Current Biology

Queen Killing Is Linked to High Worker-Worker Relatedness in a Social Wasp

Highlights

- Social insect workers can compete with the queen for reproduction

- In the wasp *Dolichovespula arenaria*, workers kill queens who have mated with few males

- Workers also kill multiply-mated queens that skew their sperm use

- Matricidal workers benefit, since the colony rears the sons of full-sibling workers

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In Brief

Social insect workers can lay male eggs and compete with the queen for reproduction. Here, Loope shows that worker wasps sometimes kill their mother queen, allowing worker reproduction. Workers do so only in colonies with high worker relatedness, where they are more related to other workers' sons than to the queen's sons, as predicted by theory.

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Queen Killing Is Linked to High Worker-Worker Relatedness in a Social Wasp

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SUMMARY

Social insect colonies are pinnacles of evolved altruism but also exhibit dramatic conflict among relatives [1, 2]. In many species, a colony's workers compete with the queen and each other over the production of males. Interspecific comparisons demonstrate the importance of within-colony relatedness in determining the outcome of such conflicts [3, 4], but facultative responses to within-colony relatedness are rarely reported [5–7]. Here, I report facultative matricide (worker killing of a colony's queen) in the social wasp *Dolichovespula arenaria.* Matricide is strongly associated with high worker-worker relatedness, as predicted by theory, because closely related workers value nephews more than brothers [8]. This pattern is the result of variation in both paternity frequency and the paternity skew of colonies with multiple patrilines, implicating worker-worker relatedness rather than a direct effect of multiple mating on queen survival. Furthermore, occasional inbreeding can explain why some multiple-patriline colonies exhibit high paternity skew associated with matricide. In general, these results support the hypothesis that workers can facultatively respond to intracolony relatedness determined by queen mating behavior and demonstrate a novel benefit of polyandry in annual social insects. Facultative matricide shows dramatically how workers are evolutionary actors with interests that can diverge from the queen's, rather than being "extrasomatic projections of her personal genome" [9].

RESULTS AND DISCUSSION

In many species of social wasps and bees, colonies are founded in the spring by a single queen, produce several cohorts of female workers, rear new queens and males, and finally senesce in late summer or autumn. Workers do not mate but, due to haplodiploidy, can lay unfertilized male eggs (Figure 1). In colonies with the queen present, worker reproduction is inhibited through egg eating by the queen and other workers [10–12]. It has been proposed that workers of some species kill their mother queen in order to evade this reproductive suppression, and matricide has

been observed or inferred in numerous species of wasps and bees [4, 8, 13]. Queen killing is potentially costly: in many social insects, the single, irreplaceable queen is the only colony member capable of producing the fertilized eggs that become new workers and queens [14]. However, matricide during the reproductive phase, at the end of an annual colony's life, could benefit aspiring reproductive workers because it stops the queen from eating worker-laid eggs and attacking ovipositing workers, as well as removing a competing source of male eggs [4, 8]. In large colonies, a single potentially matricidal worker is unlikely to dominate reproduction after the queen is dead. Thus, the gain from queen killing would largely come from replacing the queen's sons with other workers' sons [8]. Workers are more related to their fellow workers' sons than to the queen's sons if the queen has fewer than two effective mates [8, 15]. Therefore, matricide should be most common in colonies with effective paternity less than 2.0, when colony resources are redirected mostly to the sons of full siblings. Interspecific comparisons support this prediction [4, 8], though it remains untested within a species that has varying levels of paternity.

To determine the role of worker-worker relatedness in the evolution of matricide, I studied *Dolichovespula arenaria*, a yellowjacket wasp. In this species, workers can produce large numbers of males after the death of the queen ([16]; unpublished data; F.L.W. Ratnieks, personal communication). I genotyped workers from 21 colonies, predicting that matricide colonies would have higher worker-worker relatedness than would colonies that retained their queens. Video observations of two of these colonies revealed matricide directly (see Movie S1 and Supplemental Results for a description of observed matricides). The remaining 19 analyzed colonies were wild-collected, mature colonies: ten were collected with a queen present, and nine were queenless with inferred matricide. To infer matricide from wild colonies, I collected mature, pre-decline colonies, 42% (13/31) of which were queenless. I estimated the non-matricide queen mortality rate (4.7%) from published colony collection data from species in the sister genus *Vespula*, a similar group of wasps that lack matricide. Assuming a non-matricide queen mortality rate equal to that of *Vespula* spp., and using the estimate of overall queen mortality, one can estimate that the fraction of collected, queenless colonies that experienced matricide is approximately 89%, which is high enough to use queenlessness as an indicator of matricide (see Supplemental Experimental Procedures for a complete explanation of these assumptions).

