

The copulation biology of ants (Hymenoptera: Formicidae)

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Abstract

As part of a copulation, males transfer ejaculates to the female in internally fertilising species. Ejaculates consist of sperm and glandular secretions referred to as seminal fluid or seminal plasma. In ants, the latter typically consists of at least two distinct parts, a soluble component mixed with sperm and a non-soluble component referred to as a mating plug or a spermatophore. Recent work has provided fascinating examples of the various effects of seminal fluid and sperm on reproductive success. Here I overview our current knowledge about ant copulations and place this information into a broader context of evolutionary biology to exemplify how natural and sexual selection have shaped ant mating systems. Ant copulations have been described for more than 100 species and queen multiple mating is widespread suggesting that postcopulatory sexual selection such as sperm competition or cryptic female choice might be an important selective force in ants. As I point out newly available technologies from the molecular sciences can be used to better understand ejaculate transfer, sperm storage and sperm use, thereby offering exciting opportunities for future work. I here encourage more research into the copulation biology of ants to investigate questions that are of general interest in the fields of evolutionary, reproductive and systems biology.

Key words: Sperm, seminal fluid, accessory glands, kin selection, sexual reproduction, sperm competition, cryptic female choice, review.

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Males in the Aculeata are often maligned in science and fable as drones, characterized as "greedy, cowardly, and stupid"
Chris Starr, 1984

Introduction

Sexual reproduction is the most widespread form of animal reproduction and under natural selection for traits that optimize fertilisation success. Sexual selection shaped polyandrous animal mating systems, either before copulation as male-male competition or female mate choice or after copulation as sperm competition or cryptic female choice (EBERHARD 1996, BIRKHEAD & MOLLER 1998, SIMMONS 2001, BIRKHEAD & al. 2009). Sexual reproduction has been intensively studied since Darwinian times although precopulatory aspects initially received much more attention than postcopulatory effects. Paternity was assumed to be determined once females accepted to copulate with a chosen male. As a consequence, ejaculates were regarded to consist of sperm as propelled pieces of DNA being surrounded by a support medium termed seminal fluid or seminal plasma. The potency of postcopulatory effects on paternity, whether naturally or sexually selected, was ignored until the seminal work of PARKER (1970) and EBERHARD (1996). Their contributions initiated a substantial shift in thinking, and resulted in a rapidly growing field of research studying all aspects of sperm and ejaculate biology (AINSWORTH 2005, BIRKHEAD & al. 2009), albeit with a specific focus on postcopulatory sexual selection (EBER-

HARD 1996, BIRKHEAD & MOLLER 1998, SIMMONS 2001). A major challenge in the study of ejaculate characteristics and their effects on reproductive success is that the traits of interest are less accessible to study than precopulatory traits, because they operate inside the body of the male or the female and on a molecular scale.

The spectacular advances of modern biochemistry and molecular biology gave rise to a new field of research known as systems biology, unifying the fields of genomics, transcriptomics, proteomics and metabolomics. Systems biology now provides a number of tools that generate global views of molecules in ejaculates while also offering spectacular new opportunities to study sperm form and function. For example, transgenic *Drosophila melanogaster* (MEIGEN, 1830) males with green (GFP) or red (RFP) sperm heads recently allowed – for the first time – the observation of competing sperm *in vivo* inside the female's sexual tract (MANIER & al. 2010). Fluorescence labelling has now also been used in mice (FISHER & HOEKSTRA 2010) to study sperm behaviour revealing that sperm seem capable of self-nonsel self recognition, a result that was likewise inferred to affect sperm competition in ants and bees (DEN BOER & al. 2010).

Reproductive characteristics are remarkably variable, starting from the molecular complexity of sperm (DORUS & al. 2006, OLIVA & al. 2009) and seminal fluid (WOLFNER 1997, RAM & WOLFNER 2007, BAER & al. 2009c) to the large variation detected in the morphology of sperm or the sexual organs that produce, assemble and transfer them to the female (EBERHARD 1985, 2004). In fact, sperm cells are the morphologically most diverse cell type known (BIRKHEAD & al. 2009), and male genitalia are often variable enough to be used as taxonomic markers to distinguish closely related species (CLAUSEN 1938, EBERHARD 1985, 2004). The first proteomic studies in humans have identified more than 1760 proteins in sperm (JOHNSTON & al. 2005) and more than 800 different proteins in seminal fluid (PILCH & MANN 2006). Despite all this intense research and a substantial body of data and theory, there are still remarkably big gaps in our knowledge: We still miss a reliable method to estimate sperm or ejaculate quality (see for example HOLMAN 2009) despite its importance for evolutionary and reproductive biology. The morphological ultrastructure of sperm has been studied and described for hundreds of species (see JAMIESON 1987, JAMIESON & al. 1999, BIRKHEAD & al. 2009 and references therein), but very little is known about the biological relevance and functioning of sperm components and how they contribute to sperm quality or paternity success. This is true for even the most simple sperm characters such as total sperm length (BAER & al. 2006b, HUMPHRIES & al. 2008), which differs more than 600,000 fold in insects from a 8 μm in *Meteorus* sp. (QUICKE & al. 1992) to more than 5 cm in *Drosophila subobscura* (COLLIN, 1936) (PITNICK & al. 1995).

Here I provide an overview of what is known about the copulation biology of ants and elaborate that ants make excellent model systems to study ejaculate and copulation traits. I will start with a brief introduction into the general mating characteristics of hymenopteran social insects (the ants, social bees and social wasps). I kept this part brief, because these aspects have been covered elsewhere (STARR 1984, HÖLLDOBLER & BARTZ 1985, PAGE 1986, BOOMSMA & RATNIEKS 1996, BAER 2003, 2005, BOOMSMA & al. 2005, BOOMSMA 2007). I will then focus on our current knowledge about the copulation biology of ants as this area is particularly understudied and provides excellent opportunities for future research.

