



REVIEW

An Updated Guide to the Study of Polyandry in Social Insects

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Abstract

In spite of the importance of understanding the adaptive significance of polyandry in the social Hymenoptera (ants, bees and wasps), little consensus exists regarding the terminology employed, the use of different paternity estimates, the calculation of such estimates and their associated error measures, and the way paternity should be treated in comparative studies. Here I summarize previous methodological contributions to the study of polyandry in social insects, hoping that such a compendium will serve as an updated guide to future researchers. I first revise the estimates describing queen mating behavior and paternity outcomes in polyandrous social insects, outlining appropriate methods for calculating them. I then address the errors associated to paternity estimates and explain how to account for them. Finally I discuss in which cases paternity should be treated as a continuous or a categorical variable, and provide an insight into the distribution of paternity across the social Hymenoptera. This technical review highlights the importance of standardizing research methods to prevent common errors, raise confidence in the reported data, and facilitate comparisons between studies, to help shed light into many unanswered questions.

1. Why study polyandry in social insects?

Understanding the adaptive significance of multiple mating by social insect queens (polyandry) has been a central goal of many social insect researchers for the past three decades (Page & Metcalf 1982; Crozier & Fjerdingstad 2001; Boomsma et al. 2009; Kraus & Moritz 2010; Palmer & Oldroyd 2000). Single paternity resulting from monandry (single mating) is currently regarded as a crucial precondition for the evolution of eusociality in the Hymenoptera (ants, bees and wasps), since it maximizes genetic relatedness between colony members (Boomsma 2009; Hughes et al. 2008a). Polyandry, 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 sterile worker behaviors (Page & Metcalf 1982). Polyandry has nevertheless evolved independently in ants, in bees and in wasps (Hughes et al. 2008a).

Among the many hypotheses that have been proposed to explain the evolution of polyandry in the social Hymenoptera, the genetic diversity or genetic variance hypothesis enjoys most current support (Palmer & Oldroyd 2000; Crozier & Fjerdingstad 2001). The increase in genetic diversity within colonies, resulting from the co-occurrence of worker offspring from different fathers, has been suggested as the most plausible explanation for the evolution and maintenance of polyandry in this group of insects. High genetic diversity among colony members has been shown to increase productivity and broaden tolerance to environmental changes (Mattila & Seeley 2007; Oldroyd & Fewell 2007), increase resistance to pathogens (Hughes & Boomsma 2006; Seeley & Tarpay 2007; Schmid-Hempel 1998; Baer & Schmid-Hempel 1999), and enhance an efficient division of labor (Hughes et al. 2003; Jaffé et al. 2007; Smith et al. 2008). Paternity frequency has been found to be negatively correlated with the number of queens per colony (Hughes et al. 2008b; Keller & Reeve 1994), which suggests polyandry and polygyny (multiple queens)



are alternative mechanisms to increase genetic diversity in social insect colonies. Colony size, has also been found positively correlated to paternity frequency, indicating that larger colonies might profit more from genetic diversity (Schmid-Hempel 1998; Bourke 1999), or alternatively, that queens heading large colonies need to mate with several males to obtain enough sperm (sperm limitation hypothesis) (Cole 1983; Kraus et al. 2004; but see also Jaffé et al. 2014). Finally, a recent study found a negative association between paternity frequency and paternity biases, showing that queens of highly polyandrous species maximize genetic diversity by equalizing paternity (Jaffé et al. 2012).

In addition to increasing within-colony genetic diversity, 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). Post-copulatory sexual selection is known to be a significant evolutionary force, shaping the evolution of male and female traits across taxa (Andersson & Simmons 2006). Understanding the consequences of polyandry for the evolution of male and female traits is thus crucial to gain a complete understanding of the reproductive biology of social insects (Kvarnemo and Simmons 2013). Moreover, this knowledge is essential to design effective breeding programs for commercial species.

2. An updated guide to the study of polyandry in social insects

In spite of the importance of understanding the adaptive significance of polyandry in social insects, little consensus exists regarding the terminology employed, the use of different paternity estimates, the calculation of such estimates and their associated error measures, and the way paternity should be treated in comparative studies. Methodological consensus and standardization is important, because it could prevent

common errors, raise confidence in the reported data, and facilitate comparisons between studies. Thus, my aim here is to gather and summarize previous methodological contributions to the study of polyandry in social insects, hoping that such a compendium will serve as an updated guide to future researchers. I first revise the estimates describing queen mating behavior and paternity outcomes in polyandrous social insects, providing definitions and estimation methods. I then address the errors associated to paternity estimates and explain how to account for them. Finally, I discuss in which cases paternity should be treated as a continuous or a categorical variable, and provide an insight into the distribution of paternity across the social Hymenoptera.

