PATTERNS OF PATERNITY SKEW AMONG POLYANDROUS SOCIAL INSECTS: WHAT CAN THEY TELL US ABOUT THE POTENTIAL FOR SEXUAL SELECTION?

Rodolfo Jaffé,[1,2,3] **Francisco Garcia-Gonzalez,**[1] **Susanne P. A. den Boer,**[1,2,4] **Leigh W. Simmons,**[1] **and Boris Baer**[1,2]

[1]Centre for Evolutionary Biology, The University of Western Australia, M092, 35 Stirling Highway, 6009 Crawley, Australia

[2]ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, M316, 35 Stirling Highway, 6009 Crawley, Australia

[3]E-mail: rojaff@gmail.com

[4]Centre for Social Evolution, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark

Monogamy results in high genetic relatedness among offspring and thus it is generally assumed to be favored by kin selection. Female multiple mating (polyandry) has nevertheless evolved several times in the social Hymenoptera (ants, bees, and wasps), and a substantial amount of work has been conducted to understand its costs and benefits. Relatedness and inclusive fitness benefits are, however, not only influenced by queen mating frequency but also by paternity skew, which is a quantitative measure of paternity biases among the offspring of polyandrous females. We performed a large-scale phylogenetic analysis of paternity skew across polyandrous social Hymenoptera. We found a general and significant negative association between paternity frequency and paternity skew. High paternity skew, which increases relatedness among colony members and thus maximizes inclusive fitness gains, characterized species with low paternity frequency. However, species with highly polyandrous queens had low paternity skew, with paternity equalized among potential sires. Equal paternity shares among fathers are expected to maximize fitness benefits derived from genetic diversity among offspring. We discuss the potential for postcopulatory sexual selection to influence patterns of paternity in social insects, and suggest that sexual selection may have played a key, yet overlooked role in social evolution.

KEY WORDS: Cryptic female choice, genetic diversity, kin selection, sexual conflict, social Hymenoptera, sperm competition.

Understanding the adaptive significance of polyandry in the social Hymenoptera (ants, bees, and wasps) has been a central goal in kin selection research for the past three decades (Page and Metcalf 1982; Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001; Hughes et al. 2008b; Boomsma et al. 2009). Single paternity, the ancestral mating system in the social Hymenoptera (Hughes et al. 2008a), is currently regarded as a crucial precondition for the evolution of eusociality in this group, because it maximizes relatedness between colony members [(Boomsma 2009), but see also other studies (Fromhage and Kokko 2011; Nonacs 2011)]. Multiple paternity, on the other hand, dilutes the relatedness between group members because it generates half-sib families within a colony. In consequence, the benefits gained through inclusive fitness can be reduced (Hamilton 1964), and may not outweigh the cost associated with the maintenance of helper behaviors (Page and Metcalf 1982). Polyandry has

nevertheless evolved independently in several lineages of eusocial Hymenoptera (Boomsma et al. 2009), and genetic benefits have been suggested as the most plausible explanation for the evolution and maintenance of polyandry in these groups (Crozier and Fjerdingstad 2001). High genetic diversity among colony members has been shown to increase productivity and broaden tolerance to environmental changes (Mattila and Seeley 2007; Oldroyd and Fewell 2007), increase resistance to pathogens (Schmid-Hempel 1998; Baer and Schmid-Hempel 1999; Hughes and Boomsma 2006; Seeley and Tarpy 2007), and improve task performance through a more efficient division of labor (Hughes et al. 2003; Jaffé et al. 2007; Smith et al. 2008). Moreover, queen mating frequency has been found to be negatively correlated with the number of queens per colony (Hughes et al. 2008b), which suggests polyandry and polygyny (multiple queens) are alternative mechanisms to increase genetic diversity in social insect colonies.

In many animal taxa, polyandry causes the co-occurrence of different ejaculates in the female's reproductive tract. This allows sexual selection to operate after copulation, either through the competition of ejaculates from different males to fertilize an egg (sperm competition) (Simmons 2001) or through the ability of females to influence which sperm fertilize their eggs (cryptic female choice) (Eberhard 1996). Postcopulatory sexual selection is known to be a significant force shaping the evolution of traits that allow males to monopolize paternity (Parker 1970; Simmons 2001), and thus is expected to act on variation of male traits, such as ejaculate size or genital morphology, that determine fertilization success [e.g., (Simmons and García-González 2008, 2011)]. Postcopulatory sexual selection has been found to favor adaptations in males that generate high paternity skew, as the best adapted males will sire the majority of offspring (Simmons 2001). In odonates for example, females are highly polyandrous, but the removal of rival sperm by copulating males ensures complete paternity for the last male to copulate before oviposition (Siva-Jothy and Tsubaki 1989). In social insects, high paternity skew can bias paternity toward one or a few males, thus increasing genetic relatedness among colony members (Boomsma and Ratnieks 1996; Sundström and Boomsma 2000).