Sexual reproduction in eusocial systems

A theoretical framework for understanding the ways in which sexual reproduction and sexual selection affect the establishment and kin structure of societies of ants, bees, wasps and termites has been developed since the 1980s (HÖLLDOBLER & BARTZ 1985) and further elaborated during the last decade (BAER 2003, 2005, BOOMSMA & al. 2005, BOOMSMA 2007). These reviews have indicated that eusocial living has shaped the mating systems of ants and selected for a number of characteristics that are otherwise rare or absent in non-social species:

(1) Kin selected hymenopteran social insects have closed mating systems where sexual reproduction is restricted to a few individuals that find their mates during a brief period early in life.

(2) Females (queens or gamergates) acquire a lifetime supply of sperm but never remate once they have started to lay eggs. As a consequence queens are capable to store large

amounts of sperm over prolonged periods of time, keep them viable, and use them prudently for fertilisations (TSCHINKEL & PORTER 1988, BAER & al. 2006a, DEN BOER & al. 2009a). This implies that social insects would make perfect model systems for studies on sperm senescence (PIZZARI & al. 2008), underlining that more work is required to understand the proximate and ultimate basis of sperm longevity in social insects (HEINZE & SCHREMPF 2008).

(3) Males are typically short lived and die either during or shortly after copulating but survive as stored ejaculates inside their mates for up to several decades (WEBER 1972, PAMILO 1991, BAER & al. 2006a). The spermatogenesis of hymenopteran social insects is discontinued in adult life (HÖLLDOBLER & WILSON 1990) and, similar to females, males are not able to replenish sperm throughout their adult life. As males originate from non-fertilised eggs, they are haploid and their sperm are therefore clonal. This might have promoted the evolution of molecular nonself recognition systems resulting in altruistic sperm behaviours (STARR 1984) similar to those recently reported in mice (FISHER & HOEKSTRA 2010), but experimental work is needed to test this idea. The only known exceptions are several species of *Cardiocondyla* ants, where a wingless fighting male morph has evolved (HEINZE & al. 1998, ANDERSON & al. 2003). These males stay inside their maternal nest over prolonged periods of time and their continuous spermatogenesis (HEINZE & HÖLLDOBLER 1993) allows them to monopolize copulations with emerging virgin queens and to kill hatching rival fighter males in some species.

(4) Obligatory multiple mating by females evolved independently in several clades of ants, bees and wasps (HUGHES & al. 2008), resulting in opportunities for postcopulatory sexual selection such as sperm competition or cryptic female choice (BAER 2003, 2005, DEN BOER & al. 2010). However, the general importance of sexual selection for social insect mating systems is controversial. Queen mating frequencies are typically derived from molecular data quantifying the number of patriline present in worker offspring. A substantial body of molecular work revealed that a single male sires most if not all worker offspring in many hymenopteran social insects. Consequently monandry was proposed as the most widespread mating system in the social hymenopterans (STRASSMANN 2001). However, whereas molecular markers are reliable tools to confirm polyandrous mating systems, they are inaccurate predictors for the number of queen copulations because postcopulatory sexual selection either biases paternities towards one male (for example the first or the last male) or exclude some male ejaculates completely (see for example Table 2.3 in SIMMONS 2001). There is a fundamental discrepancy in the social hymenopteran insect literature because observational studies consistently report higher queen mating frequencies than molecular studies identifying the number of contributing fathers in worker offspring (PAGE & METCALF 1982, STARR 1984, KELLER & REEVE 1994, BOURKE & FRANKS 1995, BOOMSMA & RATNIEKS 1996).

As part of this review, I searched the literature collecting information for all ant species where copulations have been observed and described. An overview of these species is provided in Table 1 with additional information on specific mating characteristics – if available – such as copulation duration, queen remating frequencies or the transfer of mating plugs or mating signs and whether copulations

were observed in the field or in the lab. A look at the ant species in Table 1 reveals that queen multiple mating was reported for 51 different ant species or in 78% of species where queen remating behaviour was recorded. Even if we assume all remaining species with no available remating data to have strictly single mating queens, this still leaves us with 50% of all species in Table 1 to be polyandrous. Furthermore, behavioural studies on copulation behaviour in the Argentine ant *Linepithema humile* (MAYR, 1868) (KELLER & PASSERA 1992) reported that 60% of queens mated multiply, some of them with up to 20 males although allozyme markers revealed single paternity in worker offspring (BOOMSMA & RATNIEKS 1996). In most but not all cases only one male seemed to get his sperm stored in the spermatheca, but no information is provided on whether a specific male such as the first or last one monopolized sperm storage success. Similar results have been obtained from *Leptothorax gredleri* MAYR, 1855 (OBERSTADT & HEINZE 2003), where queens mate with up to four males but microsatellite work revealed the presence of only a single male in worker offspring. Interestingly, either the first or the second or the third male copulating could monopolize paternity, depending on the queen investigated. In the wood ant *Formica aquilonia* YARROW, 1955, queens mate up to 6 times but not all copulations result in sperm getting stored and the first male to copulate seems to father most but not all offspring (FORTELIUS 2005). All these examples illustrate that molecular analysis of worker offspring are not suitable predictors for the number of copulations of a queen or the presence / absence of postcopulatory sexual selection.

Eusocial species such as ants can be expected to promote postcopulatory sexual selection so that only the most competitive (in the case of sperm competition) or the most preferred male (in the case of cryptic female choice) monopolizes paternity thereby maximizing worker relatedness and therefore inclusive fitness while minimizing kin related conflicts. Experimental evidence in bumblebees supports the idea that selection favours single male paternity, because colonies of artificially inseminated queens with moderate levels of polyandry had significantly reduced fitness compared to monandrous queens. This "adaptive fitness valley" was only overcome when queens were mated with many males so that disease resistance benefits became significant (BAER & SCHMID-HEMPEL 2001). Selection promoting postcopulatory sexual selection to generate a single winner could therefore explain the discrepancy between observational and molecular studies on eusocial female mating frequencies, but would imply that polyandry and postcopulatory sexual selection is much more widespread in hymenopteran social insects than generally assumed. What we need is to understand the dynamics of sperm transfer and sperm storage in social insects. This will allow us to isolate the factors determining paternity and to quantify the influence of natural and sexual selection on these mating systems.