2.1. Mating frequency, paternity frequency, effective paternity or paternity skew?

Paternity in the social Hymenoptera is usually reported as observed and effective paternity. While observed paternity (K_{obs}), or paternity frequency, is the number of males siring offspring of a single queen, effective paternity (m_e) is paternity weighted by the proportion of offspring sired by each male (Nielsen et al. 2003). Mating frequency, often confused with paternity frequency, measures the actual number of males that copulated with a single queen, even if some of them failed to sire any offspring (Table 1). The distinction between these terms is important, because they reflect the outcome of different evolutionary processes.

Social insect research has focused on the study of effective paternity (m_e), because this estimate reflects the average genetic relatedness among the workers of colonies headed by a single queen (Pamilo 1993; Boomsma & Ratnieks 1996). Effective paternity is indeed the most informative estimate for studies addressing the impact of polyandry on colony relatedness and reproductive conflicts (Wenseleers & Ratnieks 2006; Sanetra & Crozier 2001). However, effective paternity is not a good indicator of the actual number of males the queens mated with. Observed paternity provides a more accurate estimate of the queen's real mating frequency, even

Table 1: Estimates describing queen mating behavior and paternity outcomes in polyandrous social insects.

Estimate	Definition	Estimation method
Copulation frequency	Number of times a single queen copulates *	Observation
Mating frequency	Number of males copulating with a single queen †	Observation
Insemination frequency	Number of males inseminating a single queen	Genotyping of sperm from spermatheca
Observed paternity (K_{obs}) or paternity frequency	Number of males fertilizing eggs and siring offspring of a single queen	Genotyping of eggs, pupae or worker offspring
Effective paternity (m_e)	Observed paternity weighted by the proportion of offspring sired by each male.	See \hat{k}_{e3} estimate (Nielsen et al. 2003)
Paternity skew	Degree of paternity bias among the offspring of polyandrous queens.	See B -index (Nonacs 2000)

* Note that a queen may copulate several times with the same male.

† Note that insemination may or may not be involved.

though processes of post-copulatory sexual selection (sperm competition and cryptic female choice) might prevent some sperm from fertilizing eggs and siring offspring (Simmons 2001), and thus the actual mating frequency of a queen might be higher than the observed paternity. Because of this, observed paternity should always be provided along with effective paternity estimates. Mating frequency and insemination frequency should also be provided if available, although they are usually more difficult to assess (Baer 2011).

The degree of paternity biases among the offspring of polyandrous females (paternity skew), is another key quantitative measure needed to address mechanisms of post-copulatory sexual selection and sexual conflict (Den Boer et al. 2010; Jaffé et al. 2012; Baer et al. 2006). Levels of paternity skew may also be the outcome of kin-selection processes, as high paternity skew can bias paternity toward one or a few males, thus increasing genetic relatedness among the offspring of polyandrous queens (Jaffé et al. 2012; Cole 1983). Providing paternity skew along with paternity estimates, is thus essential.

2.2. General methodological considerations and useful software

Paternity is most commonly deduced from molecular markers, by grouping offspring sharing the same father and assigning them to patriline. A large body of data from studies employing genetic markers have accumulated during the past decade (Boomsma et al. 2009), and this trend is likely to remain or increase with new technological developments that allow massively parallel and multiplexed sample sequencing (Ellegren 2013; Allendorf et al. 2010). Studies estimating paternity from worker genotypes should be careful not to sample offspring from different colonies occurring together in one colony. For instance, there is growing awareness of “worker drifting” between colonies of social Hymenoptera (Lopez-Vaamonde et al. 2004). A sample of honeybee workers collected at a nest entrance, for example, is usually composed of workers from the study colony as well as drifter workers from other colonies (Neumann et al. 2000). Depending on the genotypes of these samples, drifter workers could be misinterpreted as workers from a different patriline, thus inflating paternity estimates. It is therefore important to avoid sampling drifter workers, as this would simplify analyses and yield accurate paternity estimates. An easy way to avoid sampling drifter workers is to collect freshly emerged workers inside the colonies, or to sample pupae or even eggs (Paxton et al. 2003).