Paternity analysis of workers showed that worker-worker relatedness was strongly associated with whether or not a queen is killed (Figure 2A). Queen mating frequency explains part of this
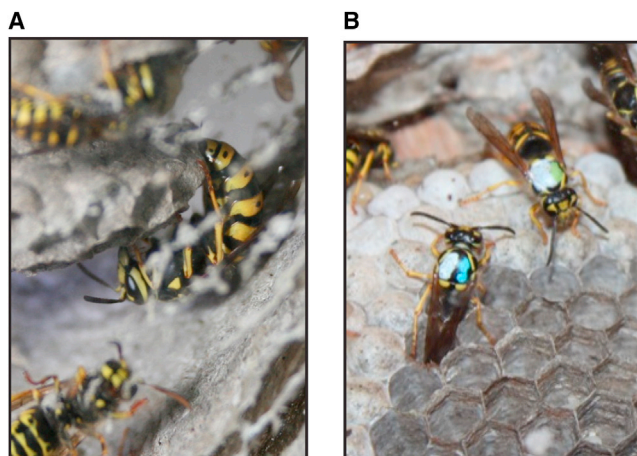
Figure 1. Queen and Worker Reproduction in *Dolichovespula arenaria*

(A) A queen lays an egg while workers tend brood. Photo by Barrett Klein.
(B) A paint-marked worker lays a male egg the day after her sister, another reproductive worker, stung the queen to death (see Movie S1 and Supplemental Results for description).

Figure 2. Matricide, Paternity, and Inbreeding in *D. arenaria*

(A) Each point represents the average worker-worker relatedness of a colony. Relatedness within colonies that are queenless due to matricide (gray circles: observed matricide colonies, n = 2; black circles: inferred matricide colonies, n = 9) is higher than within colonies that remain queenright (all queenless colonies: 0.69 ± 0.030 mean ± SEM, n = 11; queenright: 0.52 ± 0.025, open circles: n = 10; unequal variances t test on ranks: t = 3.40, df = 18.95, p = 0.003; observed matricide only versus queenright: t = 4.04, df = 5.86, p = 0.007). This difference is predicted by kin selection theory [8], because when worker-worker relatedness is greater than 0.5 (horizontal dotted line), workers value other workers' sons more than they value the queen's sons. The only monandrous queenright colony (asterisk) had just entered the reproductive stage when it was collected, unlike the nine other queenright colonies, suggesting that this queen may yet have been killed (unpublished data). For detailed paternity data, see Table S1.

(B) Points of the same shade connected by lines represent males mated to the same queen; four queens are double mated and one is triple mated. Only males mated to queens of polyandrous, queenless colonies are shown (fathers of polyandrous, queenright colonies shown in Figure S1B). Males with a low paternity share are more related to their mates in queenless colonies. Male-queen relatedness, estimated using inferred parental genotypes, is an index of inbreeding. Inbreeding avoidance or depression could therefore explain high paternity skew associated with matricide in these colonies. This pattern is not present in queenright colonies, which typically have low paternity skew (see Figure S1B).

association: queens from 6 of 7 single-paternity colonies did not survive, and 9 of 10 colonies with surviving queens had multiple patrilines. In addition, among the colonies with multiple patrilines, paternity skew was significantly greater in queenless colonies (B index [17]; queenless: 0.24 ± 0.069 mean ± SEM; queenright: 0.014 ± 0.020; unequal variances t test: $n_{1,2}$ = 5,9; t = 3.10; df = 4.69; p = 0.029; Figure S1), though relatedness was not significantly higher in queenless multiple-patriline colonies (queenless: 0.61 ± 0.047; queenright: 0.50 ± 0.0041; unequal variances t test: $n_{1,2}$ = 5,9; t = 2.33; df = 4.06; p = 0.079). Given the association between queenlessness and skew, workers also appeared to distinguish among multiply-mated queens, suggesting that they were directly assessing the level of worker-worker relatedness (influenced by paternity skew) rather than some cue indicating the number of times a queen had mated.

