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Workers respond to unequal likelihood of future reproductive opportunities in an ant

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In cooperatively breeding or eusocial societies, opportunities may arise for helper individuals to gain direct fitness by reproducing. However, the extent to which helpers respond differentially, in terms of their reproductive behaviour, to the probability that reproductive opportunities will arise is not fully known. In many eusocial Hymenoptera, workers lay eggs only in queenless conditions following the death of the queen or queens. Relative to polygyny (multiple queens per colony), monogyny (single queen per colony) increases the probability that queenless conditions arise. We therefore tested the hypothesis that ant workers respond differentially to queenless conditions as a function of the probability of queenlessness. We compared worker behaviour and reproduction before and after removal of queens from monogynous and polygynous colonies of the ant *Leptothorax acervorum*. We found that, in queenless conditions, workers from monogynous colonies were significantly more likely to lay eggs, showed a significantly reduced latency to egg laying and laid eggs at a significantly higher rate per capita than workers from polygynous colonies. In addition, before queen removal, workers that laid eggs in queenless conditions across both monogynous and polygynous colonies performed a range of behaviours associated with reproduction at significantly higher rates than nonreproductive, control workers. These 'future reproductive' workers also significantly reduced their rates of brood care following queen removal. These findings show that workers under monogyny reproduce more readily in queenless conditions than workers under polygyny, and that would-be reproductive workers alter their behaviour before they experience the opportunity for future reproduction. They therefore suggest that workers adaptively modulate their reproductive behaviour as a function of the likelihood of opportunities for direct reproduction arising, and that workers' behaviour is affected by the ability to gain direct fitness even when reproduction is currently not occurring.

Many vertebrate and invertebrate animals live in cooperatively breeding or eusocial societies characterized by a reproductive division of labour. In such societies, reproductive individuals gain evolutionary fitness directly through their own reproduction, whereas nonreproductive individuals (helpers) can gain evolutionary fitness indirectly through aiding the reproduction of related groupmates (Hamilton, 1964a, 1964b). In many cases, helpers are physiologically capable of reproducing but do not do so because of social control or self-restraint (Cant, 2000; Faulkes, Abbott, & Jarvis, 1991; Moore & Liebig, 2013; Ratnieks, 1988; Wenseleers, Hart, & Ratnieks, 2004; Wenseleers, Helanterä, Hart, & Ratnieks, 2004). However, opportunities (e.g. death of a dominant individual) may arise for helpers to produce offspring in the absence of social control or self-restraint (Wenseleers & Ratnieks, 2006). Subordinate individuals have been found to modulate their helping effort before such reproductive opportunities arise (Cant & Field, 2001). But the full effect of the existence of future reproductive opportunities on helper behaviour is unclear and, overall, the relative roles of direct and indirect means of gaining fitness in shaping animal societies is not fully resolved (Clutton-Brock, 2002; Wright, 2007). In the present study, we therefore investigated whether helpers maximize their chances of gaining direct fitness from future reproduction.

The eusocial Hymenoptera (ants, bees and wasps) often exhibit morphological female castes, with reproductive queens and nonreproductive, or less reproductive, helper workers. Workers of many species possess ovaries but lack the ability to mate, which allows them (via haplodiploid sex determination) to lay viable male eggs (Bourke, 1988a). Workers are more related to their own sons

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than to any other class of male relative, and hence, according to inclusive fitness theory, should value the production of their own sons most highly (Hamilton, 1964a, 1964b; Ratnieks, 1988). However, in colonies containing queens ('queenright' colonies), workers rarely successfully reproduce, either because their reproduction is 'policed' by queens and other workers (e.g. Kikuta & Tsuji, 1999; Ratnieks & Visscher, 1989; Wenseleers, Tofilski, & Ratnieks, 2005; Zanette et al., 2012), or because workers exhibit reproductive self-restraint (Moore & Liebig, 2013; Ratnieks, 1988; Wenseleers, Hart, et al., 2004; Wenseleers, Helanterä, et al., 2004). Workers are thought to exhibit reproductive self-restraint when the costs of direct reproduction outweigh the benefits of acting as nonreproductive helpers (Cole, 1986; Moore & Liebig, 2013; Ratnieks, 1988). For example, worker reproduction is thought to reduce colony efficiency (and therefore colony productivity) by causing reproductive workers to engage in frequent aggressive interactions (Cole, 1986) and to avoid risky but essential tasks such as foraging (Franks & Scovell, 1983).