In cases where it is not possible to avoid sampling drifter offspring, or where colonies have more than one queen, sibship reconstruction analyses can be performed to assign workers into queens and patrilines within queens. A particularly useful software to perform this kind of analyses is COLONY, a free program implementing maximum likelihood method to

assign sibship and parentage jointly, using individual multi-locus genotypes at a number of co-dominant or dominant marker loci (Jones & Wang, 2010). COLONY can be found here: <http://www.zsl.org/science/software/colony>

MateSoft (Moilanen et al. 2004) is a free software developed to estimate paternity statistics in haplodiploid organisms (like the social Hymenoptera), based on co-dominant genetic marker data. The genetic data can be queen genotypes, genotypes of worker offspring from a single queen, or genotypes of sperm stored in the queen’s spermatheca. A particularly appealing feature of this program is that it can also deduce parental genotypes and provide a likelihood probability for alternative genotypes. MateSoft can be found here: <http://www.bi.ku.dk/staff/jspedersen/matesoft/>

Another approach to deduce paternity from worker genotypes is to estimate effective paternity based on the genetic relatedness between workers (r_{ww}). Relatedness between worker offspring of a queen that mated with a single male is $r_{ww} = 0.75$, while relatedness between worker offspring of a highly polyandrous queen approaches $r_{ww} = 0.25$. Hence, effective paternity (m_e) can be obtained from the relatedness between the workers of a single queen, following Pamilo (1993): $m_e = 0.5 / (g_{ww} - 0.25)$, where g_{ww} is the pedigree relatedness. This approach assumes that the regression worker-worker relatedness (r_{ww}) is identical to the pedigree relatedness (g_{ww}), and hence should only be applied under no inbreeding. KINGROUP is a free open source program implementing a maximum likelihood approach to pedigree relationships reconstruction and kin group assignment (Konovalov et al. 2004). It allows estimating relatedness between offspring and includes a number of features originally found in the program KINSHIP (Goodnight & Queller 1999), which is no longer updated and only runs in the Classic Macintosh OS platform. KINGROUP can be found here: <https://code.google.com/p/kingroup/>

Among the different skew indexes, the B -index (Nonacs 2000) has been proposed as the standard estimate to be used in future studies, since it can be easily calculated from paternity data and shows very robust statistical properties (Nonacs 2003). The B -index can be calculated using the skew calculator available here: <https://www.eeb.ucla.edu/Faculty/Nonacs/PI.html>

2.3. Non-detection and non-sampling errors

Paternity estimates from genetic data are affected by two main types of error: Non-detection and non-sampling errors. The Non-detection Error (NDE) is the probability of two fathering males having identical haplotypes by chance. NDE is determined by the number of markers employed and their level of polymorphism and is an indicator of the resolution of these markers. It should always be reported along with paternity estimates to provide a quantitative measure of accuracy (high NDE s imply a low detection power, and thus paternity estimates might be underestimated). NDE can also be calcu-

lated when estimating the number of matriline in a colony (or the number of reproductive females). Formulae for calculating NDEs are summarized in Table 2.

The Non-sampling Error (*NSE*) estimates the number of males siring offspring remaining undetected because of an insufficient sampling. *NSE* will be affected by the level of paternity skew, and hence needs to be estimated based on the paternity shares of each male and the number of workers analyzed. In species with an observed paternity $K_{obs} = 1$, *NSE* should be calculated to indicate the probability of failing to detect a second male, which fertilized some of the queen's eggs but remained undetected because none of its offspring were sampled. A low *NSE* would indicate that queens are indeed monandrous, as no further males remained undetected because of sampling effects. In species with an observed paternity $K_{obs} > 1$, *NSE* should be calculated to estimate the number of males remaining undetected due to insufficient sampling. To this end, a given frequency distribution (Binomial, Poisson, etc.) can be fitted to the real distribution of workers among patriline. The expected frequencies for each category (number of sired workers) can then be computed, and the number of males remaining undetected because of an insufficient sampling estimated as the expected frequency for the zero or less than one category (Cornuet & Aries 1980; Human et al. 2013). In this case, *NSE* can be accounted for by adding the number of undetected males to observed paternity estimates. Nielsen's effective paternity estimate (Nielsen et al. 2003) is already corrected for sample size, so there is no need for additional corrections for that estimator. Approaches for calculating NSEs are summarized in Table 2.