Although polyandry in social insects offers the potential for both sperm competition and cryptic female choice to operate, the study of sexually selected processes in social insects has received little attention (Boomsma et al. 2005). Social insect research has focused on the study of effective paternity frequency (m_e) (Nielsen et al. 2003), because this estimate is weighted by the proportion of offspring sired by each male and thus determines the genetic relatedness among the workers of colonies headed by a single queen (Pamilo 1993). In consequence there is a wealth of molecular data available from social insect species on the number of males represented among a given queen's offspring, and the distributions

of paternity among those males. Interestingly, the number of potential sires represented in a brood or in a queen's sperm storage organ has often been reported to be greater than the effective paternity (Fernández-Escudero et al. 2002; Boomsma et al. 2009; Haapaniemi and Pamilo 2012). Moreover, many studies have revealed a mismatch between observed queen mating frequencies and actual numbers of sires (Boomsma and Ratnieks 1996; Baer 2011). These discrepancies could arise from (1) stochastic events, such as insemination failure or male infertility (Garcia-Gonzalez 2004); (2) factors leading to inequalities between fertilization success and paternity success, which include embryo or juvenile mortality resulting from intrinsic sire effects or genetic incompatibilities between a queen and potential sires (García-González 2008); or (3) as is common in other animal taxa, an outcome of sperm competition, cryptic female choice, or conflicting interests over paternity (Sundström and Boomsma 2000; Haapaniemi and Pamilo 2012). Currently however, no attempt has been made to assess variation in paternity skew across all the major social Hymenoptera clades where polyandry has evolved independently (Boomsma and Sundström 1998; Schlüns et al. 2005), or to explicitly relate paternity skew to the number of potential sires. Here, we fill this gap by investigating the evolutionary relationships between paternity skew and paternity frequency across 72 species of polyandrous social Hymenoptera for which reliable paternity data are available.

If polyandry provides direct or indirect benefits to queens or offspring (Arnqvist and Nilsson 2000; Crozier and Fjerdingstad 2001; Simmons 2005; Oldroyd and Fewell 2007), a trade-off is expected between the benefits gained through multiple mating and the inclusive fitness losses derived from the reduction in colony relatedness. Kin selection is expected to drive the evolution of mechanisms to maintain high paternity skew and thus high colony relatedness, particularly in species where queens mate with just a few males, because relatedness drops sharply with every additional male the queens mate with (Page and Metcalf 1982). In consequence, sexual selection and kin selection are both likely to influence paternity skew in species showing low queen mating frequencies. On the other hand, because the reduction in relatedness between the members of a colony diminishes as paternity frequency increases (Page and Metcalf 1982), selection to maintain high colony relatedness via paternity skew is expected to be weaker in highly polyandrous species. In this case, selection should maximize the benefits of genetic diversity. In social insects, genetic diversity benefits are not only determined by the number of allelic combinations at a given locus, but also by the mutualistically advantageous expression of multiple alleles at a single locus across interacting individuals (Nonacs and Kapheim 2007). Highly polyandrous queens should thus promote equal paternity among offspring to maximize genetic diversity gains. To test these predictions, we compared the observed association

between paternity skew and paternity frequency to the expected association between these two variables under random paternity allocation. We envisaged three possible scenarios: (1) observed paternity skew does not depart from the expected skew under random paternity allocation; (2) observed paternity skew is higher than that expected by chance, suggesting the action of processes of postcopulatory sexual selection that increase skew and colony relatedness; and (3) observed paternity skew is lower than that expected by chance, suggesting postcopulatory sexual selection to equalize paternities and thus increase genetic diversity. Given that colony size and queen number are known to be correlated with paternity frequency (Schmid-Hempel 1998; Hughes et al. 2008b), we also included these variables as predictors. Finally, we related colony size and paternity frequency across our entire dataset to test weather larger colonies have more polyandrous queens.

Methods

Briefly, to describe the association between paternity skew and paternity frequency across polyandrous social Hymenoptera, we first gathered all available paternity data. Based on a sample of 72 species of ants, bees, and wasps, we ran simple and multiple regressions correcting for phylogenetic effects, with paternity skew being the dependent variable and paternity frequency, colony size and queen number as predictor variables. The slopes of these regressions were then compared to those of a null model assuming random paternity allocation, constructed using Monte Carlo simulations mirroring the structure of the data. Finally, we ran simple and multiple regressions correcting for phylogenetic effects, using paternity frequency as the dependent variable and colony size and queen number as predictors.

DATASET

We conducted an extensive literature search and collected all available data on paternity in social Hymenoptera from studies employing genetic markers published by early 2011. Paternity in social insects is reported as paternity frequency or observed paternity (K_{obs}), which is the number of different fathers detected in a sample of worker offspring, as well as effective paternity (m_e), which is paternity weighted by the proportion of offspring sired by each male (Nielsen et al. 2003). Paternity frequency informs on the minimum realized mating frequency of a queen, whereas mating frequency sets the maximum value that paternity frequency can take. We then selected the polyandrous species (with $K_{obs} > 1$), classified the different m_e estimates according to the method by which they were calculated (Nielsen et al. 2003), and collected information on the number of offspring screened to determine paternity, as well as average queen number per colony

and colony size. Our search resulted in complete records for 87 species of polyandrous ants, bees, and wasps (Table S1). We then used this dataset to calculate the effective number index of paternity skew (S) proposed by Pamilo and Crozier (1996) as $S = (K_{obs} - m_e)/(K_{obs} - 1)$, which provides a measurement for the relative contributions of each father within offspring. If paternity skew is 0, all fathers contribute equally to offspring, whereas if paternity is monopolized by a single male skew is 1. We employed this index because it has been widely used in social insect studies (Fernández-Escudero et al. 2002; Goodisman et al. 2002; Wenseleers et al. 2005; Bonckaert et al. 2008; Pearcy et al. 2009), and also because it allows the estimation of paternity skew from observed and effective paternity estimates, which have progressively become available in many social insect species (Boomsma et al. 2009). There are other paternity skew indexes with robust statistical properties (Nonacs 2000, 2003), but their calculation requires access to raw data on paternity shares. These data are not often published, and are only available in a reduced number of species.