Why would a multiply-mated queen skew the paternity in her colony, and thereby increase worker-worker relatedness, if workers kill queens that do so? One possibility is that she has no control over paternity, and that male-male sperm competition determines paternity skew. Alternatively, partial inbreeding (queens mating to both a relative and a non-relative) could result in the observed paternity skew measured in adult workers. Inbreeding in haplodiploid organisms with a single-locus complementary sex determination system can result in the production of diploid males, often killed by workers [18]. If an inbred father's genotype matches the queen's, 50% of diploid offspring will be male, reducing the number of females he sires, possibly resulting in biased paternity. A similar pattern could arise if queens bias sperm use to avoid using sperm from relatives [19]. Given that some yellowjacket wasps readily copulate with siblings [20], I looked for an effect of inbreeding on paternity share in multiple-patriline, matricide colonies. Consistent with inbreeding-driven paternity skew, the minority father was significantly more related to the queen than was the majority father
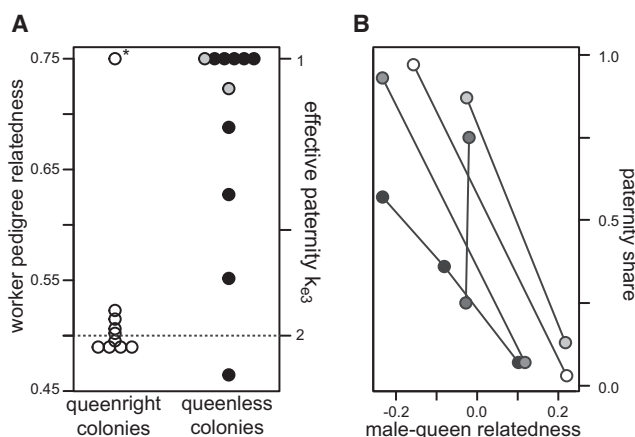
(paired t test; mean difference = 0.26; n = 5, t = 3.68, df = 4, p = 0.021; Figure 2B), and these minority-patriline fathers from queenless colonies were significantly more related to their mates than were the remaining males across all colonies (unequal variances t test: $n_{1,2}$ = 5,32; t = 2.77, df = 8.14, p = 0.024). Because of the low paternity skew among males mated to polyandrous queens in queenright colonies (n = 9; mean B skew index = 0.014 ± 0.020 SEM; Figure S1A), the hypothesis that partial inbreeding causes high paternity skew predicts that such males are not related to their mates, and thus we should see no association between inbreeding and paternity skew in this group. As predicted, there was no difference in inbreeding coefficients between majority and minority fathers in queenright colonies (paired t test; df = 9, p > 0.05; Figure S1B), and overall, relatedness between fathers and queens was not different from zero (relatedness: −0.03 ± 0.07, mean ± 95% confidence interval, jackknifing over loci), consistent with a lack of inbreeding in low-skew, queenright colonies. These patterns suggest that occasional inbreeding may cause the high paternity skew associated with the killing of some multiply-mated queens.

Table 1. Binomial Regression Models of Queenlessness

Response	Predictor	Estimate	SE	z	p
Queenlessness	relatedness	13.84	5.92	2.34	0.019*
	worker number	−0.01	0.01	−1.44	0.15
Queenlessness	relatedness	18.18	7.39	2.46	0.014*
	comb ratio	0.62	0.72	0.86	0.39
Queenlessness	relatedness	15.4	5.86	2.63	0.0087*
	calendar date	−0.01	0.06	−0.24	0.81

Worker-worker relatedness, but none of three indices of colony stage, predicts queenlessness in 21 study colonies. Worker number refers to the total worker population in each colony at time of collection. Comb ratio refers to the ratio of the area of large cells (for producing reproductive queens and males) to the area of small cells (for producing workers) in the nest, which increases as colonies mature. Calendar date represents how late in the season the colony was collected. Significant predictors are indicated by asterisks (*$p < 0.05$).

