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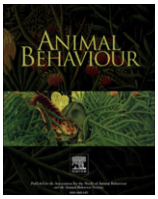

Kinship in a social bee mediates ovarian differentiation and has implications for reproductive skew theories

Sally L. Harradine, Michael G. Gardner, Michael P. Schwarz*

School of Biological Sciences, Flinders University, Adelaide, Australia

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Understanding how social species differentially allocate reproduction within groups is a major problem in evolutionary biology. A key issue is whether relatedness among nestmates can be assessed by group members and then used to determine levels of skew. Several studies have shown a relationship between skew and relatedness, but this might indicate correlational rather than causal relationships. For example, if fecundity has a genetic component, then more closely related females might have similar reproductive outputs, and hence low skew, owing to genetic similarity in genes controlling reproduction, rather than relatedness per se. Here we show that in a facultatively social bee ovarian differentiation between queens and secondary reproductives, prior to egg-laying declines with increasing relatedness. This finding could not be explained by genetically determined similarities in fecundity, by variation in body size or age, or by selective execution of brood by workers. Our results indicate that females are able to assess pairwise relatedness, either directly or indirectly, and use this information to mediate ovarian development. This points a way forward for future developments in skew theory by throwing attention onto what has been largely treated as a 'black box': the ability of organisms to acquire and process the kinds of information needed for skew theories to operate.

Understanding how sociality can evolve where some members of a group have very low or zero direct fitness has been a major challenge for evolutionary biology. Kin selection provides one paradigm for approaching this challenge and, despite some recent contention (Wilson & Hölldobler 2005; Nowak et al. 2010), it remains our best theoretical tool for understanding social evolution (Bourke 2011). Nevertheless, the complexity of factors that determine how social systems might evolve is profound. Although early kin selection approaches that emphasized Hamilton's rule have been heuristic (e.g. Krebs & Davies 1984), most animal systems are characterized by a complex interplay of genetic, ecological, behavioural, physical and signalling factors. Understanding how these have combined ultimately to lead to the forms of social organization that we see in the real world is daunting.

Reproductive skew theories (e.g. Reeve et al. 1998; Crespi & Ragsdale 2001; Buston et al. 2007; Johnston 2008; Johnston & Cant 2008; Buston & Zink 2009) attempt to model key parameters that are used by individuals to arrive at decisions about group membership and reproductive apportionment within groups. However, skew theories have been remarkably difficult to test, and for reasons of tractability many models contain assumptions that may be unrealistic or difficult to assess for real animal systems (e.g. Nonacs 2011; Nonacs & Hager 2011). Many attempted assessments of skew models have taken a qualitative approach that only permits a conclusion that supports one skew model over others, rather than a possible conclusion that skew models are not applicable for the case at hand (Nonacs 2011). For example, finding a positive or negative relationship between skew and relatedness could be used to support transactional or tug-of-war models, but such relationships in themselves do not tell us whether any kind of skew model is indeed operating. A further problem is that skew models assume that animals are able to acquire the kinds of information that are assumed in the models, and then act on that information in a way that maximizes their inclusive fitness.

For most empirical skew studies, the ability of subject species to acquire and then use information that indicates relatedness has been treated as a 'black box'. With few exceptions (e.g. Hannonen & Sundström 2003) demonstrated relationships between skew and relatedness have been implicitly assumed to entail the ability to assess kinship (e.g. Langer et al. 2004, 2006; Lucas et al. 2011). This

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* Correspondence: M. P. Schwarz, School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia.
E-mail address: Michael.Schwarz@flinders.edu.au (M. P. Schwarz).

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is concerning for two reasons: first, there are no unambiguous studies showing that true kin recognition, as opposed to discrimination between familiar and unfamiliar individuals, occurs in social insects; and second, alternative explanations for a correlation between relatedness and skew are available. We now discuss three of these alternative explanations and return to the issue of recognition at the end of our paper.

(1) In species in which reproductive skew involves multiple queens but in which brood are reared by workers, those workers may be able to execute brood they are least related to selectively, so that the ultimate production of mature offspring may reflect a complex outcome of competing queens along with a third-party worker 'collective' (Reeve & Keller 2001; Hannonen & Sundström 2003; Reeve & Jeanne 2003). For example, if queens are closely related, workers may execute fewer brood, leading to a situation in which it appears that queens adjust their reproductive output more equitably, but in which the agents of skew are in fact the workers, rather than the queens.

(2) If relatedness varies with age structure (for example matrifilial versus sororal colonies), skew might reflect differences in age rather than relatedness. For haplodiploids this becomes especially important, because sisters are more closely related to each other than mother–daughter dyads, but differences in age could influence skew in ways that are related only to age. For example, mothers may have greater power than their daughters, or younger females may be able to adopt a 'hopeful inheritor' strategy (e.g. Sumner et al. 2010; Schwarz et al. 2011). In these cases skew would be higher in matrifilial associations, where $r = 0.5$, than in sororal colonies, where $r = 0.75$, leading to a false conclusion that skew is negatively associated with relatedness.

