

## Proximate and ultimate consequences of polyandry in ants (Hymenoptera: Formicidae)

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### Abstract

The process of partner choice and mating is of fundamental importance in ants, because queens copulate only during a very short time window early in their lives prior to egg-laying and eusocial life. As a consequence, several key characteristics of a queen's later emerging colony are defined during these short and early mating events. For example, the number of sperm a queen initially stores determines her total fecundity and therefore limits the size and longevity of monogynous ant societies. A key reproductive behaviour of some queens is that they copulate with more than one male, resulting in postcopulatory sexual selection if ejaculates compete against each other for access to the limited sperm storage space in the spermatheca. Furthermore, polyandrous queens could discriminate against unwanted males and their ejaculates, and thereby manipulate paternities in their own interest, for example, to increase worker relatedness. If sperm from more than a single male becomes stored and used, genetic heterogeneity among helpers increases, which has a number of well documented beneficial effects. However, multiple paternity can also generate costs if helping incentive decreases due to lower inclusive fitness returns for workers. Polyandry can also alter conflicts within insect societies, for example over the sex ratios preferred by queens and workers. Here I consolidate our current knowledge of how early decisions of partner choice and mating in polyandrous ants impact the life history of queens and males as well as their influence on the performance and fitness of the later emerging colony.

**Key words:** Multiple mating, sperm competition, female choice, genetic diversity, social evolution, kin selection, review.

Myrmecol. News 22: 1-9 (online 24 September 2015)

ISSN 1994-4136 (print), ISSN 1997-3500 (online)

Received 7 April 2015; revision received 6 July 2015; accepted 8 July 2015

Subject Editor: Timothy A. Linksvayer

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## Introduction

### The reproductive biology of social insects

Together with the eusocial bees, wasps and termites, ants are a remarkably successful group of insects. Their social life style evolved quite a long time ago in the early Cretaceous period more than 100 million years ago (PERRICHOT & al. 2008). Since then, ants have become a phylogenetically large group consisting of more than 12,000 species, found on all continents except the Antarctic. Furthermore, some ants have become ecological key species or are able to dominate entire ecosystems (WEBER 1972, HÖLLDOBLER & WILSON 1990). This success story of ants is undoubtedly linked to their social life style, which is found in all species known to date. Sociality allows ants to live in groups comprising a higher level of organization, similar to the cells of a metazoan body. Individual colony members benefit from helping and giving up their own reproduction if they support related individuals and thereby increase their inclusive fitness (HAMILTON 1964, TRIVERS & HARE 1976). Ant societies are characterized by the presence of only one or very few reproductive animals, referred to as queens or gamergates, which increases relatedness and thereby helping incentive among colony members. Furthermore, ant queens perform only a single round of partner choice and copulation(s) prior to egg laying, and the resulting "marriages for life" make ant societies closed

genetic systems where no or very little additional genetic contributions are accepted after this initial mating episode (BOOMSMA & al. 2005, BAER 2011, 2014). These reproductive characteristics have two remarkable consequences:

First, queens had to evolve a number of reproductive traits that are rarely or never found in other animals (BAER 2011), such as the ability to store large numbers of sperm and to keep them alive for up to several decades (PAMILO 1991, KELLER & GENOUD 1997, DEN BOER & al. 2009). Furthermore, because sperm is never replenished later in life, ant queens had to become highly economic in their usage of sperm in order to produce a large number of female offspring. To achieve this, they had to maximize their fecundity by minimizing the number of sperm used per egg fertilisation (DEN BOER & al. 2009). Ant males, on the other hand, had to evolve ejaculates consisting of large numbers of high quality sperm to accommodate the fertility demands imposed by queens and to enhance their chance to sire new gynes (HUNTER & BIRKHEAD 2002, STURUP & al. 2014a).

Secondly, key parameters of the later emerging society are determined during a brief initial mating process, which cannot be modified once partner choice and matings have been concluded. I find this to be one of the most intriguing characteristics of social insects: It implies that the colonies consisting of millions of workers as found

Box 1: Definition of selected terms.

**Cryptic female choice:** Females biasing paternity by discriminating against ejaculates of unwanted males. The processes are cryptic because they occur inside the female's sexual tract and typically after copulations, so in the absence of males.