By contrast, workers in queenless colonies (i.e. those containing no queens) tend to reproduce readily (Bourke, 1988a), because, under queenless conditions, queen policing cannot occur and worker policing is relaxed (d'Ettorre, Heinze & Ratnieks, 2004; Miller & Ratnieks, 2001; Wenseleers & Ratnieks, 2006). Furthermore, in queenless colonies, worker reproduction often provides the final opportunity for colony members to gain any form of fitness (Bourke, 1988a; Ratnieks, 1988). However, it is unknown whether workers show differential responses to queenlessness as a function of differences in the likelihood of queenlessness arising. Differences in gyny status (colony queen number) provide the opportunity to test for such differential responses. This is because the death of only one queen brings about queenlessness in monogynous colonies (having one queen per colony), whereas the death of more than one queen is required to bring about queenlessness in polygynous colonies (having multiple queens per colony); workers under monogyny therefore experience a greater probability of becoming queenless than workers under polygyny (Bourke, 1988a). This difference predicts that workers under monogyny should express higher levels of reproduction when queenless than workers under polygyny. Consistent with this prediction, interspecific comparisons in ants suggest that the frequency of species in which worker reproduction is confined to queenless conditions is greater in monogynous than in polygynous species (Bourke, 1988a). However, an intraspecific comparison would offer a more powerful test of the prediction, because it eliminates confounding factors associated with other differences between species.

In several ant species in which most worker reproduction occurs in queenless conditions, workers establish dominance hierarchies before the queen's death (e.g. Bourke, 1988b; Brunner & Heinze, 2009; Heinze & Oberstadt, 1999). Workers in these species therefore apparently modulate their behaviour in advance of the occurrence of direct reproduction. This is the case even when very little or no egg laying occurs in the presence of the queens. For example, in the ant *Temnothorax unifasciatus*, workers that were dominant in queenless conditions had significantly more contact with queens and engaged in significantly more brood care before queen removal than other workers (Brunner & Heinze, 2009). Therefore, it is of interest to investigate the extent to which workers have undergone selection to modify their behaviour in queenright conditions in advance of opportunities for direct reproduction arising.

The ant *Leptothorax acervorum* provides an excellent study system in which to address these issues because workers lay very few eggs in queenright conditions (Bourke, 1991; Hammond, Bruford, & Bourke, 2003; Heinze, Puchinger, & Hölldobler, 1997) and gyny status varies intraspecifically, with some populations containing both monogynous and polygynous colonies (facultative polygyny; Heinze, Lipski, Hölldobler, & Bourke, 1995). In addition, workers in *L. acervorum* have been shown to behave in an evolutionarily self-interested manner as a function of gyny status in several contexts, including sex allocation (Chan, Hingle, & Bourke, 1999) and queen replacement (Gill & Hammond, 2011). We therefore tested two hypotheses in a facultatively polygynous population of *L. acervorum*. The first hypothesis was that, when queenless, workers from monogynous colonies express higher levels of reproduction than workers from polygynous colonies. We tested this hypothesis by removing all queens from monogynous and polygynous *L. acervorum* colonies and measuring levels of worker reproduction under queenless conditions. The second hypothesis was that, irrespective of gyny status, workers in queenright conditions that go on to lay eggs in queenless conditions (hereafter, 'future reproductive workers') differ behaviourally from other workers in ways that prepare them for future reproduction. We tested this hypothesis by comparing the rates of selected behaviours in future reproductive workers and control workers under queenright conditions.

METHODS

Colony Collections and Sample Sizes

Colonies of *L. acervorum* were collected from a facultatively polygynous population in Thetford Forest, Norfolk, U.K., in June and October 2009 (37 and 43 colonies, respectively). In this population, colonies are monodomous (single colonies occupy single nests), 20–50% of colonies are polygynous (with a mean of 2–5 related, egg-laying queens per polygynous colony) and 95% of all queens are singly mated (Bourke, Green, & Bruford, 1997; Chan et al., 1999; Friend & Bourke, 2012; Hammond, Bourke, & Bruford, 2001; Hammond, Bruford, & Bourke, 2006; Heinze et al., 1995). Mean worker–worker relatedness is 0.50–0.71 in monogynous colonies and 0.26–0.56 in polygynous colonies (Bourke et al., 1997; Friend & Bourke, 2012; Hammond et al., 2001; Hammond, Bruford, & Bourke, 2002; Hammond et al., 2003; Heinze et al., 1995). The mean (range) percentage of queenless colonies is 12% (2–22%; Chan et al., 1999). Both monogynous and queenless colonies exhibit female-biased sex allocation, whereas polygynous colonies exhibit male-biased sex allocation (Chan et al., 1999), consistent with queenless colonies arising mainly from monogynous ones. Once in the laboratory, colonies were kept inside artificial nests composed of two microscope slides (as in Bourke, 1991). During the experiment, all colonies were kept under an environmental regime of 23 °C, 14 h light/13 °C, 10 h darkness and were fed frozen adult *Drosophila* and diluted honey every 2–3 days. Humidity was maintained using moistened cotton wool placed in the nest arena.

One replicate of the experiment was performed in July–August 2009 (using the June 2009-collected colonies) and a second replicate in March–May 2010 (using the October 2009-collected colonies, following an artificial hibernation). Across the two replicates, 59 'experimental colonies' (36 monogynous and 23 polygynous) were selected for use in the experiment on the basis of their being queenright and containing more than 15 adult workers on collection (Appendix Table A1).

Comparison of Reproduction by Queenless Workers

To test the first hypothesis, all dealate queens and eggs were carefully removed (the eggs being completely removed from nests rather than being destroyed in situ) from the experimental colonies to create queenless conditions and allow the detection of newly laid, worker-derived eggs. (Dealate queens, hereafter 'queens', are