The copulation biology of ants

Studying the mating biology of ants is often assumed to be challenging because many ants mate on the wing (STARR 1984, HÖLLDOBLER & BARTZ 1985, HÖLLDOBLER & WILSON 1990, BOOMSMA & al. 2005) limiting the possibilities to observe copulations or to conduct experimental work. An

intense body of literature has become available to describe precopulatory mating behaviour in ants such as characteristics of swarming and precopulatory behaviour, which are discussed elsewhere (HÖLLDOBLER & BARTZ 1985, HÖLLDOBLER & WILSON 1990, BOURKE & FRANKS 1995, BOOMSMA & al. 2005). As swarming seems an important trigger to initiate mating behaviour (MINTZER 1982, REICHARDT & WHEELER 1996), ants are typically less willing to copulate under laboratory conditions. Furthermore, the small body size of many ants limits the possibilities for observational and / or experimental studies. Due to these technical problems the copulation biology of ants remains remarkably little investigated to date and is better understood in other social hymenopterans such as honeybees (KOENIGER 1986, KOENIGER & al. 1979, 2000, BAER 2005) or bumblebees (ALFORD 1975, BAER 2003, BROWN & BAER 2005). Because our knowledge of postcopulatory dynamics in social hymenopteran insects is in its infancy, I decided to concentrate this review on what is known about ant ejaculates, their transfer to the female and the fundamental influence that these processes may have on reproductive success. This aspect of ant reproduction received remarkably little attention so far, in contrast to research on non-social organisms over the last decades that provided a solid body of literature, illustrating that postcopulatory effects are major drivers of reproductive success throughout the animal and plant kingdoms (BIRKHEAD & MOLLER 1998, SIMMONS & SIVA-JOTHY 1998, SIMMONS 2001, BERNASCONI & al. 2004, BIRKHEAD & al. 2009).

During my work on this paper, I found a remarkably large number of papers that report observations of copulations in ants. As summarized in Table 1 ant copulations have been observed in more than 100 different ant species. There are very detailed descriptions available for some species although sample sizes are often low. Apart from these initial observations hardly any follow-up work has been performed in these species to explore sperm transfer and sperm storage in more detail. The mating biology of ants has also been studied using comparative approaches investigating ant-mating systems in a phylogenetic context, studies that revealed novel insights into the evolutionary history of mating characteristics in ants (BAER & BOOMSMA 2004, HUGHES & al. 2008, BAER & al. 2009a, DEN BOER & al. 2010).

Apart from direct observations of ant copulations a technique for artificially induced copulation has been documented for *Solenopsis invicta* BUREN, 1972 and *Solenopsis richteri* FOREL, 1909 (CUPP & al. 1973), where decapitated males were induced to successfully copulate and ejaculate. Artificial insemination techniques have further been developed for *S. invicta* (see BALL & al. 1983) and *Atta* leaf cutter ants (DEN BOER & al. 2010), offering opportunities for experimental manipulations as in honeybees (TARPY 2003) and bumblebees (BAER & SCHMID-HEMPEL 1999, BAER & al. 2001, BAER & SCHMID-HEMPEL 2001, 2005). Obviously ant species as listed in Table 1 provide ample opportunities for experimental studies on the copulation biology of ants – both in the field and in the lab.

The descriptions of ant mating as found in the literature cited in Table 1 also reveal that there is a basic copulation biology of ants that follows a general sequence of events, likely to be the ancestral mode of ant reproduction although no phylogenetic analyses are available. A general overview

Tab. 1: A list of species (adapted to current taxonomy) where copulations have been observed and described in the literature. The copulation duration is provided as a range (if available, otherwise means are presented) and is given in the second column. Observations of females accepting / rejecting multiple copulations are provided in the third column with maximal numbers of queen copulations reported in brackets. Reported transfers of spermatophores (S) or mating plugs (M) are given in the fourth column. The origin of the observations (laboratory, field or both) is mentioned in the fifth column, followed by the original references.

¹ Copulation with same male; ² Alate males; ³ Ergatoid males; ⁴ Matings shorter in the field; ⁵ Suicidal males; ⁶ Cocoon mating; ⁷ Two sizes of sexuals; ⁸ Interspecific copulations; ⁹ Forced copulation; ¹⁰ Sperm counts in bursa and spermatheca.