2.4. Is paternity a continuous or a categorical variable?

Traditionally, paternity has been treated as a categorical variable in the social Hymenoptera, with species being grouped into different paternity or polyandry categories. Boomsma and Ratnieks (1996) first proposed four paternity categories, based on the frequency of multiple paternity among study queens and the mean value of effective paternity

in the study population. Later studies proposed variations of these original categories, and employed either observed paternity (referred to as mating frequency), effective paternity or the frequency of multiple paternity among study queens, as grouping characteristics (see Table 3). To date, however, there is no consensus on how many categories should be used, how to establish the limits between them or which characteristics to use for assigning species into paternity categories.

A recent study retrieved paternity data for 87 polyandrous species of social Hymenoptera (Jaffé et al. 2012). This data set shows that paternity is not normally distributed, with nearly half of all polyandrous species ($N = 46$) showing mean observed paternities below 2 (Fig. 1). Two polyandry categories could be created based on this distribution: low polyandry (mean observed paternity below 2) and high polyandry (mean observed paternity above 2). By so doing, the high polyandry category would merge about half of all polyandrous species ($N = 41$), with mean observed paternities ranging from 2 to 55. Clearly, informative variance would be lost by this grouping, as selective forces differ considerably between species with small colonies and queens that mate with a few males (such as bumble bees), and species with huge colonies and highly polyandrous queens (such as honeybees). Nevertheless, such categorization based on the real frequency distribution of paternity across species is more parsimonious than the creation of categories based on arbitrary assumptions and lacking consensus across studies.

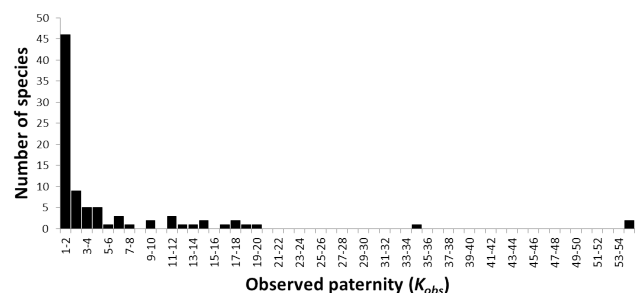


Fig. 1: Frequency distribution of mean observed paternity (K_{obs}) in 87 polyandrous species of social Hymenoptera (data taken from Jaffé et al. 2012).

Table 2: Non-detection and non-sampling errors.

Type of error	Level	Formula	Reference
Patriline non-detection	Population	$(\sum q_i^2)(\sum r_i^2)\dots(\sum z_i^2)$	Boomsma & Ratnieks 1996
Patriline non-detection	Colony	$(q_i)(r_i)\dots(z_i)^\dagger$	Foster et al. 1999
Matriline detection probability	Locus	N/A [€]	Richards et al. 2005
Patriline non-sampling for $K_{obs} = 1$	Colony	$(1 - p)^n \ddagger$	Foster et al. 1999
Patriline non-sampling for $K_{obs} > 1$	Colony	N/A [£]	Human et al. 2013

* q_i are the allele frequencies at the first locus, r_i the allele frequencies at the second locus, and z_i are the allele frequencies at the last locus. This calculation assumes all loci are unlinked and under Hardy-Weinberg equilibrium.

† See corrections for ambiguity in identification of paternal alleles (Foster et al. 1999).

€ Not applicable. See different cases depending on the inheritance of distinct grandparental alleles (Richards et al. 2005).

‡ p is the proportion of offspring sired by the second male (usually set to three values: $p = 0.50$, $p = 0.25$ and $p = 0.10$) and n is the number of worker offspring analyzed.

£ Not applicable. Frequency distribution fitting. For an example see section 4.3.3.5 in (Human et al. 2013).

Table 3: Paternity categories as reported in the literature.

