 16 linkage groups. More importantly, we have shown for the first time that QTLs affecting worker sterility via ovary activation in honey bees can be statistically detected. These QTLs suggest a number of candidate genes that influence cheating behavior – the first for a social animal.

Further, we modify our initial hypothesis for a single recessive gene that controls ovary activation (sensu BARRON *et al.* 2001) to that of a single locus of moderate to high dominance, combined with incomplete penetrance. If correct, then a future QTL study of the reciprocal backcross to that used here should see the *An* locus segregate directly with ovary activation in queenright workers. The anarchistic line therefore remains an ideal model for the continued exploration of the evolution of cooperation among social insects at the molecular level.

ACKNOWLEDGEMENTS

We thank M. Solignac for advice; J. Lim, J. Paar and M. Duncan for assistance. Funding for this project was provided by an Australian Research Council grant to BPO.

LITERATURE CITED

- BARRON, A. B., R. MALESZKA, R. K. VAN DER MEER and G. E. ROBINSON, 2007 Octopamine modulates honey bee dance behavior. *PNAS*: 0610506104.
- BARRON, A. B., and B. P. OLDROYD, 2001 Social regulation of ovary activation in 'anarchistic' honey-bees (*Apis mellifera*). *Behavioral Ecology and Sociobiology* **49**: 214-219.
- BARRON, A. B., B. P. OLDROYD and F. L. W. RATNIEKS, 2001 Worker reproduction in honey-bees (*Apis*) and the anarchic syndrome: A review. *Behavioral Ecology and Sociobiology* **50**: 199-208.
- BARRON, A. B., D. J. SCHULZ and G. E. ROBINSON, 2002 Octopamine modulates responsiveness to foraging-related stimuli in honey bees (*Apis mellifera*). *Journal of Comparative Physiology A* **188**: 603-610.
- BENJAMINI, Y., and Y. HOCHBERG, 1995 Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society* **57**: 289-300.
- CHÂLINE, N., F. L. W. RATNIEKS and T. BOURKE, 2002 Anarchy in the UK: Detailed genetic analysis of worker reproduction in a naturally-occurring British anarchistic honeybee, *Apis mellifera*, colony using DNA microsatellites. *Molecular Ecology* **11**: 1795-1803.
- CHEN, L., and J. D. STOREY, 2006 Relaxed Significance Criteria for Linkage Analysis. *Genetics* **137**: 2371-2381.
- CHURCHILL, G. A., and R. W. DOERGE, 1994 Empirical threshold values for Quantitative Trait Mapping. *Genetics* **138**: 963-971.
- DADE, H. A., 1977 *Anatomy and dissection of the honeybee*. International Bee Research Association, London.
- DAWKINS, R., 1989 *The Selfish Gene*. Oxford University Press.
- DOMBROSKI, T. C. D., Z. L. P. SIMÕES and M. M. G. BITONDI, 2003 Dietary dopamine causes ovary activation in queenless *Apis mellifera* workers. *Apidologie* **34**: 281-289.
- ENNIS, H. L., D. N. DAO, S. U. PUKATZKI and R. H. KESSIN, 2000 *Dictyostelium* amoebae lacking an F-box protein form spores rather than stalk in chimeras with wild type. *Proceedings of the National Academy of Sciences of the United States of America* **97**: 3292-3297.
- FOSTER, K. R., G. SHAULSKY, J. E. STRASSMANN, D. C. QUELLER and C. R. L. THOMPSON, 2004 Pleiotropy as a mechanism to stabilise cooperation. *Nature* **431**: 693-696.
- GILBERT, O., K. R. FOSTER, N. MEHDIABADI, J. E. STRASSMANN and D. C. QUELLER, 2007 High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 8913-8917.
- HAIG, D., 1996 Gestational drive and the green-bearded placenta. *Proceedings of the National Academy of Sciences, USA* **93**: 6547-6551.
- HAMILTON, W. D., 1972 Altruism and related phenomena, mainly in social insects. *Annual Review of Ecology and Systematics* **3**: 193-232.
- HOOVER, S. E. R., B. P. OLDROYD, T. C. WOSSLER and M. L. WINSTON, 2005a Anarchistic queen honey bees have normal queen mandibular pheromones. *Insectes Sociaux* **52**: 6-10.