**Genetic diversity:** Genetically determined variation in worker offspring, measured as the average relatedness among all helpers within a colony.

**Monandry:** A female (queen) copulating once and with a single male.

**Multiple paternity:** Worker offspring is sired by more than one male.

**Paternity skew:** The relative contributions of different fathers to offspring. Paternity skew can range between 0 and 1, where 0 indicates that all males are equally represented in offspring and 1 that a single male monopolises paternity (i.e., single paternity).

**Polyandry:** A female (queen) copulating with more than one male.

**Postcopulatory sexual selection:** Processes that bias paternity after the successful transfer of ejaculates to females.

**Sexual conflict:** Conflicts arising within and between the sexes over paternity.

**Single paternity:** All offspring within a colony is sired by a single male.

**Sperm competition:** The competition between ejaculates of several males for access to sperm storage organs and eggs.

for example in leaf-cutter or army ants (WEBER 1972, KRONAUER 2009) are all headed by a single queen that engaged in a mating event years to decades earlier when the total number of colony members and their relatedness became fixed within a time span of minutes to a few hours. As we will see later, this fundamentally impacts the performance and fitness of these colonies.

Given the obvious importance of mating for ant queens and their societies, we can therefore define the events occurring during the nuptial flight(s) as a series of proximate processes that first affect the life history of queens and males but ultimately impact the later emerging colony through helper offspring. The latter is driven by paternity, which has been intensively studied in ants and other eusocial insects, especially to test and elaborate inclusive fitness theory (BOOMSMA & RATNIEKS 1996, BOOMSMA & al. 2005, BOOMSMA 2009, 2013). The reproductive biology of social insects, i.e., the processes that initially determine paternity during mating events received less attention although several key studies published in recent years provided intriguing new insights into the processes that occur during and shortly after copulations (DUVOISIN & al. 1999, ALLARD & al. 2002, BAER 2005, ALLARD & al. 2006, ALLARD & al. 2007, OPPELT & HEINZE 2007, BAER 2011, IZZO & TIBBETTS 2012) offering first insights into the links between mating system – and social evolution.

### Polyandry

A key reproductive feature of social insect queens that has received intense scientific attention is their mating frequency, i.e., the number of copulations they accept with different males. Polyandry can – but does not have to – result in multiple paternity among helpers, which reduces their inclusive fitness and helping incentive. A substantial amount of experimental and theoretical work therefore investigated the potential costs and benefits resulting from multiple paternity in social insects (PAGE 1986). As a result, polyandry became recognized as a fundamental game changer for social insects influencing the life history of

queens and their societies. Polyandry is remarkably common in social insects and has been found in about a third of all species (HUGHES & al. 2008, BAER 2011), but paternities detected in offspring are often substantially lower than expected from observed copulation frequencies of queens (BOOMSMA & RATNIEKS 1996, BAER 2011, 2014). This mismatch implies the presence of sexual selection in these species, excluding or biasing individual paternal contributions during and shortly after mating (JAFFE & al. 2012).

I start this review by defining key technical terms used throughout this manuscript, because I found them to be used inconsistently in the published literature (Box 1, see also JAFFE 2014). I then summarise our present knowledge about the consequences of polyandry for social insects, both on the proximate level impacting the reproductives as well as on the ultimate level in eusocially living offspring (Tab. 1). Finally I link these two chapters and point out some broader opportunities for future research.

### Consequences of polyandry for males and queens:

A key consequence of polyandry is that ejaculates of competing males coexist within the sexual tract of the female where competition can arise among sperm for access to storage organs or eggs, which is known as sperm competition (PARKER 1970, BIRKHEAD & MOLLER 1998, SIMMONS 2001, BIRKHEAD & al. 2009, SHUKER & SIMMONS 2014). Compared to other insects, sperm competition remains understudied in social insects despite good evidence that it evolved in a number of species including ants (see BAER 2014: tab. 1). For example, in the leaf-cutting ants *Atta colombica* GUÉRIN-MÉNEVILLE, 1844 and *Acromyrmex echinator* (FOREL, 1899), ejaculates contain glandular secretions, known as seminal fluid, which are able to recognise and kill sperm of rival males (DEN BOER & al. 2010). This is known as sperm incapacitation and is also found in the polyandrous honeybee *Apis mellifera* LINNAEUS, 1758 but – as can be expected – is absent in closely related monandrous ant and bee species (DEN BOER & al. 2010). Sperm competition seems to have a substantial impact on male reproductive investments, such as for example