Species	Duration	Remate	S / M	Origin	References
<i>Acanthomyops interjectus</i> MAYR, 1866	1 min			Field	TALBOT (1963)
<i>Acromyrmex landolti</i> (FOREL, 1885)		Yes			PAGE (1986)
<i>Acromyrmex hundi</i> (GUÉRIN-MÉNEVILLE, 1838)	1.5 min				WEBER (1972)
<i>Acromyrmex versicolor</i> (PERGANDE, 1894)	4 - 5 min	Yes (4)		Field	REICHARDT & WHEELER (1996)
<i>Acropyga</i> sp.	5 min			Field	EBERHARD (1978)
<i>Anergates atratulus</i> (SCHENCK, 1852)					MEYER (1955)
<i>Brachymyrmex depilis</i> EMERY, 1893		Yes (3)		Field	PAGE (1982)
<i>Cardiocondyla batesii</i> FOREL, 1894	5 - 15 s			Lab	MERCIER & al. (2007)
<i>Cardiocondyla elegans</i> EMERY, 1869	3 - 55 s	Yes (9)		Lab	LENOIR & al. (2007), MERCIER & al. (2007)
<i>Cardiocondyla emeryi</i> FOREL, 1881	2 - 34 s			Lab	MERCIER & al. (2007)
<i>Cardiocondyla kagutsuchi</i> TERAYAMA, 1999	13 - 26 s			Lab	MERCIER & al. (2007)
<i>Cardiocondyla mauritanica</i> FOREL, 1890	5 s 1 - 10 s	Yes (3) ¹		Lab Lab	CREIGHTON & SNELLING (1974) MERCIER & al. (2007)
<i>Cardiocondyla minutior</i> FOREL, 1899	3 - 24 s 16 - 21 s			Lab Lab	MERCIER & al. (2007) YAMAUCHI & al. (1996)
<i>Cardiocondyla obscurior</i> WHEELER, 1929	32 s			Lab	SCHREMPF & al. (2005)
<i>Cardiocondyla venustula</i> WHEELER, 1908	17 s			Lab	MERCIER & al. (2007)
<i>Cardiocondyla wroughtonii</i> (FOREL, 1890)	11.1 ± 4.6 s ² 25.4 ± 23.5 s ³	Yes Yes		Lab Lab	KINOMURA & YAMAUCHI (1987) KINOMURA & YAMAUCHI (1987)
<i>Carebara vidua</i> SMITH, 1858	4 min	Yes (4)	M	Lab	ROBERTSON & VILLET (1989), HEINZE & HÖLLDOBLER (1993)
<i>Cataglyphis cursor</i> (FONSCOLOMBE, 1846)	40 s - 16 min ⁴	Yes		Both	LENOIR & al. (1988)
<i>Chalepoxenus brunneus</i> CAGNIANT, 1985				Lab	BUSCHINGER & al. (1989)
<i>Crematogaster</i> sp.	1.32 min				ROBERTSON & VILLET (1989)
<i>Diacamma australe</i> (FABRICIUS, 1775)					MONNIN & PEETERS (1998)
<i>Diacamma pallidum</i> (SMITH, 1858)	15 h ⁵		S	Lab	ALLARD & al. (2007)
<i>Diacamma rugosum</i> (LE GUILLOU, 1842)	2 days ⁵			Lab	FUKUMOTO & al. (1989)
<i>Diacamma</i> sp.	24 h ⁵	No	S	Lab	NAKATA & al. (1998), ALLARD & al. (2002)
<i>Dinoponera quadriceps</i> KEMPF, 1971	30 min ⁵	No		Lab	MONNIN & PEETERS (1998)
<i>Dorylus molestus</i> (GERSTÄCKER, 1859)	5 - 10 h	Yes		Field	KRONAUER & BOOMSMA (2007)
<i>Eciton burchellii</i> (WESTWOOD, 1842)	1 h	Yes (5)		Field	KRONAUER & BOOMSMA (2007)
<i>Eciton hamatum</i> (FABRICIUS, 1782)	10 h				KRONAUER & BOOMSMA (2007)
<i>Formica aquilonia</i> YARROW, 1955	55 s - 1.4 min	Yes (6)		Field	FORTELIUS (2005), PAMILO & al. (1978)
<i>Formica bradleyi</i> WHEELER, 1913	30 s - 1.5 min	Yes (3)		Field	HALVERSON & al. (1976)
<i>Formica dakotensis</i> EMERY, 1893		Yes		Field	TALBOT (1972b)
<i>Formica japonica</i> MOTSCHOUJSKY, 1866				Field	KAMIMURA (2008)
<i>Formica lugubris</i> ZETTERSTEDT, 1838		Yes		Field	CHERIX & al. (1991)
<i>Formica montana</i> WHEELER, 1910		No		Field	KANNOVSKI & JOHNSON (1969)
<i>Formica nitidiventris</i> EMERY, 1893				Field	TALBOT (1948)
<i>Formica obscuripes</i> (FOREL, 1886)	1 - 5 min			Field	TALBOT (1972a)
<i>Formica opaciventris</i> EMERY, 1893	40 s - 2 min	Yes		Field	SCHERBA (1961)
<i>Formica paralugubris</i> SEIFERT, 1996		Yes (3)			CASTELLA & al. (2009)
<i>Formica pergandei</i> EMERY, 1893		Yes		Field	KANNOVSKI & JOHNSON (1969)
<i>Formica polycytena</i> FOERSTER, 1850	1 s - 1.8 min				YAMAUCHI & al. (1994)
<i>Formica rufa</i> LINNAEUS, 1761		Yes ⁵		Field	MARIKOVSKY (1961)
<i>Formica sanguinea</i> LATREILLE, 1798					PAMILO & al. (1978)
<i>Formica subintegra</i> WHEELER, 1908	6 - 30 s	Yes		Field	ITO & IMAMURA (1974), KELLER & REEVE (1994)
<i>Formica subpolita</i> MAYR, 1886	28 s - 1.6 min	Yes (4)		Field	O'NEILL (1994)
<i>Formica ulkei</i> EMERY, 1893					KANNOVSKI & JOHNSON (1969)
<i>Formica uralensis</i> RUZSKY, 1895		Yes		Field	PAMILO & al. (1978)
<i>Formica yessensis</i> WHEELER, 1913	30 s - 9 min	Yes (2)		Field	ITO & IMAMURA (1974), HIGASHI (1983)