- HOOVER, S. E. R., M. L. WINSTON and B. P. OLDROYD, 2005b Retinue attraction and ovary activation: responses of wild type and anarchistic honey bees (*Apis mellifera*) to queen pheromones. *Behavioral Ecology and Sociobiology* **59**: 278-284.
- JAY, S. C., 1970 The effect of various combinations of immature queen and worker bees on the ovary development of worker honeybees in colonies with and without queens. *Canadian Journal of Zoology* **48**: 169--173.
- JAY, S. C., and E. V. NELSON, 1973 The effects of laying worker honeybees (*Apis mellifera* L.) and their brood on the ovary development of other worker honeybees. *Canadian Journal of Zoology* **51**: 629-632.
- JONES, A. K., V. RAYMOND-DELPECH, S. H. THANY, M. GAUTHIER and D. B. SATTELLE, 2006 The nicotinic acetylcholine receptor gene family of the honey bee, *Apis mellifera*. *Genome Research* **16**: 1422-1430.
- MANLY, K. F., J. CUDMORE, R.H. and J. M. MEER, 2001 Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome* **12**: 930-932.
- MARTIN, C. J., B. P. OLDROYD and M. BEEKMAN, 2004 Differential reproductive success among subfamilies in queenless honey bee colonies (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* **56**: 42-49.
- MONTAGUE, C. E., and B. P. OLDROYD, 1998 The evolution of worker sterility in honey bees: an investigation into a behavioral mutant causing a failure of worker policing. *Evolution* **52**: 1408-1415.
- OLDROYD, B. P., L. HALLING and T. E. RINDERER, 1999 Development and behaviour of anarchistic honeybees. *Proceedings of the Royal Society of London B* **266**: 1875-1878.
- OLDROYD, B. P., and K. E. OSBORNE, 1999 The evolution of worker sterility in honeybees: the genetic basis of failure of worker policing. *Proceedings of the Royal Society of London B* **266**: 1335-1339.
- OLDROYD, B. P., A. J. SMOLENSKI, J.-M. CORNUET and R. H. CROZIER, 1994 Anarchy in the beehive. *Nature* **371**: 749.
- OLDROYD, B. P., T. C. WOSSLER and F. L. W. RATNIEKS, 2001 Regulation of ovary activation in worker honey-bees (*Apis mellifera*): larval signal production and adult response thresholds differ between anarchistic and wild-type bees. *Behavioral Ecology and Sociobiology* **50**: 366-377.
- QUELLER, D. C., E. PONTE, S. BAZZARO and J. E. STRASSMANN, 2003 Single-gene greenbeard effects in the social amoeba *Dictyostelium discoideum*. *Science* **299**: 105-106.
- RATNIEKS, F. L. W., 1988 Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *American Naturalist* **132**: 217-236.
- RATNIEKS, F. L. W., 1993 Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. *Behavioral Ecology and Sociobiology* **32**: 191-198.
- ROBINSON, G. E., C. M. GROZINGER and C. W. WHITFIELD, 2005 Sociogenomics: social life in molecular terms. *Nature Reviews Genetics* **6**: 257-270.
- ROBINSON, G. E., R. E. PAGE and M. K. FONDRK, 1990 Intracolony behavior in worker oviposition, oophagy, and larval care in queenless honey-bee colonies. *Behavioral Ecology and Sociobiology* **26**: 315-323.
- SCHULZ, D. J., A. B. BARRON and G. E. ROBINSON, 2002 A role for octopamine in honey bee division of labor. *Brain Behavior and Evolution* **60**: 350-359.

- SOLIGNAC, M., F. MOUGEL, D. VAUTRIN, M. MONNEROT and J.-M. CORNUET, 2007 A third-generation microsatellite-based linkage map of the honey bee, *Apis mellifera*, and its comparison with the sequence-based physical map. *Genome Biology* **8**.
- SPIVAK, M., R. MASTERMAN, R. ROSS and K. A. MESCE, 2003 Hygienic behavior in the honey bee (*Apis mellifera* L.) and the modulatory role of octopamine. *Journal of Neurobiology* **55**: 341-354.
- THANY, S. H., CROZATIER, V. RAYMOND-DELPECH, M. GAUTHIER and LENAERS, 2005 Apisa2, Apisa7-1 and Apisa7-2: three new neuronal nicotinic acetylcholine receptor α -subunits in the honeybee brain. *Genome Research* **16**: 1422-1430.
- THOMPSON, G. J., R. KUCHARSKI, R. MALESZKA and B. P. OLDROYD, 2006 Towards a molecular definition of worker sterility: differential gene expression and reproductive plasticity in honey bees. *Insect Molecular Biology* **15**: 637-644.
- VELICER, G. J., L. KROOS and R. E. LENSKI, 2000 Developmental cheating in the social bacterium *Myxococcus xanthus*. *Nature* **404**: 598-601.
- VERHOEVEN, K. J. F., K. L. SIMONSEN and L. M. MCINTYRE, 2005 Implementing false discovery rate control: increasing your power. *Oikos* **108**: 643-647.
- WALSH, P. S., D. A. METZGER and R. HIGUCHI, 1991 Chelex (R)100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**: 507.
- WILSON, E. O., 1971 *The insect societies*. Harvard University Press, Cambridge.
- WINSTON, M. L., 1987 *The biology of the honey bee*. Harvard University Press, Cambridge.
- WINSTON, M. L., and K. N. SLESSOR, 1998 Honey bee primer pheromones and colony organization: gaps in our knowledge. *Apidologie* **29**: 81-95.