Tab. 1: Consequences of polyandry for social insects. I here distinguish between proximate effects that occur prior, during, or shortly after mating and influence paternity distributions, and ultimate effects, being long term consequences of mating that become effective in offspring and impact colony performance.

<b>Proximate effects of polyandry</b>	Sexual selection	Precopulatory	Male-male competition
			Female choice
		Postcopulatory	Sperm competition
			Cryptic female choice
	Queen life history	Survival	Male influence on queen survival during or shortly after mating
		Ecology	Fitness costs for search of additional partners
Colony foundation		Sperm storage costs trade off with other life history traits such as immunity	
<b>Ultimate effects of polyandry</b>	Genetic diversity in helpers	Parasitism	Reduced parasitism in colonies with multiple paternity
		Division of labor	Genetically based differences in task performance between patrines
		Kin conflicts	Nepotism
		Colony homeostasis	Increased abilities to adapt to ecological perturbations
		Queen / worker production	Inter-lineage mating required for the production of workers in <i>Pogonomyrmex</i> ants
	Inbreeding	Diploid male production	Reduced costs of matched matings for individual queens
	Parasite load	Sexually transmitted diseases	Sexually transmitted diseases are more frequent in polyandrous species, and queens increase risk of infection with every additional copulation
	Reproductive success	Sex ratios	Changes in queen worker conflict over sex ratios in reproductive offspring

their accessory glands that produce mating plugs as part of the seminal fluid (MIKHEYEV 2004). In attine ants for example, males of polyandrous species have significantly smaller glands and larger sperm containing accessory testes (BAER & BOOMSMA 2006) compared to males of monandrous species. This implies that the loss of male power to control female mating frequencies through mating plugs resulted in increased investments into sperm numbers within ejaculates. This can intensify sperm competition and / or provide queens with more sperm and hence allow them to maintain larger and longer-lived colonies. The presence of sperm competition is also implicated by the presence of cooperative sperm behaviours such as the formation of bundles and / or trains, which have most recently been reported in several ant species such as in *Crematogaster victima* SMITH, 1858 (see OLIVEIRA & al. 2014) and *Lasius pallitarsis* (PROVANCHER, 1881) (see BURNETT & HEINZE 2014). In the desert ant *Cataglyphis savignyi* (DUFOR, 1862), males produce sperm in bundles which have a 51% faster swimming speed compared to individual sperm (PEARCY & al. 2014). Such cooperative behaviours might in fact be much more widespread than acknowledged so far, as haploid males produce clonal sperm and any form of intra-ejaculatory competition is therefore absent. Sperm competition can also result in males transferring substantially more sperm to queens than can be stored. The best example known so far is the honeybee *Apis mellifera*, where queens copulate with large numbers of males in quick succession, but only 3 - 5% of the sperm will eventually be transferred to the spermatheca (BAER 2005).

The presence of multiple ejaculates within the female's sexual tract also offers queens opportunities to manipulate paternity – and consequently worker relatedness. This is known as cryptic female choice (EBERHARD 1996), and similar to sperm competition, has not been studied in detail in social insects. Available data indicate that it could be a widespread phenomenon because it can provide queens with the necessary mechanisms to manipulate paternity and helper relatedness (see for example BAER 2015: tab. 1). In the leaf-cutting ant *Atta colombica* for example, glandular secretions that queens provide to ejaculates target those molecules in the seminal fluid that are responsible for sperm incapacitation (DEN BOER & al. 2008). Consequently, queens have ultimate control over sperm competition and storage in this species. Ant queens also possess morphological structures in their sexual tract that allow them to control sperm access to the spermatheca. In the ant *Leptothorax gredleri* MAYR, 1855 for example, the spermathecal duct consists of a very narrow duct that only allows single sperm to pass (OPPELT & HEINZE 2007) and the filling of the spermatheca therefore requires several hours. This morphological structure could be used to reduce the number of males siring offspring, because queens are polyandrous but only sperm of a single male is used to sire offspring (OBERSTADT & HEINZE 2003). Mechanisms allowing queens to select sperm can also be expected in some populations of *Pogonomyrmex* harvester ants (HOSKEN & PITNICK 2003), because colonies depend on queens storing sperm from males of their own as well as a different genetic lineage (HERRMANN & CAHAN 2014). Because