<i>Gnamptogenys menadensis</i> (MAYR, 1887)	30 s			Lab	GOBIN & al. (2001)
<i>Harpagoxenus canadensis</i> SMITH, 1939	40 s - 1.5 min	No		Lab	BUSCHINGER & ALLOWAY (1979)
<i>Harpagoxenus sublaevis</i> (NYLANDER, 1849)	15 - 45 s	No		Lab	BUSCHINGER (1971b)
<i>Hypoponera nubatama</i> TERAYAMA & HASHIMOTO, 1996	3.4 min - 2.08 h ⁶			Lab	YAMAUCHI & al. (2001)
<i>Hypoponera opacior</i> (FOREL, 1893)	2 h - 1.71 days ⁶	Yes		Lab	FOITZIK & al. (2002)
<i>Hypoponera schauinslandi</i> (EMERY, 1899)	5 - 14 min			Lab	YAMAUCHI & al. (1996)
<i>Lasius alienus</i> (FOERSTER, 1850)	7 - 18 min	Yes (2)		Field	BARTELS (1985)
<i>Lasius flavus</i> (FABRICIUS, 1782)		Yes			STARR (1984)
<i>Lasius niger</i> (LINNAEUS, 1758)		Yes (> 2)			IMAI (1966)
<i>Lepisiota frauenfeldi</i> (MAYR, 1855)					TOHMÉ & TOHMÉ (1975)
<i>Leptothorax acervorum</i> (FABRICIUS, 1793)	10 - 15 s				BUSCHINGER (1971a)
<i>Leptothorax gredleri</i> (MAYR, 1855)	13 s - 2.4 min	Yes (4)	S	Lab	OBERSTADT & HEINZE (2003), OPPELT & HEINZE (2007)
<i>Leptothorax kutteri</i> BUSCHINGER, 1966	30 - 50 s	No		Lab	BUSCHINGER (1971a)
<i>Letothorax pacis</i> (KUTTER, 1945)	15 - 45 s	No		Lab	BUSCHINGER (1971b)
<i>Linepithema humile</i> (MAYR, 1868)	6 min	Yes (20)		Lab	KELLER & PASSERA (1992)
<i>Meranoplus peringueyi</i> EMERY, 1886	19 - 26 s	Yes			FUKUMOTO & al. (1989)
<i>Messor ebeninus</i> SANTSCHI, 1927	14 s	Yes (3)		Field	TOHMÉ (1975)
<i>Monomorium floricola</i> (JERDON, 1851)	20 - 40 s	Yes		Lab	BARTH (1953)
<i>Monomorium minimum</i> (BUCKLEY, 1867)	10 - 13 min ⁵⁷	No		both	BHATKAR (1992)
<i>Monomorium pharaonis</i> (LINNAEUS, 1758)	30 - 60 s	No	S	Lab	ALLARD & al. (2006)
<i>Monomorium salomonis</i> (LINNAEUS, 1758)		Yes			PAGE (1986)
<i>Mycetophylax emeryi arenicola</i> (FOREL, 1912)					WEBER (1972)
<i>Myceopurus goeldii</i> (FOREL, 1893)		Yes (4)			KERR (1961), STARR (1984)
<i>Myrmecia pyriformis</i> (SMITH, 1858)					HÖLLDOBLER & WILSON (1990)
<i>Myrmecina graminicola</i> (LATREILLE, 1802)	40 - 60 s			Lab	BUSCHINGER (2003)
<i>Myrmica americana</i> WEBER, 1939	30 - 60 s	No		Field	KANNOWSKI & KANNOWSKI (1957)
<i>Myrmica ruginodis</i> NYLANDER, 1846 ⁷		No			ELMES (1991), SEPPÄ (1992)
<i>Myrmica scabrinodis</i> NYLANDER, 1846				Field	CLAUSEN (1938)
<i>Myrmicaria opaciventris</i> EMERY, 1893		Yes			KENNE & DEJEAN (1998)
<i>Myrmica rubra</i> (LINNAEUS, 1758)	10 s - 22.1 min	Yes (7)		both	CLAUSEN (1938), STARR (1984), HÖLLDOBLER & WILSON (1990), WOYCIECHOWSKI (1990)
<i>Pachycondyla tarsata</i> (FABRICIUS, 1798)	4 - 5 min	Yes		Field	VILLET & al. (1989)
<i>Paratrechina flavipes</i> (SMITH, 1874)	49 s - 12.2 min	Yes		both	ICHINOSE (1994)
<i>Pheidole megacephala</i> (FABRICIUS, 1793)					WILLIAMS (1935)
<i>Pheidole soritis</i> WHEELER, 1908	1 - 2 min	No		Field	WILSON (1957)
<i>Pheidole</i> sp. (<i>fervens</i> ?) SMITH, 1858					LITTLE (1980)
<i>Plagiolepis pygmaea</i> (LATREILLE, 1798)		Yes (3)		Lab	THURIN & ARON (2009)
<i>Pogonomyrmex badius</i> (LATREILLE, 1802)	59 s - 1.5 min	Yes (4)		Field	HARMON (1993), STARR (1984)
<i>Pogonomyrmex barbatus</i> (SMITH, 1858)	28 s ⁸	Yes (5)		Field	MICHENER (1948), HÖLLDOBLER (1976)
<i>Pogonomyrmex californicus</i> (BUCKLEY, 1867)	1.6 min	Yes (6)		Field	MINTZER (1982)
<i>Pogonomyrmex desertorum</i> (WHEELER, 1902)		Yes (3)		Field	HÖLLDOBLER (1976), HÖLLDOBLER & WILSON (1990)
<i>Pogonomyrmex maricopa</i> WHEELER, 1914		Yes (3)		Field	HÖLLDOBLER (1976), HÖLLDOBLER & WILSON (1990)
<i>Pogonomyrmex occidentalis</i> (CRESSON, 1865)	6 - 12 min ⁸	Yes		Field	NAGEL & RETTENMEYER (1973)
<i>Pogonomyrmex rugosus</i> EMERY, 1895	30 s - 22.3 min ⁸	Yes (6)		Field	HÖLLDOBLER (1976)
<i>Polyergus breviceps</i> EMERY, 1893		No			TOPOFF & GREENBERG (1988)
<i>Polyergus lucidus</i> MAYR, 1870		Yes (6)		Field	MARLIN (1971)
<i>Polyergus rufescens</i> (LATREILLE, 1798)	10 s - 3 min	Yes (8)		Field	MORI & al. (1994)
<i>Prenolepis imparis</i> (SAY, 1836)	2 - 5 min	Yes (2)		Field	TALBOT (1945), TARPLEY (1965)
<i>Rossomyrmex minuchae</i> TINAUT, 1981	12 ± 4.8 s	No		Field	RUANO & TINAUT (2005)
<i>Solenopsis invicta</i> BUREN, 1972	30 - 45 s	Yes ¹⁰		Lab ⁹ Field	CUPP & al. (1973) BALL & VINSON (1983)
<i>Solenopsis lou</i> FOREL, 1902		Yes			PAGE (1986)
<i>Solenopsis richteri</i> FOREL, 1909	10 s	No (?)		Lab ⁹ Field	CUPP & al. (1973) WUELLNER (2000)
<i>Technomyrmex albipes</i> (SMITH, 1861)	4.9 - 15 min			Lab	YAMAUCHI & al. (1991)
<i>Temnothorax carinatus</i> (COLE, 1957)					BUSCHINGER (1971a)
<i>Temnothorax nylanderi</i> (FOERSTER, 1850)		Yes			BUSCHINGER (1971a), PLATEAUX (1978)
<i>Temnothorax pergandei</i> (EMERY, 1895)				Field	HEINZE & al. (1995)