Negative values of skew were not considered in the analyses, as they appear when the effective paternity is higher than the observed paternity due to sample size corrections (11 species in our dataset), and hence indicate that the sample size used in a particular study did not allow a reliable estimation of paternity skew. Furthermore, because paternity skew is not an informative measure in species where queens mate with a single male, we excluded all S estimates from chiefly monandrous species (showing a mean $K_{obs} < 1.10$; four additional species). Our analyses are therefore conservative, because these species may actually be truly monandrous or mate multiply but show a very high paternity skew. All analyses were performed either using the whole dataset (72 species) or a subset of the data, including only the species that showed skew values significantly different from those expected by chance (56 species; see Paternity skew analyses within species below and Table S1). Analyses were performed on all species as well as within groups of species (ants, bees, and wasps). Analyzing the data by groups allowed us to test whether the observed pattern was driven by the relationship between skew and paternity frequency in one or two groups, or if it was a general pattern across the social Hymenoptera.

PHYLOGENETICALLY CONTROLLED REGRESSIONS

We analyzed our data using generalized least-squared (GLS) regressions controlling for phylogenetic effects (Pagel 1999; Freckleton et al. 2002). The scaling parameter λ was calculated to assess the degree of phylogenetic dependence in the data (Pagel 1999). This approach is considered more informative than phylogenetically independent contrasts, because it allows a variable degree of phylogenetic correction according to each model tested (Freckleton et al. 2002). Moreover, although Bayesian
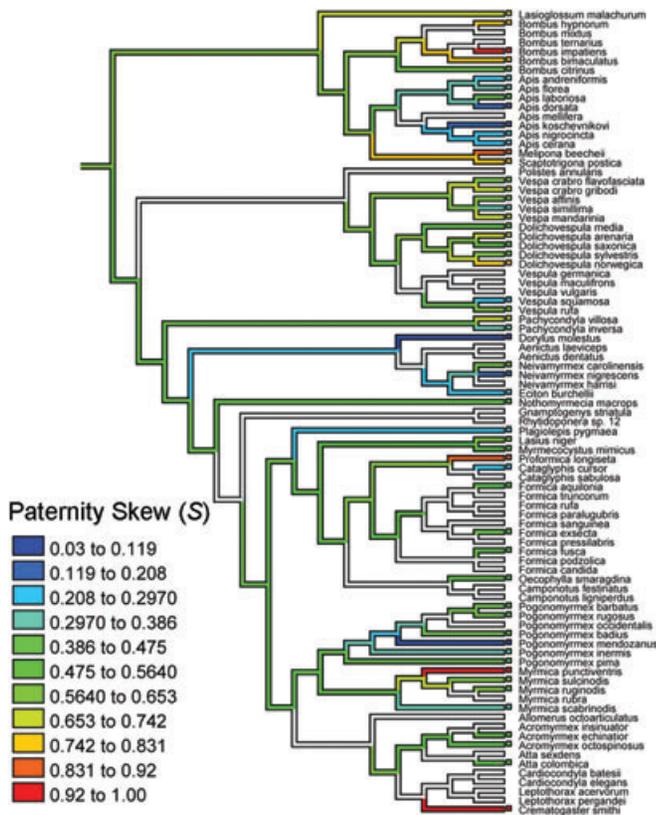
Figure 1. Phylogenetic tree for 87 polyandrous species of social Hymenoptera. Significant skew values are shown in colors (see Supporting Information Table S1 for details). The most parsimonious ancestral skew levels were reconstructed in each node using the Mesquite package (Maddison and Maddison 2010).

phylogenetic mix models have recently been used to analyze binary traits (Cornwallis et al. 2010), phylogenetically controlled GLS regressions are the appropriate method to assess associations between continuous traits (Freckleton et al. 2002). We constructed a phylogenetic tree based on a recently published phylogeny (Hughes et al. 2008a) (Fig. 1). The phylogenetic tree was built using the Mesquite package (Maddison and Maddison 2010) and then saved as text file. To normalize residual distributions and homogenize residual variances, all continuous variables (paternity skew, colony size, observed and effective paternity, and queen number) were log transformed prior to phylogenetic analyses (Harvey and Pagel 2000), which were performed using the statistical package R (R Development Core Team 2011). In our main analyses, where paternity skew was the dependent variable, a $\log_{10}(x+1)$ transformation was used to compare the observed slopes with the simulated slopes (which were obtained from datasets containing zeros, see below). In the regressions aimed at testing the relationship between paternity frequency and colony size, paternity frequency was used as the dependent variable and colony size and queen number as the predictors (only species with a known queen number were included in these

analyses, $n = 85$). A simple $\log_{10}(x)$ transformation was used in this case. To account for the different methods employed to estimate m_e (Nielsen et al. 2003), we included method as a two-level categorical predictor of paternity skew, grouping the estimates that correct for sample size (pp and pn) and those that do not (r and ps, see Table S1 for details). Because the same variables were used repeatedly in different phylogenetically controlled regressions, we calculated effect sizes (r) to determine the strength of the relations between the traits of interest. We calculated r from the t values generated in the phylogenetic GLS models, and used the noncentral 95% confidence interval (CI) for r to determine statistical significance (Nakagawa and Cuthill 2007).