intra-lineage sperm is used to produce queen offspring and inter-lineage sperm sires worker offspring, queens need not only sperm from both lineages, but also in those specific proportions that ensure the required ratios of worker and queen offspring.

Apart from sexual selection, polyandry has a range of other effects on males and queens, which substantially influence their life history, but remarkably little empirical work has been conducted to study these in more detail. For example, polyandry can increase the total number of sperm stored in the spermatheca, so queens are able to produce more fertilised eggs. In the leaf-cutter ant *Atta colombica* and the honeybee *Apis mellifera*, the number of sperm found in the storage organ is higher in queens that mated with more males (FJERDINGSTAD & BOOMSMA 1998, SCHLÜNS & al. 2005, BAER & al. 2006). However, the number of matings was a much better predictor for queen immunity in *A. colombica* (BAER & al. 2006) and queen hibernation success and survival in *Bombus terrestris* (BAER & SCHMID-HEMPEL 2005) than sperm number, indicating that polyandry per se induces fitness costs for queens. Such costs could for example be caused by a queen's response to control competition between rival ejaculates. Consequently, sperm number and polyandry both incur physiological costs for queens that trade off with other life history traits (Fig. 1) (BAER & al. 2006). Consequently polyandry can result in substantial short-term mating costs for queens that impact their survival and colony foundation success.

Apart from physiological costs resulting from storing and maintaining sperm, polyandry can also inflict copulation costs, for example if males physically damage females. Such physical injuries are known from other insects (see, for example, CRUDGINGTON & SIVA-JOTHY 2000, SIVA-JOTHY & STUTT 2003) and could evolve in social insects under sexual selection if males fight for access to females and therefore impose fitness costs for copulations on queens to lower their incentive to re-mate. However, such damages could also be a by-product resulting from natural selection to maximise the transfer of large amounts of high quality sperm. In attine ants for example, male genitalia possess two rows of sclerotized sharp teeth (Fig. 2) that they use to get firmly attached to a specialised organ in the queen's sexual tract, known as the mussel organ (BAER & BOOMSMA 2006, HIMLER & al. 2009). As males do not access the queen's bursa copulatrix with their reproductive organs, a successful transfer of sperm depends on a correct and firm positioning of the genitals of the male and the queen. Mussel organs of mated queens show marks of the males' sclerotized teeth and an immune response by the queen (Fig. 2). Unfortunately, the fitness costs associated with these damages have not been quantified, and it is unknown whether these mating costs increase with additional copulations.

Mating costs could also increase for polyandrous queens if pathogens hitchhike on the copulation process for host transmission (KNELL & WEBBERLEY 2004). Polyandry is known to increase a host's risk to get infected with a sexually transmitted disease, and such diseases are indeed more abundant in polyandrous compared to monandrous species (KNELL & WEBBERLEY 2004). Sexually transmitted diseases are still poorly studied in social insects, but several pathogens have been described in the honeybee *Apis melli-*



Fig. 1: Too much love will kill you: Mating is associated with substantial physiological costs for queens in the leaf-cutter ant *Atta colombica* that trade off with other life history traits. As queens copulate with more males and store more sperm they become increasingly unable to upregulate their immune systems during colony foundation and – as pictured here – fall victim to parasites and pathogens attacking them (Photo by the author).



Fig. 2: (a) During mating, males of most species of leaf-cutting ants use specific rows of sclerotized teeth that are part of their external sclerotized genitalia (for more details, see BAER & BOOMSMA 2006) to establish a firm contact with the queen during copulation. To achieve this, queens possess a specific organ known as the mussel organ. (b) The mussel organs of mated queens show marks of copulation and signs of a female's immune response as indicated by the presence of lines of black dots in underlying tissue indicating melanization, as is typically found after wound healing in insects. Photos taken by the author (*Atta colombica*).