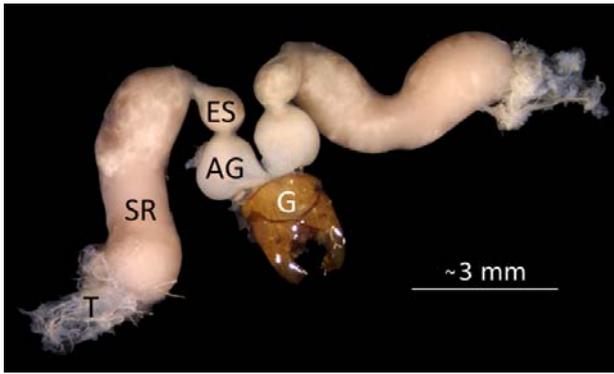


Fig. 1: The sexual organs of a mature *Acromyrmex echinator* male collected shortly before the nuptial flight in Panama. Sexual maturity is indicated by the degenerated testes (T) and the fully developed accessory testes (also referred to as vasa seminales or vasa deferentia in the literature) that consist of the ejaculatory sections (ES) and the sperm reservoirs (SR). The accessory glands (AG) as the main contributors of seminal fluid produce a small mating plug in this species. The external sclerotized genitalia (G) contain the ejaculatory duct (not visible) and are used to establish a firm link with the queen using two rows of spiny teeth, which fit into a specialized pouch organ (mussel organ) in the female's sexual tract. Photograph taken by the author using a digital Canon camera connected to a Leica dissection microscope.

of the sexual organs typically seen in ants is presented in Figure 1. Ant males possess sclerotized outer genitalia and use different parts of these to establish a firm link with the female. This is either achieved by grasping the female sting apparatus or by linking-up with specialized parts in the female sexual tract, such as thick and soft cuticular layers covering the bursa copulatrix (ALLARD & al. 2006) or a specialised pouch, termed the mussel organ (JANET 1902, CLAUSEN 1938, BAER & BOOMSMA 2006, HIMLER & al. 2009). Ejaculates are assembled before or during ejaculation when sperm passes the accessory glands in order to reach the ejaculatory duct (ROBERTSON 1995). They consist of sperm from the accessory testes (vasa deferentia) and seminal fluid. Similar to honeybees (BAER & al. 2009c) and fruit flies (CHAPMAN 2008) the male accessory glands can be assumed to be the main contributors of seminal fluid (DEN BOER & al. 2008) and mating plugs or spermatophores that have been reported in several ant species (Tab. 1). The distinction is that spermatophores completely surround sperm and form a compartment for sperm transfer (MADEL & al. 1990, ROBERTSON 1995, ALLARD & al. 2002, OPPELT & HEINZE 2007) whereas mating plugs or mating signs are transferred separately from sperm into the females' sexual tract and only have a partial contact to sperm (DUVOISIN & al. 1999). Both spermatophores and mating plugs can make up a substantial proportion of the entire ejaculate (ALLARD & al. 2002). In the fire ant *S. invicta* a chemical analysis of the mating plug revealed that it consists of similar fatty acids as found in the bumblebee *Bombus terrestris* (LINNAEUS, 1758) (MIKHEYEV 2003). Linoleic acid is found in the mating plug of both species and is known to act as an anti-aphrodisiacum in *B. terrestris*, reducing the willingness of queens to remate (DUVOISIN & al. 1999, BAER & al. 2001, SAUTER & al. 2001). The ex-

act biological functioning of spermatophores and mating plugs in ants remains unknown so that studies identifying the molecular components (see below) and testing for their biological relevance for paternity success would be highly welcome. Also, further experimental work on ant mating plugs seems timely and feasible to conduct.

Apart from accessory gland secretions, some ant males also seem to use their entire bodies as an extended form of a mating plug (MONNIN & PEETERS 1998), resulting in long copulation durations in some species (Tab. 1). This form of mate guarding seems to have resulted in the evolution of suicidal males in some species (Tab. 1), because queens or workers decapitate the male in copula and only the gaster remains attached to the female. Males being capable to perform only a single copulation could also occur in the army ant *Eciton burchellii* (WESTWOOD, 1842), because males are unable to walk properly after disintegration from the queen and seem to die soon after (KRONAUER & BOOMSMA 2007).

Males usually transfer their ejaculates into a specialised section of the female sexual tract, often referred to as bursa copulatrix or oviducts. Sperm will normally become stored in the spermatheca only as a second step and – similar to most honeybees and bumblebees (DUVOISIN & al. 1999, BAER 2005) – the process of sperm storage often continues until well after the termination of the copulation (REICHARDT & WHEELER 1996, OPPELT & HEINZE 2007). As shown in Table 1, the time ants spend in copula is highly variable and ranges from a few seconds up to several hours or even days, but more work is required to understand the evolutionary causes and consequences of variation in copulation duration for male and female reproductive success.

Copulation biology of attine ants

The reproductive biology of fungus growing ants has been intensively studied over the last two decades. As a consequence attine ants are now, by a large margin, the clade of ants where the mating biology is best understood. This is remarkable, because for most species investigated copulations have not been observed (Tab. 1). Attine ants are therefore a good example to illustrate that the study of the mating biology of ants does not necessarily depend on opportunities to observe copulations. Fungus growing ants have a number of key characteristics that made them attractive model species to study reproduction:

(1) Obligatory female multiple mating evolved in the ancestor of *Acromyrmex* and *Atta* leaf cutting ants (VILLESEN & al. 1999, MURAKAMI & al. 2000, VILLESSEN & al. 2002) making fungus growing ants one of the very few social insect clades where obligatory polyandry evolved (HUGHES & al. 2008) and where a sharp transition from a single to multiple paternity has been documented. Interestingly, the social parasite *Acromyrmex insinuator* SCHULTZ, BEKKEVOLD & BOOMSMA, 1998 appears to have secondarily reverted to a mating system where paternity is dominated by a single male (SUMNER & al. 2004), offering opportunities for comparative studies on closely related sister species with different mating systems. Various forms of sperm competition or cryptic female choice are likely to have evolved in *Acromyrmex* and *Atta* species (DEN BOER & al. 2010). The availability of a well-resolved phylogeny (SCHULTZ & MEIER 1995, WETTERER & al. 1998, SCHULTZ & BRADY 2008, MEHDIABADI & SCHULTZ 2010) allows

comparative studies of polyandrous fungus growing ants with closely related monandrous sister species (BAER & BOOMSMA 2004, BAER & al. 2009a). Finally, complete asexuality evolved in at least one fungus growing ant species, *Mycocepurus smithii* (FOREL, 1893) (HIMLER & al. 2009), making attine ants the only social insects that allow to compare three different reproductive systems within the same tribe.