PATERNITY SKEW ANALYSES WITHIN SPECIES

Because some level of paternity skew is expected from entirely random processes, such as sampling effects, we determined the statistical significance of the skew values estimated for each species using case-specific null hypotheses generated with Monte Carlo methods (Nonacs 2003). The null expectation is based on the assumption that each individual offspring, among those screened for a particular species to calculate observed and effective paternity, has the same probability of being sired by any of the fathers. Thus, for any given species i, for which an observed paternity K_{obsi} has been reported after determining paternity in k_i number of workers, the paternity of each individual worker from 1 to k_i is set at random among all the sires from 1 to K_{obsi}. For each iteration of the simulation process, sires are allocated at random among the offspring provided that all males sire at least one offspring (the number of males represented in the offspring always needs to be equal to K_{obs}). Next, effective paternity (m_e) is calculated according to the method used in the particular study of that species (either ps, pp, or pn; for studies reporting relatedness-based estimates m_e was calculated following ps, which conservatively yields broader confidence limits; see Table S1 for details). To allow for random allocation of sires among offspring in those cases in which the reported K_{obs} value is fractional (arising from averaging estimates from different colonies or studies), we rounded up the value (e.g., a reported K_{obs} value of 4.3 was rounded up to 5). This measure conservatively produces wider confidence limits. The simulation protocol was iterated 3000 times for each species to create species-specific and sample size-specific (i.e., adjusted to the particular sample size in terms of number of workers analyzed) distributions of paternity skew values with which to test the observed values of skew. The significance of a given skew value was determined on the basis of whether the value fell outside the 95% confidence limits of its null model distribution. Results are shown in Table S1. Nonsignificant skew values were obtained for 16 species of the 72 species included in the analyses. We note that skew values that do not differ from the random skew expectation might nonetheless

represent the true skew value for a given species. For this reason, we performed all further analyses in this study twice, using the full dataset (S_{all}, $n = 72$ species) and the subset dataset, including only those species showing significant skew values (S_{sig}, $n = 56$ species).

PATERNITY SKEW ANALYSES ACROSS SPECIES

Because paternity skew is a function of observed paternity (see formula above), a correlation is expected a priori between these two variables. To control for this dependency when performing the regressions across species, we again constructed appropriate null hypotheses using Monte Carlo methods, and compared the observed slopes of the relationship between paternity skew and paternity frequency with a distribution of slopes obtained in simulations where paternity allocation was random. The simulation protocol takes into account the particular details of each species involved. In a first group of simulations, we focused, as in the main analysis in our study, on species with positive skew values and $K_{obs} > 1.10$ ($n = 72$ species; see Table S1). The protocol consisted in generating sets of skew values with a sample size equal to the sample size in our main analysis ($n = 72$ species or values of skew). For each of the 72 species included in the analysis, we randomly extracted a single value of skew (associated to its specific value of paternity frequency, K_{obs}) from among those produced in the intraspecific simulations (based on random paternity allocation, detailed above in the Paternity skew analyses within species section). We then calculated the slope of the regression line between skew and paternity frequency across these 72 values (species). We iterated this protocol 10,000 times, each time calculating the slope, to obtain the distribution of slopes expected from the relationship between skew and paternity frequency when paternity allocation is random (Table 3). This distribution provided the 95% confidence limits (calculated as the slopes marking the percentiles 2.5% and 97.5% of the distribution) with which to test the significance of the observed slope. The advantage of this protocol is that it produces sets of simulated data that precisely mirror the structure of the real data not only in terms of sample size but also in terms of the different methods used to calculate effective paternity across species and the different number of workers analyzed in each species. This is important because these are the factors that determine the values that skew can take by stochastic processes.

In the simulations, instances of negative skew may be common in some cases, depending on the method used to calculate effective paternity, on the value of K_{obs} and on the number of workers screened. We dealt with negative skew values in two ways. First, in a set of simulations, negative skew values arising from the sets of simulated data in the intraspecific simulations were excluded. It can be argued, however, that these negative values represent zero skew. For this reason, we also carried out

conservative simulations in which negative skew values were set to zero. We also carried out different sets of simulations adapted to each group of species (ants, bees, and wasps; 10,000 iterations for each group; Table 3).

In addition, we carried out simulations adapted to the dataset where only species with significant skew values are included. The slope simulations follow the same protocol as those for the complete dataset ($n = 72$ species), but the number of species (and therefore the values in the calculation of the slope in each iteration) is 56 (see above). As with the complete species dataset, we carried out sets of simulations excluding negative skew values, setting negative skew values to zero, and adapting the dataset to each group of species (ants, bees, and wasps; 10,000 iterations in each case; Table 3).

All the above simulations produced slopes calculated using linear regressions that do not control for phylogenetic effects. However, we also confirmed that our observed slopes for the relationships between skew and paternity frequency did not overlap with the 95% confidence limits of the distributions of random slopes calculated using multiple regressions, accounting for variations in colony size, and controlling for phylogenetic effects. To this end, we extracted 100 independent sets of data from the simulation protocols described above and ran GLS multiple regressions controlling for phylogenetic effects (see above). Due to the logistical challenges of implementing the phylogenetic correction in the across-species simulations, these analyses are based on a reduced number of iterations (100). Notwithstanding, these later analyses confirm our previous results (Table 3). Paternity frequency (K_{obs}) and paternity skew were $\log_{10}(x+1)$ transformed prior to calculating the slopes in all simulations. All simulations were carried out with PopTools 3.1.1 (Hood 2010).

Results

Using phylogenetically controlled multiple regression analyses, we show that paternity skew is negatively associated with paternity frequency (Table 1; Fig. 2). The negative association between paternity frequency and paternity skew is not a statistical artifact, as the observed slope is significantly more negative than the slope from the expected association between these two variables under random paternity allocation (lower 95% confidence limits for slopes calculated from randomly generated values of skew ranged from -0.07 to -0.04 and did not overlap with the observed slopes which ranged from -0.13 to -0.09) (Tables 1 and 3; Fig. 2). Moreover, this association remains significant when we analyzed ants, bees, and wasps separately (Tables 2 and 3; Fig. S1). Queen number and the method employed to estimate paternity did not have a significant effect on paternity skew in any of the multiple regression models (Table 1).

Table 1. Results of simple and multiple regression analyses across all species, using GLS and controlling for phylogeny.