The main result, that workers facultatively kill queens based on worker-worker relatedness determined by colony paternity, is difficult to explain via alternative hypotheses. Multiple mating typically *decreases* female lifespan in insects [21], including annual social Hymenoptera [22], and a direct benefit of re-mating (e.g., a nuptial gift or nutrient transfer via seminal fluid) cannot explain the association between paternity skew and polyandrous queen death. Multiple mating increases female egg production rate in some insects [21], and this could, under some circumstances, disfavor matricide behavior [8], but an experiment that reduced queen fecundity did not induce matricide (unpublished data). Given that some killed queens were partially inbred, it is possible that workers kill such queens because they produce diploid (sterile) males, as has been suggested in a stingless bee [23]. While this hypothesis could explain the killing of the polyandrous matricide queens that were partially inbred (Figure 2B), it cannot explain the remaining six monandrous queen matricides, for which the fathers are not more related to their mates than are the fathers of queenright colonies (mean relatedness for monandrous matricide colony fathers = −0.02, mean relatedness for queenright fathers = −0.03, t = 0.16, df = 9.906, p = 0.88). The probability that, strictly due to chance, 6 of 6 remaining (outbred) killed queens are singly mated, while only 1 of 10 (outbred) surviving queens is singly mated, is 0.0009 (Fisher's exact test). This pattern is not predicted if inbreeding alone drives matricide, but it is predicted by the kin selection hypothesis. Furthermore, the inbreeding hypothesis does not predict that the matricidal worker is reproductive (observed in one colony; see Supplemental Results), though this is predicted by the kin selection hypothesis [8]. Finally, the relationship between queenlessness and relatedness is not the result of a confound with the stage of colony development, as has been suggested for a similar association between relatedness and worker policing in a related species of wasp [5, 7]. Logistic regression models show that queenlessness is predicted by worker-worker relatedness but not by calendar date, worker number, or the ratio of reproductive comb area to worker comb area, three indices of colony development (Table 1). Thus, after considering these alternatives, the hypothesis most consistent with the results reported here is that workers kill their queen when they are more related to nephews than brothers, as predicted by kin selection theory [8]. Further, manipulative tests will be important in confirming the critical role of worker-worker relatedness in facultative matricide.

Most examples of facultative responses to intracolony relatedness come from studies of conflict over sex allocation [24], though only a few involve paternity variation in species with single-queen societies [25, 26]. Other than these studies of sex-ratio biasing in ants, the only example of a facultative worker behavioral response to natural variation in colony paternity is facultative policing of worker-laid eggs in *Dolichovespula saxonica* [5], a wasp very similar to *D. arenaria*, though this finding was not replicated in a different population [7]. Why would facultative responses to relatedness determined by queen mating behavior not be widespread? First, natural variation in effective paternity among colonies must straddle 2.0, the threshold above which workers favor queen's sons over worker's sons. Furthermore, information available to workers on a colony's paternity can be limited [27], and kin-informative recognition cues may be evolutionarily unstable [28], though this depends on the situation [29, 30]. Responses to intracolony relatedness may arise and exist until the loss of recognition cues make them maladaptive, producing a patchwork of populations, some of which have sufficient information to assess and respond to relatedness variation. Although the mechanism by which workers detect relatedness is unknown, facultative matricide in *D. arenaria* reiterates the importance of kin structure in social evolution and suggests an additional conflict-driven benefit of polyandry [31, 32] for queens of annual social insects.

REFERENCES

1. Hamilton, W.D. (1972). Altruism and related phenomena, mainly in social insects. Annu. Rev. Ecol. Syst. *3*, 193–232.

2. Ratnieks, F.L.W., Foster, K.R., and Wenseleers, T. (2006). Conflict resolution in insect societies. Annu. Rev. Entomol. *51*, 581–608.

3. Wenseleers, T., and Ratnieks, F.L.W. (2006). Comparative analysis of worker reproduction and policing in eusocial hymenoptera supports relatedness theory. Am. Nat. *168*, E163–E179.

4. Foster, K.R., and Ratnieks, F.L.W. (2001). Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions. Behav. Ecol. Sociobiol. *50*, 1–8.

5. Foster, K.R., and Ratnieks, F.L.W. (2000). Facultative worker policing in a wasp. Nature *407*, 692–693.

6. Hammond, R.L., Bruford, M.W., and Bourke, A.F.G. (2003). Male parentage does not vary with colony kin structure in a multiple-queen ant. J. Evol. Biol. *16*, 446–455.

7. Bonckaert, W., van Zweden, J.S., d'Ettorre, P., Billen, J., and Wenseleers, T. (2011). Colony stage and not facultative policing explains pattern of worker reproduction in the Saxon wasp. Mol. Ecol. *20*, 3455–3468.