(3) If reproductive output is influenced by genes controlling either fecundity or how reproductive effort is distributed over time, then genetically similar individuals should have similar reproductive profiles at any one time, and this could result in low skew (Keller 1993; Keller & Ross 1993; Hannonen & Sundström 2003). In this case, lower levels of skew among closely related females may be caused by the sharing of genetically encoded reproductive strategies, rather than the operation of any dynamics related to skew theories.

In a comprehensive review of skew studies (Nonacs 2011), only six of 27 studies, in which skew and relatedness were examined across groups within populations, found a significant relationship. None of these studies ruled out the possibility that such relationships could be explained by the three confounding factors above. In at least one case (Hogendoorn & Velthuis 1993), involving skew in carpenter bees, the relationship of skew to relatedness strongly closely coincided with differences in matrifilial/sororal composition of colonies. In another case, involving skew among ant queens, the possibility of genetic correlation was raised but could not be ruled out. For two studies involving allodapine bees (Langer et al. 2004, 2006), no attempts were made to assess any of the three above possibilities. In particular, both studies involved an unknown proportion of colonies in which the ultimate composition was affected by migration of individuals between nests, and neither study was able to address how or when skew was mediated.

The inability of previous studies to show unambiguously that relationships between skew and relatedness can be explained by skew models, rather than alternative and confounding processes, does not falsify skew models per se. Instead, it indicates the need to examine these relationships more closely and ask whether subject species may indeed have the capacities to acquire and act on the kinds of information that are assumed in skew models.

A previous study showed that reproductive skew, measured in terms of the maternity of mature brood at the end of the brooding period, declines with increasing relatedness (Langer et al. 2006), but the study did not examine how that pattern may have arisen. In this study we examined the effect of relatedness on differential ovarian enlargement among nestmates in the allodapine bee *Exoneura robusta* in mid-winter, when dominant/subordinate reproductive hierarchies have developed, but before egg laying has commenced. We did not attempt to assess reproductive skew theories. Instead, we asked whether nestmates have the ability to acquire information about relatedness and used this to determine reproductive strategies in ways that might allow skew models to apply. Our study was able to address this question because it was carried out at a time in the life cycle where: (1) reproductive differentiation among nestmates was well developed, but selective execution of brood by workers was not possible; (2) differences in the age of nestmates could be assessed at the time when reproductive hierarchies were established and before the process of brood rearing could obscure those differences (for example, by execution of brood by workers); and (3) genetic correlations in fecundity could be examined when females were reproductive rather than at the end of brood maturation when oviposition had long since finished.

The social behaviour and ecology of *E. robusta* has been studied extensively (Schwarz et al. 2007). There is one generation per year and social nesting is near-mandatory because of severe brood predation threats in single female colonies (Schwarz 1986, 1988a, b, 1994; Schwarz et al. 2007). Per capita brood production increases strongly with colony size, up to about four to six females per brood-rearing nest (Schwarz 1986, 1994). During mid-winter, when our study was carried out, each colony contains a 'winter queen', which has large ovaries and goes on to lay a large clutch of eggs in late winter and early spring. Colonies at this time also contain a secondary reproductive with smaller ovaries, which adds further eggs to the queen's existing clutch in mid–late spring (Smith & Schwarz 2006; Kayaalp & Schwarz 2009). Winter colonies also contain a variable number of additional females, all with small ovaries, which either ultimately disperse in late spring and early summer as reproductive foundresses, or else remain in their natal nest effectively as nonreproductive workers (Schwarz 1986). During autumn and winter, queens also act as guards (Hogendoorn & Schwarz 1998) and are able to prevent nestmates from re-entering the colony if they pose a reproductive threat to the queen (Bull et al. 1998); ovarian development of subordinate females during this time is mediated by pheromones produced by the queen (Schwarz & O'Keefe 1991). Previous studies of *E. robusta* therefore indicate that there are large benefits to social nesting and strong constraints to independent nesting, and that winter queens are able to signal their dominance pheromonally and evict nestmates if those females become potential reproductive rivals.

METHODS

Whole *E. robusta* colonies were collected on 2 July 2006 from Toolangi State Forest, Victoria, and transported on ice to Flinders University, where adults were preserved in 100% ethanol until genotyping and dissection. Females from multifemale colonies were dissected in 70% ethanol as per Schwarz (1986) after microsatellite genotyping, but before genotype analyses were undertaken. Ovary size was measured as the average length of the three largest terminal oocytes. Wing length was used as a measure of body size (Schwarz 1986) and measured from the apex of the submarginal cell to the base of the forewing.

Total DNA was extracted from two to three legs of each female using the fresh tissue protocol in the Gentra Puregene Cell Kit (Qiagen Inc., Valencia, CA, U.S.A.). We amplified five polymorphic