Category	Original description *	Reference
Single paternity	Double mating absent or very rare; population-wide effective mating frequency < 1.05	Boomsma & Ratnieks 1996
Single-Double paternity	Double mating occurs in ca 20%-50% of queens; effective mating frequency 1.05-1.25	
Single-Multiple paternity	Mating frequency above two occurs regularly; effective mating frequency 1.4-2	
Multiple paternity	Mating frequency usually greater than two; effective mating frequency > 2	Oldroyd & Fewell 2007
Polyandry	Multiple mating	
Extreme polyandry	Mating number > 6	Hughes et al. 2008b
Monandry	N/A†	
Facultative low polyandry	Effective mating frequencies of < 2	
Moderate polyandry	Effective mating frequencies of 2–10	
Extreme polyandry	Effective mating frequencies of > 10	
Monandry	N/A†	Hughes et al. 2008a
Facultative low polyandry	< 2 effective mates	
High polyandry	> 2 effective mates	
Singly mated	N/A†	Boomsma et al. 2009
Facultatively multiply mated	Usually $\geq 50\%$ Singly mated with a variable minority of queens mated to 2-5 males	
Obligately multiply mated	Almost always ≥ 2 and often ≥ 5 matings per queen	

* Note that the word “mating” in the original descriptions actually refer to paternity.

† Not applicable

Phylogenetic studies assessing the transition from monandry to polyandry could benefit from categorizing species by their paternity frequency to perform ancestral state reconstruction analyses (Hughes et al. 2008a). Similarly, studies aiming to detect adaptations to polyandry, or the consequences of polyandry for the evolution of other relevant traits, could profit from comparing the traits of interest between highly polyandrous, lowly-polyandrous and monandrous species (Den Boer et al. 2010). However, efforts aiming to detect biologically meaningful associations between paternity frequency and other continuous traits or ecological factors would maximize detection power and avoid losing meaningful variance by employing the actual paternity estimates, as most sexual selection studies do (Simmons 2001). Even though Boomsma (2013) argued that facultative and obligate polyandry appear to be mutually exclusive lineage-specific syndromes, ecological factors could still have shaped the evolution of paternity frequency within as well as across clades (Arnqvist & Nilsson 2000). Hence, treating paternity as a continuous variable could prove more informative to unravel such factors.

3. Future perspectives

The study of polyandry in social insects offers exciting opportunities for future research. Efforts are still needed to understand, for example, how paternity skew has been shaped by the interplay between kin selection and sexual selection (Jaffé et al. 2012). Likewise, the mechanisms and adaptations by which queens and males influence paternity

outcomes are still largely unknown (Baer et al. 2001; den Boer et al. 2009; Den Boer et al. 2010), because sexual selection has been considerably understudied in the social insects (Boomsma et al. 2005). Also, very little is known about the conflicts mediating paternity of sexual offspring (Moritz et al. 2005; Hughes and Boomsma 2008), as most studies have analyzed paternity in worker offspring. Finally, understanding the consequences of polyandry for the evolution of male and female traits could substantially improve current breeding programs of commercial species, such as honeybees. For instance, incorporating male selection into current honeybee breeding programs, which thus far focus exclusively on queen or colony traits (Bienefeld et al. 2008), could substantially increase breeding efficiency by improving drone and sperm quality, assuring high queen mating success, and speeding up the whole process of selecting desirable traits.

Standardizing research methods could aid such future research efforts by preventing common errors, raising confidence in the reported data, and facilitating comparisons between studies. A first step towards this standardization could be to employ a similar terminology and to report comparable paternity estimates, along with their associated error measures. The unification of available software into a common open source platform such as R (R Core Team 2013), could also facilitate analyses as well as enhance collaborative work. Finally, it is very important to make paternity data available through open access data bases or data repositories, so that they can be used in comparative studies and re-analyzed when new analytical tools become available.