8. Bourke, A.F.G. (1994). Worker matricide in social bees and wasps. J. Theor. Biol. *167*, 283–292.

9. Nowak, M.A., Tarnita, C.E., and Wilson, E.O. (2010). The evolution of eusociality. Nature *466*, 1057–1062.

10. Wenseleers, T., Tofilski, A., and Ratnieks, F.L.W. (2005). Queen and worker policing in the tree wasp *Dolichovespula sylvestris*. Behav. Ecol. Sociobiol. *58*, 80–86.

11. Zanette, L.R.S., Miller, S.D.L., Faria, C.M.A., Almond, E.J., Huggins, T.J., Jordan, W.C., and Bourke, A.F.G. (2012). Reproductive conflict in bumblebees and the evolution of worker policing. Evolution *66*, 3765–3777.

12. Foster, K.R., and Ratnieks, F.L.W. (2001). Convergent evolution of worker policing by egg eating in the honeybee and common wasp. Proc. Biol. Sci. *268*, 169–174.

13. Strassmann, J.E., Nguyen, J.S., Arévalo, E., Cervo, R., Zacchi, F., Turillazzi, S., and Queller, D.C. (2003). Worker interests and male production in *Polistes gallicus*, a Mediterranean social wasp. J. Evol. Biol. *16*, 254–259.

14. Trivers, R.L., and Hare, H. (1976). Haploidploidy and the evolution of the social insect. Science *191*, 249–263.

15. Starr, C.K. (1984). Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In Sperm competition and the evolution of animal mating systems, R. Smith, ed. (Academic Press), pp. 428–459.

16. Freiburger, B.J., Breed, M.D., and Metcalf, J.L. (2004). Mating frequency, within-colony relatedness and male production in a yellow jacket wasp, *Dolichovespula arenaria*. Mol. Ecol. *13*, 3703–3707.

17. Nonacs, P. (2000). Measuring and using skew in the study of social behavior and evolution. Am. Nat. *156*, 577–589.

18. van Wilgenburg, E., Driessen, G., and Beukeboom, L.W. (2006). Single locus complementary sex determination in Hymenoptera: an "unintelligent" design? Front. Zool. *3*, 1–15.

19. Bretman, A., Wedell, N., and Tregenza, T. (2004). Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. Proc. Biol. Sci. *271*, 159–164.

20. Kovacs, J.L., Hoffman, E.A., and Goodisman, M.A.D. (2008). Mating success in the polyandrous social wasp *Vespula maculifrons*. Ethology *114*, 340–350.

21. Arnqvist, G., and Nilsson, T. (2000). The evolution of polyandry: multiple mating and female fitness in insects. Anim. Behav. *60*, 145–164.

22. Baer, B., and Schmid-Hempel, P. (2001). Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, *Bombus terrestris*. Evolution *55*, 1639–1643.

23. de Camargo, C.A. (1979). Sex determination in bees. XI Production of diploid males and sex determination in *Melipona quadrifasciata*. J. Apic. Res. *18*, 77–84.

24. Meunier, J., West, S.A., and Chapuisat, M. (2008). Split sex ratios in the social Hymenoptera: a meta-analysis. Behav. Ecol. *19*, 382–390.

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

26. Sundström, L., Chapuisat, M., and Keller, L. (1996). Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. Science *274*, 993–995.

27. Boomsma, J.J., Nielsen, J., Sundström, L., Oldham, N.J., Tentschert, J., Petersen, H.C., and Morgan, E.D. (2003). Informational constraints on optimal sex allocation in ants. Proc. Natl. Acad. Sci. USA *100*, 8799–8804.

28. Rousset, F., and Roze, D. (2007). Constraints on the origin and maintenance of genetic kin recognition. Evolution *61*, 2320–2330.

29. Ratnieks, F.L.W. (1991). The evolution of genetic odor-cue diversity in social Hymenoptera. Am. Nat. *137*, 202–226.

30. Ratnieks, F.L.W., Helanterä, H., and Foster, K.R. (2007). Are mistakes inevitable? Sex allocation specialization by workers can reduce the genetic information needed to assess queen mating frequency. J. Theor. Biol. *244*, 470–477.

31. Mattila, H.R., Reeve, H.K., and Smith, M.L. (2012). Promiscuous honey bee queens increase colony productivity by suppressing worker selfishness. Curr. Biol. *22*, 2027–2031.

32. Crozier, R.H., and Fjerdingstad, E. (2001). Polyandry in social Hymenoptera - disunity in diversity? Ann. Zool. Fenn. *38*, 267–285.