Trait[1]	Predictor	λ[2]	$R^2_{adjusted}$	Estimate[3]	t	P[4]	r[5]	df[6]	95% CI(r)[7]
S_{all}	Paternity frequency	0.17[*,*]	0.32	**−0.13**	−5.91	1.1×10^{-07}	−0.58	70	−0.69/−0.4
	Colony size	$6.6 \times 10^{-5ns,*}$	0.25	**−0.03**	−4.93	5.3×10^{-06}	−0.51	70	−0.64/−0.32
	Paternity frequency	$8.1 \times 10^{-2ns,*}$	0.36	**−0.10**	−4.11	1.1×10^{-04}	−0.44	69	−0.6/−0.24
	Colony size			**−0.01**	−2.06	0.043	−0.24		−0.44/−0.01
	Paternity frequency	$5.2 \times 10^{-2ns,*}$	0.34	**−0.09**	−3.27	0.002	−0.38	65	−0.55/−0.15
	Colony size			−0.01	−1.99	0.051	−0.24		−0.44/0.001
	Method			−0.01	−0.74	0.46	−0.09		−0.32/0.15
	Paternity frequency	$9.2 \times 10^{-2ns,*}$	0.35	**−0.10**	−3.92	2.13×10^{-4}	−0.43	67	−0.59/−0.22
	Colony size			−0.01	−1.98	0.052	−0.24		−0.44/0.002
	Queen number			0.01	0.30	0.76	0.04		−0.2/0.27
S_{sig}	Paternity frequency	0.21[*,*]	0.50	**−0.13**	−7.44	8.06×10^{-10}	−0.71	54	−0.8/−0.56
	Colony size	$6.6 \times 10^{-5ns,*}$	0.27	**−0.02**	−4.67	2.01×10^{-5}	−0.54	54	−0.68/−0.32
	Paternity frequency	$1.8 \times 10^{-1ns,*}$	0.50	**−0.12**	−5.58	8.4×10^{-7}	−0.61	53	−0.73/−0.42
	Colony size			0.00	−0.88	0.38	−0.12		−0.36/0.15
	Paternity frequency	0.15[ns,*]	0.50	**−0.10**	−4.43	5.34×10^{-5}	−0.53	49	−0.68/−0.31
	Colony size			0.00	−0.76	0.45	−0.11		−0.36/0.17
	Method			−0.02	−1.36	0.18	−0.19		−0.43/0.09
	Paternity frequency	0.16[ns,*]	0.50	**−0.12**	−5.59	8.56×10^{-7}	−0.61	52	−0.73/−0.42
	Colony size			0.00	−0.79	0.44	**−0.11**		−0.36 /0.16
	Queen number			−0.02	−0.76	0.45	**−0.10**		−0.35/0.17
K_{obs}	Colony size	0.95[*,ns]	0.12	**0.11**	3.61	0.001	0.36	85	0.17/0.52
	Queen number	0.99[*,ns]	0.03	**−0.16**	−0.22	0.048	−0.22	83	−0.4/−0.002
	Colony size	0.99[*,ns]	0.15	**0.10**	3.47	0.001	0.36	82	0.16/0.52
	Queen number			**−0.16**	−2.13	0.04	−0.23		−0.41/−0.01

[1]Paternity skew (*S*) and paternity frequency (K_{obs}) were used as dependent variables, and colony size, paternity frequency (K_{obs}), method employed to estimate effective paternity (m_e), and queen number were used as predictor variables. Paternity skew analyses were based on the entire dataset (S_{all}, *n* = 72) and on a subset of the data including only species with skew values differing from the random skew expectation (S_{sig}, *n* = 56, see methods and Supporting Information Table S1). Note that even though the paternity frequency analyses shown were based on observed paternity (K_{obs}) as the dependent variable, similar results were obtained when using effective paternity (m_e) as the dependent variable (results not shown).

[2]The scaling parameter λ was calculated to assess the degree of phylogenetic dependence in the data. Superscripts on λ represent significance levels (α = 0.05) of likelihood ratio tests with λ compared with 0 (first position) and 1 (second position).

[3]Regression slope estimates. Significant estimates are marked in bold.

[4]A *t*-test was used to compare the observed slope against a slope of 0 (*P* values shown).

[5]Effect sizes (*r*) were calculated from *t* values.

[6]Degrees of freedom (df) were calculated based on the relation df = *n* − (1+*v*), where *n* is total sample size and *v* is the number of predictor variables used in a model.

[7]Noncentral 95% confidence intervals (CI) for effect sizes are presented. CI that do not overlap with zero represent significant relations.

Our data also show that paternity frequency is positively associated with colony size and negatively associated with queen number (Table 1; Fig. 3), confirming previous results (Schmid-Hempel 1998; Hughes et al. 2008b). These independent associations hold in a multiple regression analysis controlling for phylogeny, with colony size showing a larger effect size than queen number. Although a positive association between polyandry and colony size is known in ants (Cole 1983; Boomsma and Ratnieks 1996; Schmid-Hempel 1998; Crozier and Fjerdingstad 2001), here we show that this association holds across all social Hymenoptera and independently of variation due to queen number, as well as within the ants, bees, and wasps (see Table 2; Fig. S2).

Table 2. Results of regression analyses within ants, bees, and wasps, using GLS and controlling for phylogeny.