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References

- Allendorf, F.W., Hohenlohe, P.A. & Luikart, G. (2010) Genomics and the future of conservation genetics. *Nature Reviews; Genetics*, 11:697-709. doi:10.1038/nrg2844
- Andersson, M. & Simmons, L.W. (2006) Sexual selection and mate choice. *Trends in Ecology and Evolution*, 21 :296-302. doi:10.1016/j.tree.2006.03.015
- Arnqvist, G. & Nilsson, T. (2000) The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behavior*, 60:145-164. doi:10.1006/anbe.2000.1446
- Baer, B. (2011) The copulation biology of ants (Hymenoptera: Formicidae). *Myrmecological News*, 14:55-68
- Baer, B., Armitage, S.A.O. & Boomsma, J.J. (2006) Sperm storage induces an immunity cost in ants. *Nature*, 441 (7095):872-875. doi:10.1038/nature04698
- Baer, B., Morgan, E.D. & Schmid-Hempel, P. (2001) A non-specific fatty acid within the bumblebee mating plug prevents females from remating. *Proceedings of the National Academy of Sciences, USA*, 98 (7):3926-3928. doi:10.1073/pnas.061027998
- Baer, B. & Schmid-Hempel, P. (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumblebee. *Nature*, 397 (6715):151-154. doi:10.1038/16451
- Bienefeld, K., Ehrhardt, K. & Reinhardt, F. (2008) Bee breeding around the world - Noticeable success in honey bee selection after the introduction of genetic evaluation using BLUP. *American Bee Journal*, 148 (8):739-742
- Boomsma, J.J. (2009) Lifetime monogamy and the evolution of eusociality. *Philosophical Transactions of the Royal Society B*, 364(1533): 3191-3207. doi:10.1098/rstb.2009.0101
- Boomsma, J.J. (2013) Beyond promiscuity: mate-choice commitments in social breeding. *Philosophical Transactions of the Royal Society B*, 368(1613): 20120050 doi:10.1098/rstb.2012.0050
- Boomsma, J.J., Baer, B. & Heinze, J. (2005) The evolution of male traits in social insects. *Annual Review of Entomology*, 50:395-420. doi:10.1146/annurev.ento.50.071803.130416
- Boomsma, J.J., Kronauer, D.J.C. & Pedersen, J.S. (2009) The evolution of social insect mating systems. In: Gadau J, Fewell J, Wilson EO (eds) *Organization of insect societies: from genome to sociocomplexity*. Harvard University Press, Cambridge, Mass., pp 3-25
- Boomsma, J.J. & Ratnieks, F.L.W. (1996) Paternity in eusocial Hymenoptera. *Philosophical Transactions of the Royal Society B*, 351 (1342): 947-975. doi:10.1098/rstb.1996.0087
- Bourke, A.F.G. (1999) Colony size, social complexity and reproductive conflict in social insects. *Journal of Evolutionary Biology*, 12(2) :245-257. doi:10.1046/j.1420-9101.1999.00028.x
- Cole, B.J. (1983) Multiple mating and the evolution of social behavior in the Hymenoptera. *Behavioral Ecology and Sociobiology*, 12(3):191-201. doi:10.1007/bf00290771
- Cornuet, J.-M. & Aries, F. (1980) Number of sex alleles in a sample of honeybee colonies. *Apidologie*, 11(1):87-93. doi:10.1051/apido:19800110
- Crozier, R.H. & Fjerdingstad, E.J. (2001) Polyandry in social Hymenoptera - disunity in diversity? *Annales Zoologici Fennici*, 38 (3-4):267-285
- Den Boer, S.P.A., Baer, B. & Boomsma, J.J. (2010) Seminal fluid mediates ejaculate competition in social insects. *Science*, 327 (5972):1506-1509. doi:10.1126/science.1184709
- den Boer, S.P.A., Boomsma, J.J. & Baer, B. (2009) Honey bee males and queens use glandular secretions to enhance sperm viability before and after storage. *Journal of Insect Physiology*, 55 (6):538-543. doi:10.1016/j.jinsphys.2009.01.012
- Eberhard, W.G. (1996) *Female control: sexual selection by cryptic female choice*. Monographs in behavior and ecology. Princeton University Press, Princeton, NJ.
- Ellegren, H. (2013) Genome sequencing and population genomics in non-model organisms. *Trends in Ecology and Evolution*, in press (0). doi:10.1016/j.tree.2013.09.008
- Foster, K.R., Seppä, P., Ratnieks, F.L. & Thorén, P.A. (1999) Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps. *Behavioral Ecology and Sociobiology*, 46 (4):252-257
- Goodnight, K.F. & Queller, D.C. (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Molecular Ecology*, 8 (7):1231-1234. doi:10.1046/j.1365-294x.1999.00664.x
- Hamilton, W.D. (1964) *Genetical Evolution of Social Behaviour I & II*. *Journal of Theoretical Biology*, 7 (1):1-16, 17-52
- Hughes, W.O.H. & Boomsma, J. (2008) Genetic royal cheats in leaf-cutting ant societies. *PNAS*, 105 (13):5150-5153. doi:10.1073/pnas.0710262105
- Hughes, W.O.H. & Boomsma, J.J. (2006) Does genetic diversity hinder parasite evolution in social insect colonies? *Journal of Evolutionary Biology*, 19 (1):132-143. doi:10.1111/j.1420-9101.2005.00979.x