Figure 1: Backcross design for QTL mapping population. Queen P:2 and worker P:1 were reared from the anarchy line. Drone P:3 was collected from a wildtype colony that (typically) did not show any signs of worker-laid brood. A presumably heterozygous F₁ queen F₁:2 was mated to an F₁ anarchist drone F₁:1 to give the F₂ generation of workers. The F₂ generation was used as the mapping population.

Figure 2: Interval Maps of the four linkage groups containing significant QTLs at 5% False Discovery Rate. LOD score (Y-axis) was calculated at 1 cM intervals across each linkage group (X-axis). The 5% empirical threshold is indicated by the dashed line. 95% confidence intervals are shown by solid black bars. Microsatellite marker positions are indicated by peaks just below each X-axis. Maps A, B, C and D show linkage groups 15, 1, 7 and 13, and QTLs *OvA1*, *OvA2*, *OvA3* and *OvA4*, respectively.

Table 1: Empirically determined *P*-values for the highest LOD score recorded in each linkage group.

LG^a	LOD^b	P value^c	5% Threshold^d
1	2.7	0.024*	2.0
2	0.9	0.57	2.1
3	1.6	0.13	2.0
4	0.5	0.9	1.5
5	1.2	0.25	2.0
6	2.0	0.057	2.0
7	2.6	0.017*	2.1
8	1.1	0.33	2.0
9	1.7	0.11	2.0
10	0.5	0.84	1.9
11	1.2	0.25	1.9
12	0.9	0.38	2.0
13	2.3	0.035*	2.0
14	1.1	0.3	2.1
15	2.9	0.0049*	2.4
16	0.5	0.46	2.0

* significant with a False Discovery Rate of 5%

^a Linkage Group (LG) number refers to the NCBI assigned chromosome number in AmelMap_4 (SOLIGNAC *et al.* 2007).

^b Refers to the highest LOD score calculated in the linkage group using interval mapping

^c *P*-value was calculated empirically through 10 000 permutations (CHURCHILL and DOERGE 1994)

^d LOD threshold for 5% significance level across linkage group, calculated by permutation (10000 iterations)

Table 2: Percentage of ovary activation scores for 14-day-old workers (n = 595). Ovary activation was scored on an integer scale from 0 to 4, based on the degree of ovary activation (see Material and Methods). Workers with a score of 2, 3 or 4 were considered to have ‘active’ ovaries.

Ovary Activation	Percentage of
Score	workers classified
0	48
1	32
2	11
3	6
4	3

Table 3: Significant quantitative trait loci for ovary activation identified through interval mapping. A negative QTL effect indicates workers carrying the allele inherited from the anarchist queen have a higher average ovary activation score than workers carrying the allele inherited from the wildtype drone.

QTL	Linkage Group	Closest Marker	P-Value	QTL Effect	Effect Size (%)
<i>OvA1</i>	15	Am253	0.0049	+0.92	8
<i>OvA2</i>	7	SV272	0.017	-0.80	6
<i>OvA3</i>	1	SV084	0.024	-0.84	6
<i>OvA4</i>	13	Am074	0.035	-1.24	5