*fera* to use the copulation process for horizontal transmission (YUE & al. 2006, YUE & al. 2007, DE MIRANDA & FRIES 2008, PENG & al. 2015, ROBERTS & al. 2015). Polyandry can also invoke substantial ecological costs, if queens searching for and engaging in additional copulations expose themselves for longer periods of time to po-

tentially dangerous environments and predation. Copulation duration can be quite long in social insects including ants (BAER 2011), implying that such costs can be significant. Future work is therefore needed to quantify how mating costs are affected with increasing copulation frequencies of queens. Because mortality rates during and shortly after nuptial flights can be excessively high (WEBER 1972, HÖLLDOBLER & WILSON 1990, BAER & al. 2006), mating costs seem to be substantial in social insects and therefore worth to be studied in more detail.

In summary, the process of mating, sperm transfer and sperm storage in social insects is a highly complex process, where reproductive traits of males and queens are under natural selection to maximize fecundity and sexual selection to bias paternity, thereby generating variation in the reproductive success of males and queens. Polyandry can substantially impact costs and benefits of reproductive traits and the question arises of how polyandry eventually impacts emerging offspring and their eusocial colonies.

**Consequences of polyandry for colonies:** The hatching of the first workers marks a key transition in a queen's life to her eusocial life phase. As helpers emerge and start building the colony, the outcomes of the processes that occurred during mating become increasingly more visible. In many ants, bees and wasps, colony longevity and size are dependent on a single queen's survival and fecundity. As already pointed out, queens storing more sperm as a result of additional copulations can produce more fertilised eggs (COLE 1983, KRAUS & al. 2004) and thereby provide long-term advantages of polyandry despite the initial costs encountered (BAER & SCHMID-HEMPEL 2001). The exceptionally large colonies of monogynous ants (*Atta*, *Acromyrmex*, *Eciton*, *Dorylus*, *Pogonomyrmex*), bees (*Apis*) and wasps (*Vespa*, *Vespula*) are all headed by polyandrous queens, implying that polyandry is a key reproductive trait in these species and required to build and maintain these remarkable megasocieties (COLE 1983, KELLER & REEVE 1994).

Polyandry resulting in the storage and use of sperm from multiple males impacts the colonies' genetic architecture, which is determined by (1) the number of fathers present in worker offspring (measured as observed paternity) (BOOMSMA & RATNIEKS 1996), (2) their relative contributions to offspring (paternity skew) (JAFFE & al. 2012, JAFFE 2014), and (3) temporal variation in paternity between the different fathers (SCHLÜNS & al. 2004, STURUP & al. 2014b). All these factors influence offspring genetic diversity, which has been intensively studied and shown to entail multiple advantages that increase colony performance and fitness.

Genetic diversity seems specifically important in species that produce large colonies (COLE 1983, SCHMID-HEMPEL 1998). In the harvester ant *Pogonomyrmex occidentalis* (CRESSON, 1865) for example, low relatedness in workers correlates with faster growth of colonies and a 35-fold increase in fitness of polyandrous colonies (COLE & WIERNASZ 1999). Several studies investigated the underlying mechanisms that provide polyandrous colonies with such selective advantages, although most of this work was done in honeybees and bumblebees. There is now ample empirical evidence for beneficial effects of genetic diversity, such as for example a reduction in parasitism (SHYKOFF & SCHMID-HEMPEL 1991, BAER & SCHMID-HEMPEL 1999,