Group	Trait[1]	Predictor[2]	λ[3]	$R^2_{adjusted}$	Estimate[4]	t	P[5]	r[6]	df[7]	95% CI(r)[8]
Ants	S_{all}	Paternity frequency	$6.61 \times 10^{-5 \, ns,*}$	0.30	**−0.083**	−2.28	0.03	−0.35	38	−0.57/−0.04
		Colony size			**−0.02**	−2.44	0.02	−0.37		−0.59/−0.06
	S_{sig}	Paternity frequency	$6.61 \times 10^{-5 \, ns,*}$	0.50	**−0.13**	−3.88	6.15×10^{-4}	−0.60	27	−0.76/−0.3
		Colony size			−0.01	−1.34	0.19	−0.25		−0.54/0.13
	K_{obs}	Colony size	$1.00^{*,ns}$	0.14	**0.08**	2.38	0.02	0.32	50	0.05/0.53
		Queen number			**−0.16**	−2.19	0.03	−0.30		−0.51/−0.02
Bees	S_{all}	Paternity frequency	$6.61 \times 10^{-5 \, ns,*}$	0.49	**−0.18**	−3.24	0.01	−0.67	13	−0.84/−0.24
		Colony size			0.03	1.21	0.25	0.32		−0.22/0.66
	S_{sig}	Paternity frequency	$6.61 \times 10^{-5 \, ns,*}$	0.72	**−0.18**	−4.67	6.83×10^{-4}	−0.82	11	−0.91/−0.49
		Colony size			0.03	1.84	0.09	0.49		−0.09/0.76
	K_{obs}	Colony size	$0.99^{ns,ns}$	0.22	**0.26**	2.34	0.03	0.52	15	0.05/0.75
Wasps	S_{all}	Paternity frequency	$6.61 \times 10^{-5 \, ns,*}$	0.64	**−0.23**	−3.25	0.01	−0.68	12	−0.85/−0.24
		Colony size			−0.03	−1.14	0.28	−0.31		−0.67/0.25
	S_{sig}	Paternity frequency	$6.61 \times 10^{-5 \, ns,*}$	0.44	**−0.20**	−2.84	0.02	−0.69	9	−0.86/−0.15
		Colony size			-3.5×10^{-05}	0.00	1.00	0.00		−0.55/0.55
	K_{obs}	Colony size	$0.69^{ns,ns}$	0.28	**0.25**	2.52	0.03	0.57	13	0.08/0.79

[1]Paternity skew (S) and paternity frequency (K_{obs}) were used as dependent variables, and colony size, paternity frequency (K_{obs}), and queen number were used as predictor variables. Paternity skew analyses were based on the entire dataset (S_{all}, $n = 72$) and on a subset of the data including only species with skew values differing from the random skew expectation (S_{sig}, $n = 56$, see methods and Supporting Information Table S1). Note that even though the paternity frequency analyses shown were based on observed paternity (K_{obs}) as the dependent variable, similar results were obtained when using effective paternity (m_e) as the dependent variable (results not shown).

[2]As all bees and wasps species included had a single queen per colony, simple regressions were performed to address the relationship between paternity frequency and colony size.

[3]The scaling parameter λ was calculated to assess the degree of phylogenetic dependence in the data. Superscripts on λ represent significance levels ($\alpha = 0.05$) of likelihood ratio tests with λ compared with 0 (first position) and 1 (second position).

[4]Regression slope estimates. Significant estimates are marked in bold.

[5]A t-test was used to compare the observed slope against a slope of 0 (P values shown).

[6]Effect sizes (r) were calculated from t values.

[7]Degrees of freedom (df) were calculated based on the relation $df = n - (1+v)$, where n is total sample size and v is the number of predictor variables used in a model.

[8]Noncentral 95% confidence intervals (CI) for effect sizes are presented. CI that do not overlap with zero represent significant relations.

Discussion

We report a negative association between paternity skew and paternity frequency across polyandrous social Hymenoptera. Species where only a few males contribute to offspring showed a high level of paternity skew. In contrast, paternity shares were found to be equalized in species where offspring are sired by many males. A previous comparative analysis of seven *Formica* ant species found a similar decrease in paternity skew associated with an increase in the proportion of multiply mated queens in the population, and attributed this pattern to kin selection (Boomsma and Sundström 1998). Our findings highlight the role of sexual selection in determining paternity in polyandrous social insects, and offer a new light for the interpretation of the pattern observed in *Formica* ants. Although cause and effect cannot be established

from the patterns of covariation in paternity frequency and paternity skew observed here, the general association raises some interesting questions, particularly with respect to the potential roles of postcopulatory sexual selection and sexual conflict in social evolution.

Our analyses of polyandrous social Hymenoptera reveal that species with low paternity frequencies have a higher paternity skew than that expected under random paternity allocation, which suggests the incidence of processes of postcopulatory sexual selection that increase both skew and colony relatedness. In nonsocial insects and many animal taxa, sperm competition (Simmons 2001) and cryptic female choice (Eberhard 1996) have favored the evolution of adaptations in males and females that maximize their reproductive interests and generate paternity skew.

Table 3. Mean and 95% confidence limits for the distribution of simulated slopes describing the relationship between paternity skew and paternity frequency.