- Hughes, W.O.H., Oldroyd, B.P., Beekman, M. & Ratnieks, F.L.W. (2008a) Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science*, 320 (5880):1213-1216. doi:10.1126/science.1156108
- Hughes, W.O.H., Ratnieks, F.L.W. & Oldroyd, B.P. (2008b) Multiple paternity or multiple queens: two routes to greater intracolony genetic diversity in the eusocial Hymenoptera. *Journal of Evolutionary Biology*, 21 (4):1090-1095. doi:10.1111/j.1420-9101.2008.01532.x
- Hughes, W.O.H., Sumner, S., Van Borm, S. & Boomsma, J.J. (2003) Worker caste polymorphism has a genetic basis in *Acromyrmex* leaf-cutting ants. *PNAS*, 100 (16):9394-9397. doi:10.1073/pnas.1633701100
- Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J.D., Forsgren, E., Fries, I., Hatjina, F., Hu, F.L., Jaffé, R., Bruun Jensen, A.B., Köhler, A., Magyar, J.P., Özkýrým, A., Pirk, C.W.W., Robyn Rose, R., Strauss, U., Tanner, G., Tarpy, D.R., van der Steen, J.J.M., Vaudo, A., Fleming Vejsnæs, F., Wilde, J., Williams, G.R. & Zheng, H.Q. (2013) Miscellaneous standard methods for *Apis mellifera* research. *Journal of Apicultural Research*, 52 (4):1-56. doi:10.3896/IBRA.1.52.4.10
- Jaffé, R., Garcia-Gonzalez, F., den Boer, S.P.A., Simmons, L.W. & Baer, B. (2012) Patterns of paternity skew among polyandrous social insects: What can they tell us about the potential for sexual selection? *Evolution*, 66 (12):3778-3788. doi:10.1111/j.1558-5646.2012.01721.x
- Jaffé, R., Kronauer, D.J., Kraus, F.B., Boomsma, J.J. & Moritz, R.F.A. (2007) Worker caste determination in the army ant *Eciton burchellii*. *Biology Letters*, 3 (5):513-516. doi:10.1098/rsbl.2007.0257
- Jaffé, R., Pioker-Hara, F., Santos, C., Santiago, L., Alves, D., M. P. Kleinert, A., Franco, T., Arias, M. & Imperatriz-Fonseca, V. (2014) Monogamy in large bee societies: a stingless paradox. *Naturwissenschaften*:1-4. doi:10.1007/s00114-014-1149-3
- Jones O.R. & Wang J. (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, 10: 551-555. doi:10.1111/j.1755-0998.2009.02787.x
- Keller, L. & Reeve, H.K. (1994) Genetic Variability, Queen Number, and Polyandry in Social Hymenoptera. *Evolution*, 48 (3):694-704
- Konovalov, D.A., Manning, C. & Henshaw, M.T. (2004) KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. *Molecular Ecology Notes*, 4 (4):779-782. doi:10.1111/j.1471-8286.2004.00796.x
- Kraus, F.B. & Moritz, R.F. (2010) Extreme polyandry in social Hymenoptera: evolutionary causes and consequences for colony organisation. In: *Animal Behaviour: Evolution and Mechanisms*. Springer, pp 413-439
- Kraus, F.B., Neumann, P., van Praagh, J. & Moritz, R.F.A. (2004) Sperm limitation and the evolution of extreme polyandry in honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology*, 55 (5):494-501. doi:10.1007/s00265-003-0706-0
- Kvarnemo, C. & Simmons, L.W. (2013) Polyandry as a mediator of sexual selection before and after mating. *Philosophical Transaction of the Royal Society B*, 368 (1613). doi:10.1098/rstb.2012.0042
- Lopez-Vaamonde, C., Koning, J.W., Brown, R.M., Jordan, W.C. & Bourke, A.F. (2004) Social parasitism by male-producing reproductive workers in a eusocial insect. *Nature*, 430 (6999):557-560. doi:10.1038/nature02769
- Mattila, H.R. & Seeley, T.D. (2007) Genetic diversity in honey bee colonies enhances productivity and fitness. *Science*, 317(5836): 362-364. doi:10.1126/science.1143046
- Moilanen, A., Sundstroem, L. & Pedersen, J.S. (2004) MATESOFT: a program for deducing parental genotypes and estimating mating system statistics in haplodiploid species. *Molecular Ecology Notes*, 4: 795-797. doi:10.1111/j.1471-8286.2004.00779.x
- Moritz, R.F.A., Michael, H., Lattorff, G., Neumann, P., Kraus, F.B., Radloff, S.E. & Hepburn, H.R. (2005) Rare royal families in honeybees, *Apis mellifera*. *Naturwissenschaften*, 92 (11):548-548. doi:10.1007/s00114-005-0054-1
- Neumann, P., Moritz, R.F.A. & Mautz, D. (2000) Colony evaluation is not affected by drifting of drone and worker honeybees (*Apis mellifera* L.) at a performance testing apiary. *Apidologie*, 31:67-79. doi:10.1051/apido:2000107
- Nielsen, R., Tarpy, D.R. & Reeve, H.K. (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population. *Molecular Ecology*, 12: 3157-3164. doi:10.1046/j.1365-294X.2003.01994.x
- Nonacs, P. (2000) Measuring and Using Skew in the Study of Social Behavior and Evolution. *American Naturalist*, 156 (6):577-589. doi:10.1086/316995
- Nonacs, P. (2003) Measuring the reliability of skew indices: is there one best index? *Animal Behavior*, 65 (3):615-627. doi:10.1006/anbe.2003.2096
- Oldroyd, B.P. & Fewell, J.H. (2007) Genetic diversity promotes homeostasis in insect colonies. *Trends in Ecology and Evolution*, 22:408-413. doi:10.1016/j.tree.2007.06.001
- Page, R.E. & Metcalf, R.A. (1982) Multiple mating, sperm utilization, and social evolution. *American Naturalist*, 119: 263-281
- Palmer, K.A. & Oldroyd, B.P. (2000) Evolution of multiple mating in the genus *Apis*. *Apidologie*, 31 (2):235-248.