(2) The higher attines have taken some reproductive traits to absolute extremes within the animal kingdom, as *Atta* queens store hundreds of millions of sperm (FJERDINGSTAD & BOOMSMA 1997, BAER & al. 2006a). They are then capable to keep them alive for several decades (WEBER 1972) and use them prudently to avoid sperm depletion (DEN BOER & al. 2009a). Sperm use by queens was recently studied in *Atta colombica* GUÉRIN-MÉNEVILLE, 1844 where a technique was developed to count the number of sperm on newly laid eggs (DEN BOER & al. 2009a). Queens were found to normally use 2 - 3 sperm for each egg fertilisation early in their life, but average sperm use per fertilisation increased with increasing queen age, probably because of senescence of either the sperm in storage or the queen's sexual tract. Queens seem capable to fertilize around 100 million eggs, placing them among the most fertile animal species known. The possibility to count the number of sperm on eggs offers a variety of opportunities to study female fecundity and sperm use over time and could be adapted for other (ant) species as well.

(3) As both males and females only mate during one brief episode in their lives, exceptionally strong selection is expected on males to initially provide high quality ejaculates (DEN BOER & al. 2008), so that females will not be sperm depleted later in life which can be up to several decades in the *Atta* ants (WEBER 1972). Obviously these species offer a unique opportunity to identify and study those components within an ejaculate that are decisive to keep sperm alive.

(4) In several species the sizes of bodies and sexual organs are exceptionally large for insects, which facilitates experimental work and the development of an artificial insemination technique (DEN BOER & al. 2010).

(5) Copulations and mating behaviour can be observed and manipulated in the field for at least one species, *Acromyrmex versicolor* (PERGANDE, 1894) (REICHARDT & WHEELER 1996), where sexuals collected from mating swarms copulate under semi-laboratory conditions (Tab. 1).

The typical mating biology of attine ants follows that of ants in general (see above and Fig. 1). Spermatogenesis is discontinued in adult life in all species investigated so far (BAER & BOOMSMA 2004, BAER & al. 2009a) and males consequently hatch with a fixed number of sperm. Sperm gets gradually transported to the accessory testes as part of male maturation. The size of the accessory testes increase with colony size throughout the attine phylogeny and they become substantially enlarged structures in species producing large ejaculates such as *Acromyrmex* or *Atta* (BAER & BOOMSMA 2004, BAER & BOOMSMA 2006). In these species the accessory testes consist of two different distinct parts, the ejaculatory duct and the sperm reservoir (Fig. 1). The ejaculatory ducts form the distal end of the accessory testes. They can contract forcefully and seem to function as an efficient pump to transfer sperm into the female (BAER & BOOMSMA 2006). The ejaculatory ducts

empty during ejaculation and muscular movements of the sperm reservoir transport sperm into the emptied ejaculatory duct in preparation for a subsequent copulation. Multiple male copulations are known to occur in *Acromyrmex versicolor* (see REICHARDT & WHEELER 1996) and have been inferred for Panamanian *Atta* and *Acromyrmex* (see BAER & BOOMSMA 2006) species, but it is unknown whether lower attine males can produce more than one ejaculate. Accessory glands have been found in all attine ants studied so far, although their size substantially differs between species (BAER & BOOMSMA 2004). A phylogenetic investigation of male accessory testes and accessory gland sizes in 16 different species of Panamanian fungus growing ants revealed that glands are substantially smaller in species where females mate multiply such as *Atta* leaf cutter ants compared to monandrous species (BAER & BOOMSMA 2004). This provided first evidence that these glands are likely to be under sexual selection and that males reduce investments into glands if non-soluble components such as mating plugs have lost their function to bias paternity success. This is supported by recent field data, where male accessory glands in the polyandrous *Atta* leaf cutting ants were found to contain two different fractions, a clear liquid as well as sticky silicon-like substances forming a small mating plug. In *Atta*, ejaculation can be induced either by killing the male or by gently squeezing its abdomen between two fingers similar to a procedure used to collect ejaculates in honeybees. By doing so a clear liquid, presumably the soluble part of the seminal fluid, appears at the tip of the males' external genitalia, followed by a minute mating plug and a massive amount of sperm (B. Baer & S.P.A. den Boer, unpubl.).

Attine males have large external sclerotized genitalia that are morphologically similar to those in other ants (CLAUSEN 1938, KAMIMURA 2008) and seem responsible for a successful attachment to the female during copulation. Two rows of spiny teeth on the penis valve perfectly fit into the mussel organ of the female's sexual tract. Dissections of newly mated females show specific scarring within the mussel organ (BAER & BOOMSMA 2006), indicating that the male inflicts damage on the female during copulation, which is also known from other ants (KAMIMURA 2008).

The transfer and storage process has been studied in *Acromyrmex versicolor* and inferred from morphological studies for *Atta colombica* and *Atta cephalotes* (LINNAEUS, 1758). In *Acromyrmex versicolor*, copulations can be observed during mating swarms and are initiated on the wing, but pairs then fall to the ground for the remaining time spent in copula, which lasts for about 4 - 5 minutes (REICHARDT & WHEELER 1996). Queens typically rejoin the swarm to find further mates and seem in control over the total number of copulations. Females remate up to four times when kept with males in plastic containers. Sperm is transferred to the oviducts and stored in the spermatheca within five hours after mating, although $\frac{2}{3}$ of spermatheca filling occurred already in the first hour after copulation but only about 10% of the initially received sperm becomes stored in the spermatheca. In *Acromyrmex versicolor*, mated queens start colonies in the lab, which allowed paternity analysis on worker offspring of two field-collected queens. A random amplified polymorphic DNA (RAPD) analysis revealed that all males that copulated sired offspring, but sample sizes used were too small to detect whether some

fathers were more successful than others. *Acromyrmex versicolor* is a very promising model system to study several aspects of ant copulation and reproduction, especially over time as queens store sperm for up to a decade (WHEELER & al. 1990). I was therefore surprised to find that no follow-up study has been conducted in this species over the last 14 years.