TARPY 2003, HUGHES & BOOMSMA 2005), partially driven by differences in disease susceptibility between patriline (BAER & SCHMID-HEMPEL 2003, PALMER & OLDROYD 2003, HUGHES & BOOMSMA 2005). Genetic diversity can also enhance task performance of workers, thereby increasing colony performance and fitness (CROZIER & PAGE 1985, MATTILA & SEELEY 2007, OLDROYD & FEWELL 2007), and there is empirical evidence that individual patrilines differ in task preference and / or performance (HUGHES & al. 2003, CHAPMAN & al. 2007, KRAUS & al. 2011, EYER & al. 2013). Polyandry can also reduce the impact of inbreeding depression: Mating between related individuals in ants, bees and wasps can result in the production of diploid offspring that are homozygous at the sex determining locus (HEIMPEL & DE BOER 2008). These animals are known as diploid males, and are generally assumed to be a genetic burden for their colonies because they can be non-viable, of low quality, sterile and do not provide any work, despite the fact that they originate from fertilised eggs (ROSS & FLETCHER 1986, BEYE & al. 2003, GERLOFF & al. 2003, GERLOFF & SCHMID-HEMPEL 2005, ARMITAGE & al. 2010). Polyandry can reduce the variance in diploid male production among colonies (PAGE 1980) and thereby reduce the costs of matched matings for individual queens, although this depends on non-linear costs that are associated with reduced brood viability, i.e., the relationship between brood viability and colony fitness has to be concave (TARPY & PAGE 2002).

Polyandry also alters reproductive conflicts over preferred sex ratios in offspring. In colonies with a single monandrous queen, workers are three times more related to their sisters ( $r = 0.75$ ) compared to their brothers ( $r = 0.25$ ) and therefore prefer a female (queen) biased sex ratio. Queens on the other hand are equally related to sons and daughters and therefore prefer equal sex ratios (RATNIEKS & al. 2006). In colonies that are headed by polyandrous queens, the preferred sex ratio of workers becomes increasingly less biased towards females. There is empirical evidence for sex ratio manipulations of workers to maximise their inclusive fitness in the ant *Formica truncorum* FABRICIUS, 1804, where workers alter sex ratios towards males if their queen sires offspring from multiple males (SUNDSTRÖM 1994). Finally, genetic heterogeneity in helper offspring has also been found to make colonies less susceptible to environmental changes and to increase homeostasis of these societies (OLDROYD & FEWELL 2007).

Apart from such advantages, polyandry can also incur costs as a consequence of reproductive conflicts (BAER & SCHMID-HEMPEL 2001). As polyandrous insect societies become chimeras consisting of different half-sister groups, relatedness among helpers decreases with increasing levels of multiple paternity. As a consequence of decreasing inclusive fitness returns, workers should eventually develop their own ovaries to lay non-fertilised eggs developing into males. Worker reproduction is indeed widespread in social insects, resulting in the evolution of policing, where workers remove eggs not laid by the queen (VISSCHER 1998, RATNIEKS & al. 2006). The fitness costs associated with worker reproduction and policing in polyandrous social insect colonies have never been quantified, something that should be studied in more detail in the future. They could be significant though, as in the wasp *Dolichovespula saxonica* (FABRICIUS, 1793), policing is only observed in colo-

nies with multiple paternity but is absent in colonies where only a single male sires offspring (FOSTER & RATNIEKS 2000). However, the methodology of this study was consequently questioned and a follow up investigation failed to confirm the initial findings (BONCKAERT & al. 2011). Conflicts between different sire groups can also result in nepotism, if workers are able of kin recognition and only support their (full) sisters. There is some empirical support for the presence of nepotism in social insects (VISCHER 1998, RATNIEKS & al. 2006), although a majority of studies found no or only weak effects of kin recognition and / or nepotism in social insects (RANGEL & al. 2009, KELLNER & HEINZE 2011, FRIEND & BOURKE 2012, BOOMSMA & D'ETTORRE 2013).

In summary, polyandry resulting in multiple paternity has substantial impact on eusocial life, but the fine-tuned interactions that are expected to impact costs and benefits of genetic diversity and inclusive fitness gains deserve more scientific attention. Expanding our knowledge in this area is important because trade-offs between these factors might be a major driver for the observed variation in mating systems among social insects.

## Conclusions

Relatedness is a key driver of altruistic helping behaviour (HAMILTON 1964, TRIVERS & HARE 1976) and is determined during a short mating period early in life in eusocial insects. This implies a co-evolutionary dynamics, where the social lifestyle of these insects influenced the evolution of their mating biology and vice versa and thereby favoured reproductive traits such as partner commitments for life (BOOMSMA 2007, 2009). In the hymenopteran social insects, the latter resulted in selection for large ejaculates of maximal quality; long-term sperm storage; exceptional sperm use economy and the evolution of exceptionally long life spans of reproductive females (WEBER 1972, PAMILO 1991, KELLER & GENOUD 1997). Because these traits evolved in response to the social life style of these species, it is not surprising that they are rarely or never found in non-social animals.