Dataset[1]	Group (n)[2]	Approach[3]	Simple regressions, phylogeny not accounted for[4]		Multiple regressions, phylogeny accounted for[5]	
			Mean	95% CL	Mean	95% CL
S_{all}	All species (72)	$N_{excluded}$	−0.04	−0.06/−0.02	−0.04	−0.07/−0.02
		N_{zero}	−0.03	−0.05/−0.02	−0.02	−0.04/−0.002
	Ants (41)	$N_{excluded}$	−0.04	−0.079/−0.001	−0.04	−0.081/0.004
		N_{zero}	−0.03	−0.07/−0.004	−0.02	−0.07/0.03
	Bees (16)	$N_{excluded}$	−0.06	−0.09/−0.03	−0.02	−0.11/0.04
		N_{zero}	−0.04	−0.08/−0.01	−0.01	−0.08/0.04
	Wasps (15)	$N_{excluded}$	−0.01	−0.11/0.08	−0.03	−0.16/0.11
		N_{zero}	−0.03	−0.12/0.06	−0.04	−0.16/0.07
S_{sig}	All species (56)	$N_{excluded}$	−0.03	−0.05/−0.02	−0.04	−0.07/−0.01
		N_{zero}	−0.03	−0.05/−0.01	−0.02	−0.04/−0.001
	Ants (30)	$N_{excluded}$	−0.03	−0.08/0.01	−0.04	−0.08/0.01
		N_{zero}	−0.03	−0.07/−0.001	−0.02	−0.07/0.02
	Bees (14)	$N_{excluded}$	−0.06	−0.10/−0.03	−0.02	−0.12/0.02
		N_{zero}	−0.04	−0.08/−0.01	−0.02	−0.09/0.02
	Wasps (12)	$N_{excluded}$	−0.02	−0.11/0.11	−0.03	−0.17/0.13
		N_{zero}	−0.04	−0.12/0.07	−0.03	−0.14/0.09

[1]Simulations were performed based on the entire dataset (S_{all}) and on a subset of the data including only species with skew values differing from the random skew expectation (S_{sig}).

[2]Simulations were performed based on all species or on groups of species (ants, bees, and wasps). Sample sizes (number of species) for the calculation of the slope in each simulation are given between brackets (n).

[3]Simulations were performed either excluding all the negative paternity skew values ($N_{excluded}$) or setting these negative values to zero (N_{zero}).

[4]Results of simple regressions using 10,000 simulated slopes. Paternity skew (S) was used as the dependent variable and paternity frequency (K_{obs}) as the predictor variable.

[5]Results of multiple regressions accounting for phylogenetic effects, using 100 simulated slopes. Colony size and paternity frequency (K_{obs}) were used as predictor variables.

These adaptations could explain the reported mismatch between observed copulation frequencies of queens and the number of actual sires (Boomsma and Ratnieks 1996; Baer 2011). Importantly, our results stress out that single paternity can arise either when females mate with just one male, or despite female multiple mating, through sperm competition and cryptic female choice. The realization that high paternity skew can result in single paternity has two important implications: (1) polyandry might be more widespread among social insects that the genetic data on parentage suggests (Boomsma et al. 2009) and (2) strict monogamy might not be the only precondition for assuring high colony relatedness and thus high inclusive fitness gains [(Boomsma 2009; see also Fromhage and Kokko 2011; Nonacs 2011)]. We believe that there are exciting research opportunities in examining the mechanisms of sperm transfer and utilization in social insects, and in exploring the interplay between kin selection and sexual selection in species characterized by single or low paternity. By the same token, data on paternity skew for a larger array of species could shed light into the role of paternity skew on the evolution of sterile worker casts.

Highly polyandrous species were found to have a lower paternity skew than that expected under random paternity allocation, suggesting an active mechanism that equalizes paternity among sires in these species. We suggest that highly polyandrous social insect queens might play a more critical role in the manipulation of sperm competition dynamics than previously acknowledged. Cryptic female choice in this case could serve to promote genetic diversity among offspring (Crozier and Fjerdingstad 2001), by controlling the outcome of sperm competition processes during sperm storage or sperm use. Because sexual selection shapes male adaptations that serve to monopolize paternity, male interests are expected to counter female interests in this scenario, and thus generate sexual conflict over paternity (Chapman et al. 2003; Arnqvist and Rowe 2005). Male bumble bees, for example, have been shown able to prevent female remating through a mating plug that contains an antiaphrodisiac (Baer et al. 2001). This is against the interest of females, as a study on the same species showed that queens have greater reproductive success, and colonies greater resistance against parasites, when queens
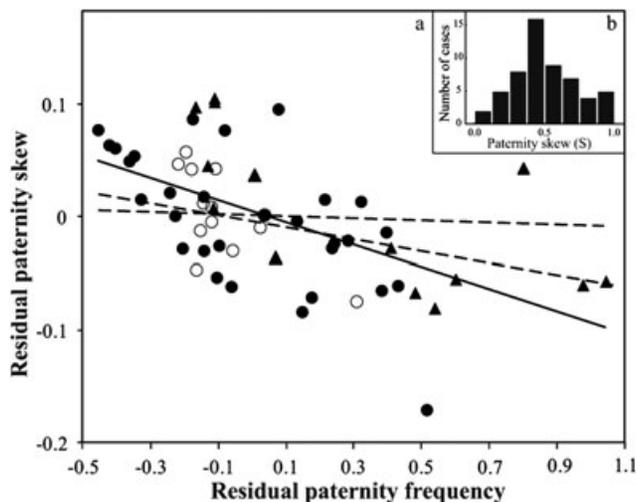
Figure 2. (a) Relationship between paternity skew and paternity frequency across 56 polyandrous species of ants (solid circles), bees (solid triangles), and wasps (open circles) after controlling for variation in colony size (only species showing significant skew values included). Residual values were obtained from a simple regression controlling for phylogenetic effects. Dashed lines represent 95% confidence limits for expected slopes under a random paternity allocation model, constructed with simulations mirroring the structure of the data (only CL for slopes obtained from simulations performed excluding negative paternity skew values are shown, as these were the most conservative; see Table 3 and methods for details). (b) Frequency distribution of paternity skew in this set of species.