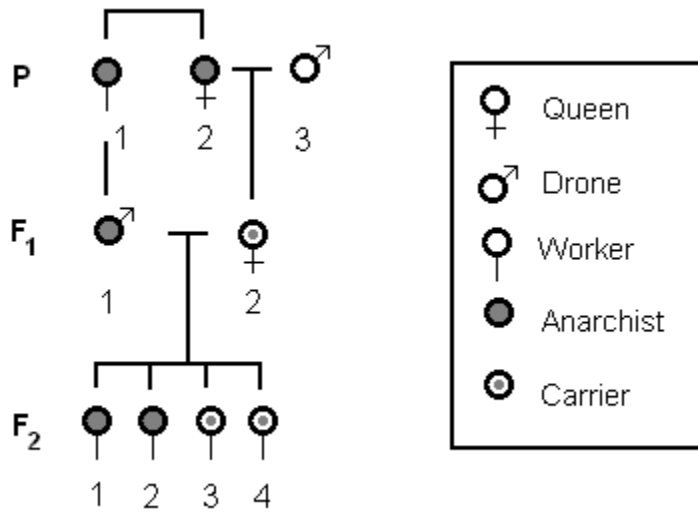


Figure 1

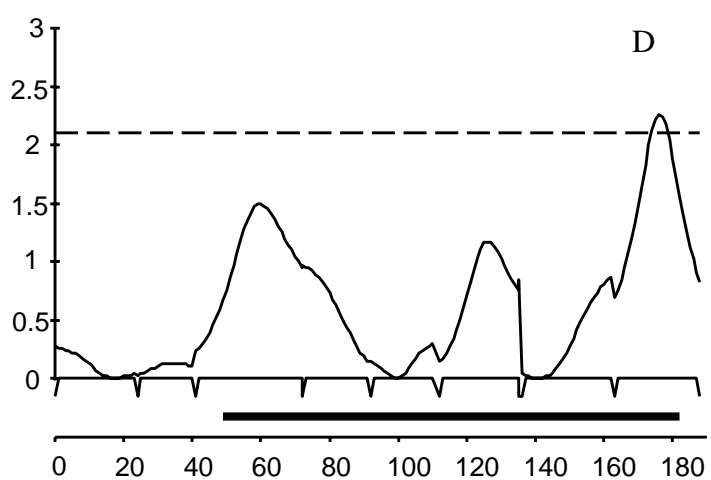
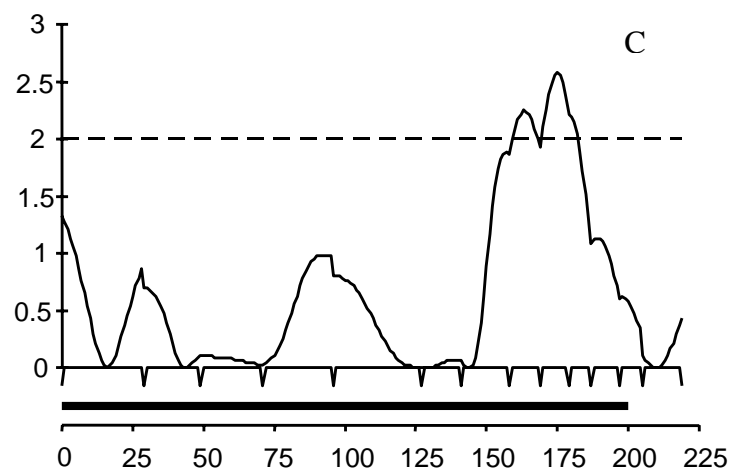
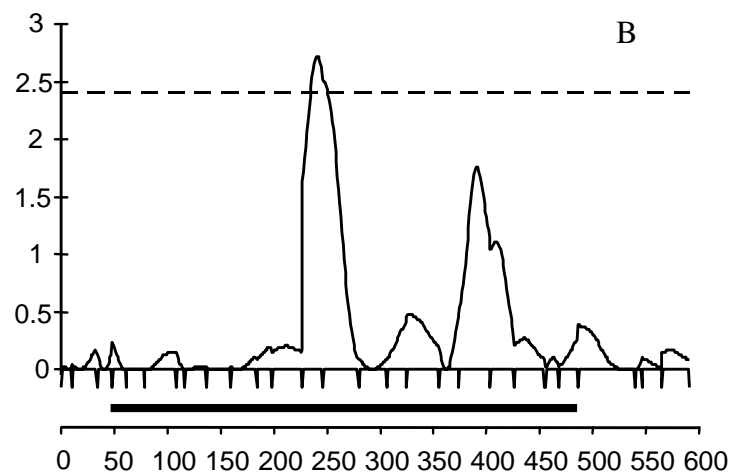
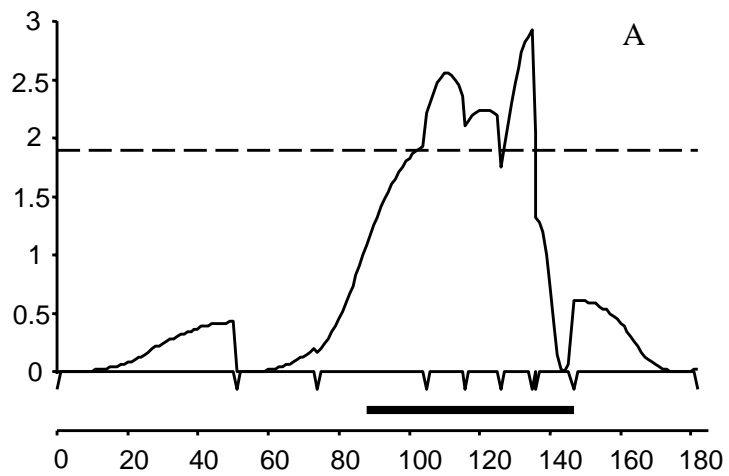


Figure 2