However, reproductive traits could also have influenced social evolution, if they offered the necessary mechanisms to store more sperm or to bias paternities, thereby determining relatedness and inclusive fitness returns of helpers. Such intimate interplay between sociality and reproduction should be studied in more detail in the future, especially on the experimental side. We have good empirical support that single paternity is an ancestral reproductive characteristic of hymenopteran social insects (HUGHES & al. 2008), which is predicted by kin selection theory (BOOMSMA & al. 2011) and maximises relatedness among helpers and their inclusive fitness returns (BOOMSMA 2009). Whether the queens of these early social insects were also singly mated remains to be investigated, because the phylogenetic analyses conducted so far studied paternity rather than queen mating frequencies. This differentiation is important because kin selection theory allows us to understand the importance of paternity (relatedness) on eusocial life, but does not predict any specific mating behaviour. Paternity distributions are indeed poor predictors for queen mating frequencies (BOOMSMA & RATNIEKS 1996, BAER 2011). Furthermore, monandry is a rare biological phenomenon (especially in insects) so we lack an explanation

for why it was widespread in ancestral hymenopterans and favoured the independent evolution of eusociality at least nine times (HUGHES & al. 2008). Obviously, there are still a number of unresolved issues concerning the mating behaviours of early social insect queens but future work could perform phylogenetic analyses on queen mating frequencies instead of paternity to test whether single or multiple mating can be identified as the ancestral mating system of early social insects.

Independently of the copulation frequencies of ancestral queens, we can define the mating systems of recent species as differential equilibriums between costs and benefits of polyandry but more empirical work is required to better understand the evolutionary interplay between the different genetic and ecological factors involved.

Recent research generated the first empirical confirmation that polyandry results in sexual selection in social insects, which could have been a key mechanism determining relatedness during social evolution. We can expect that sexual selection incurs both benefits and costs for the later emerging societies. Sexual selection biasing paternity towards a most competitive or most wanted male ultimately increases helping incentive. Alternatively, mechanisms equalising paternity shares via cryptic female choice maximise benefits derived from genetic diversity. A recent analysis of paternity skew in polyandrous ants, bees and wasps provided first empirical support that both of these effects are important (JAFFE & al. 2012): In species where queens mate with few males, paternities are biased towards a single male, thereby maximising inclusive fitness returns for helpers. However, in species with large and long-lived colonies that require the benefits of genetic diversity (SCHMID-HEMPEL 1998), queens mate with many males and paternity shares become increasingly equalised. Because sexual selection is conflict driven, it can also incur fitness costs, such as hostility within and between sexes. It would therefore be interesting to understand, how a most competitive (i.e., the winners of sperm competition) or a most wanted male (i.e., the winners of cryptic female choice) contributes towards colony performance and fitness.

Recent technological advances in systems biology such as proteomics (BAER & al. 2009a, b, KING & al. 2011) offer not only opportunities to unravel the molecular components found in ejaculates or the reproductive tract of queens. Such "-omics" knowledge can also be used to manipulate the functioning of some of these molecules. In combination with methods to manipulate the mating process such as artificial insemination (BAER & SCHMID-HEMPEL 2000, DEN BOER & al. 2013), they offer rather spectacular new avenues to unravel the interplay of mating and social life.

In summary, our knowledge about the societies and the reproductive biology of ants has substantially grown in recent years, confirming that these insects make exciting model systems for research, both in the field and in the lab. Recent contributions have changed our understanding about the potential links between mating and social evolution and created novel hypotheses and questions.

## Acknowledgements

I thank the editors of Myrmecological News for their invitation to write a review about polyandry in ants. I also thank Barbara Baer-Imhoof whose detailed comments sub-

tantially improved the manuscript. This work was supported by a Future Fellowship (DP0770050) and a Discovery grant (DP0878107) offered by the Australian Research Council.

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