Figure 3. Relationship between paternity frequency and colony size in 85 polyandrous species of ants (solid circles), bees (solid triangles), and wasps (open circles) after controlling for variation in queen number (only species with a known queen number included). Residual values were obtained from a simple regression controlling for phylogenetic effects. Dashed lines represent 95% confidence limits for fitted values.

have been inseminated with semen from multiple males (Baer and Schmid-Hempel 1999). In the leaf cutter ant *Atta colombica*, where ejaculates are transferred directly to the sperm storage organ (the spermathecae), seminal fluid is known to reduce survival of rival sperm (Den Boer et al. 2010). However, *A. colombica* queens actively move their spermathecae through muscular contractions (S.A.P den Boer et al., unpubl. data), presumably to mix the different ejaculates and distribute their own secretions, which have been shown to ameliorate the negative effects of rival ejaculates on sperm viability (S.P.A. den Boer et al. 2010). Highly polyandrous honeybee queens have also been hypothesized to manipulate the sperm storage process to reduce paternity skew (Baer 2005; Schlüns et al. 2005). Such observations provide potential mechanisms by which social insect queens might actively promote equal paternity among sires.

We also found a positive association between paternity frequency and colony size, which suggests that larger colonies might require higher levels of genetic diversity among workers, due to elevated risks of parasitism, as well as more stringent requirements for efficient division of labor (Schmid-Hempel 1998; Bourke 1999). The colony-level fitness gains arising from the synergistic interaction of genetically diverse individuals, also defined as social heterosis (Nonacs and Kapheim 2007), might in fact have
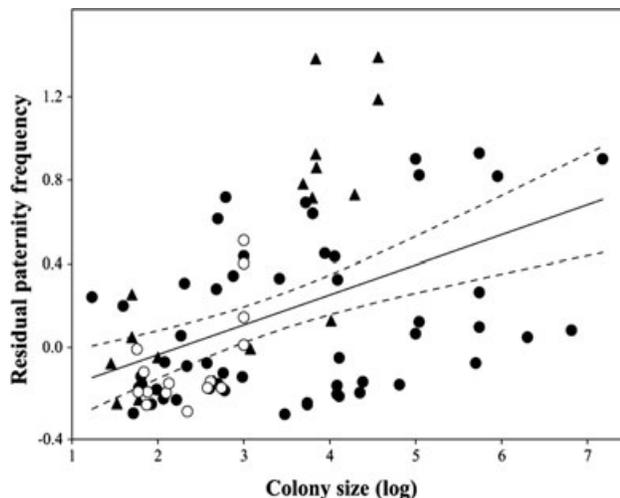
allowed the evolution of larger colony size. Alternatively, the need for more sperm has also been hypothesized as a selective force underlying queen mating frequency in species with larger colonies (Cole 1983; Boomsma and Ratnieks 1996; Kraus et al. 2004; Den Boer et al. 2009). The sperm limitation hypothesis predicts skewed paternity in species with large colonies, because queens are expected to use all available sperm and males show a considerable natural variation in sperm numbers (Koeniger et al. 2005; Stürup et al. 2011). Our findings do not support this prediction, as they reveal low paternity skew in species with highly polyandrous queens and larger colonies. However, the negative association between colony size and paternity skew was weakened or erased in the multiple regressions analyses, due to the larger explanatory power of other variables such as paternity frequency (Table 1). We therefore cannot exclude sperm limitation as an additional factor contributing to the observed association between paternity frequency and colony size.

Our analyses were based on data describing the paternity of sterile worker offspring. In honeybees (Moritz et al. 2005) and a leafcutter ant (Hughes and Boomsma 2008), paternity shares can differ between offspring destined to be sterile workers and those destined to be sexual. However, a recent study in the leafcutter ant *A. colombica* showed that paternity distribution in stored sperm match paternity in worker offspring, thus suggesting that males cannot influence the fate of their sperm cells after they have reached the queen's spermathecae (Holman et al. 2011). Paternity skew studies using sexual offspring instead of worker offspring are thus warranted to gain further insights into the consequences of sexual selection and sexual conflict in insect societies.

In conclusion, although kin selection and sexual selection have been argued to work in opposite directions (Boomsma 2007), we suggest that this need not necessarily be the case. In fact, in species with low paternity frequencies, kin selection and sexual selection seem perfectly aligned in maximizing paternity skew. At the other end of the spectrum, in species with high paternity frequencies, sexual conflict over paternity may play a predominant role. Males of highly polyandrous species are unable to monopolize paternity, as the data show that queens keep paternity skew levels below those expected by selection on males arising from sperm competition and even below those expected by random allocation. Our work thus suggests exciting opportunities for future research aimed at gaining a deeper understanding of the mechanisms and adaptations by which queens and males influence paternity outcomes, and the selection processes driving the transition from high to low skew levels. Regardless of the mechanism explaining this broad-scale pattern, our findings underscore the importance of keeping high offspring genetic diversity for female and colony fitness in polyandrous social insects. More generally, our results imply that sexual selection and sexual conflict may play a key, yet overlooked role in social evolution.

ACKNOWLEDGMENTS

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Supporting Information

The following supplementary material is available for this article:

Table S1. Observed and effective paternity frequency (K_{obs} and m_e respectively), method and sample size (n) used to calculate m_e, paternity skew (S), queen number (QN) and colony size (CS) for 87 polyandrous species of social Hymenoptera.

Figure S1. Relationship between paternity skew and paternity frequency in ants (a), bees (b), and wasps (c) after controlling for variation in colony size (only species showing significant skew values included, see Tables 2 and 3 for statistics and sample sizes).

Figure S2. Relationship between paternity frequency and colony size in ants (a), bees (b), and wasps (c). Only species with a known queen number were included (see Tables 2 and 3 for statistics and sample sizes).

Supporting Information may be found in the online version of this article.

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