doi:10.1051/apido:2000119

Pamilo, P. (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. *Heredity*, 70 (5):472-480. doi:10.1038/hdy.1993.69

Paxton, R.J., Bego, L.R., Shah, M.M. & Mateus, S. (2003) Low mating frequency of queens in the stingless bee *Scaptotrigona postica* and worker maternity of males. *Behavioral Ecology and Sociobiology*, 53:174-181. doi:10.1007/s00265-002-0561-4

R Core Team (2013) R: A Language and Environment for Statistical Computing. 3.0.2 edn. R Foundation for Statistical Computing, Vienna, Austria

Richards, M.H., French, D. & Paxton, R.J. (2005) It's good to be queen: classically eusocial colony structure and low worker fitness in an obligately social sweat bee. *Molecular Ecology*, 14 (13):4123-4133. doi:10.1111/j.1365-294X.2005.02724.x

Sanetra, M. & Crozier, R.H. (2001) Polyandry and colony genetic structure in the primitive ant *Nothomyrmecia macrops*. *Journal of Evolutionary Biology*, 14 (3):368-378. doi:10.1046/j.1420-9101.2001.00294.x

Schmid-Hempel, P. (1998) *Parasites in social insects*. Princeton University Press, Princeton, USA.

Seeley, T.D. & Tarpay, D.R. (2007) Queen promiscuity lowers disease within honeybee colonies. *Proceedings of the Royal Society of London B Biol Sci*, 274(1606):67-72. doi:10.1098/rspb.2006.3702

Simmons, L.W. (2001) *Sperm competition and its evolutionary consequences in the insects*. Monographs in behavior and ecology. Princeton University Press, Princeton, USA.

Smith, C.R., Toth, A.L., Suarez, A.V. & Robinson, G.E. (2008) Genetic and genomic analyses of the division of labour in insect societies. *Nature Reviews; Genetics*, 9 (10):735-748. doi:10.1038/Nrg2429

Wenseleers, T. & Ratnieks, Francis L.W. (2006) Comparative Analysis of Worker Reproduction and Policing in Eusocial Hymenoptera Supports Relatedness Theory. *American Naturalist*, 168 (6):E163-E179. doi:10.1086/508619