In *Atta cephalotes* and *Atta colombica*, sperm appears to be transferred directly to the spermatheca during mating (BAER & BOOMSMA 2006). This is presumably an adaptation to the exceptionally large ejaculate sizes of males resulting from the high sperm demands by females. This mode of sperm transfer is otherwise rare in insects, but has been found in two other hymenopteran social insects, the dwarf honeybees *Apis florea* FABRICIUS, 1787 and *Apis andreniformis* SMITH, 1858 (BAER 2005). Direct sperm transfer to the spermatheca reduces the opportunities for sperm competition or cryptic female choice and could explain the small size of the male accessory glands in these species (BAER & BOOMSMA 2006). *Atta* queens are therefore expected to perform a more rigorous precopulatory mate choice if their opportunities to bias paternities after copulation are restricted. A recent study found that queens of *Atta sexdens* LINNAEUS, 1758 may copulate with brothers at some frequency (ARMITAGE & al. 2010), resulting in the presumably costly production of diploid males. As this study was conducted in a population of low density, it remains to be seen whether sib-mating is common in dense populations of *Atta sexdens*. In general, however, studies on pre- or postcopulatory mate choice, especially for inbreeding avoidance, would be interesting to conduct and seems feasible in species such as *Acromyrmex versicolor*.

Attine ants are so far the only clade where physiological effects of copulations and ejaculates have been studied experimentally. Male accessory gland secretions, a major component of seminal fluid, are highly efficient in keeping sperm alive in *Trachymyrmex* cf. *zeteki* WEBER, 1940, *Acromyrmex echinator* (FOREL, 1899) and *Atta colombica* (see DEN BOER & al. 2010). In the latter species these compounds enhance sperm survival even if diluted hundreds of times and despite the relatively small size of the male accessory glands in *Atta* (BAER & BOOMSMA 2004). Similar to honeybees, the proteins within the seminal fluid seem responsible for keeping sperm alive (DEN BOER & al. 2008, BAER & al. 2009c, DEN BOER 2009, DEN BOER & al. 2010). Furthermore, male accessory gland secretions are more efficient in keeping sperm of the same males alive than that of a potential competitor (DEN BOER & al. 2010). As this effect was only found in the multiply mated species *Acromyrmex echinator* and *Atta colombica* but not in the monandrous *Trachymyrmex zeteki*, these data provide the first empirical evidence that sperm competition has evolved in social insects and likely operates through sperm incapacitation. Interestingly a male's seminal fluid seems unable to protect his own sperm from the negative effects of non-own seminal fluid (DEN BOER & al. 2010).

Physiological traits of ejaculates have been also investigated in females. Sperm was found to be costly to store for queens and to trade off with other life-history traits such as immunity (BAER & al. 2006a). In *Atta colombica*, newly inseminated queens up-regulate their immune system after mating and during colony founding, but a queen's ability to defend a possible microbial attack decreased with increas-

ing female mating frequencies and more sperm stored. This first empirical evidence for sperm storage costs could explain some of the high queen mortalities observed during colony foundation (WEBER 1972) as females are expected to provision stored sperm with costly contributions. The female spermathecal fluid in the honeybee *Apis mellifera* LINNAEUS, 1758 is indeed a complex enzymatic machinery to support sperm in storage (BAER & al. 2009b) and has also been shown to be crucial to keep sperm alive (DEN BOER & al. 2009b). A complete biochemical network of proteins indicates that sperm in storage can be compared to endosymbionts such as mitochondria – where the host (female) takes over a substantial part of the endosymbiont's (sperm) physiological needs (BAER & al. 2009b). As ejaculates are directly transferred to the spermatheca during mating, *Atta* ants offer good opportunities to investigate the influence of females on competing ejaculates. In *Atta colombica*, the spermathecal fluid is capable to terminate the negative effects of male accessory gland secretions on competing sperm (sperm incapacitation), providing first evidence that cryptic female choice has evolved in social insects as well (DEN BOER & al. 2010).

Experiments as summarized in the paragraph above mark first steps to study ejaculate characteristics on an increasingly smaller scale. However, a more complete molecular understanding of the effects of ejaculate components on own and competing sperm as well as on the female will be required to make significant further progress. I will therefore use the last section of this review to highlight that the necessary technologies to perform such work are now available, affordable and feasible.

New opportunities to study ant ejaculates: systems biology

Ejaculates are more than simple deliveries of semen as they influence paternity in many different ways. Generating a better evolutionary understanding of ejaculate-ejaculate and ejaculate-female interactions will be a challenging task. This is, because the biologically active agents are glandular secretions that contain biochemically active compounds such as proteins that are more difficult to study experimentally than the classical phenotypic traits studied by evolutionary biologists for decades. The study of such traits requires establishing links between two fields of research that are historically separated: molecular biology studying proximate relationships (asking how questions) and evolutionary biologists addressing ultimate aspects (asking why questions).

Ongoing fundamental scientific breakthroughs characterize the biochemical and molecular sciences and some of these technologies are now reliable and affordable enough to be used by non-specialists. For example, sequencing technology now allows fast compiling of genomic data using only a fraction of the time, costs and manpower compared to earlier years. A new generation of sequencing equipment now allows not only to determine the genome of a species of interest, but to quantify differences in transcript abundance and therefore to study physiological responses to stimuli of individuals on the transcript level (SULTAN & al. 2008). Furthermore, large scale sequencing, capable to produce terabytes of sequence data per run, offer the study of composition and functioning of entire ecological (micro-) habitats.

In recent years, proteomics has proven to be a very promising technology to study ejaculate characteristics, because it can identify and quantify the most abundant proteins in samples of interest. Sperm seems transcriptionally silenced (BAER & al. 2009b) and seminal fluid as a glandular secretion probably does not consist of metabolically active cells or tissues (BAER & al. 2009c). In the absence of any transcriptional activity within the ejaculate, proteins can therefore be expected to be the main drivers determining reproductive success.

Modern proteomics provides a variety of different methods that can be divided in gel-based and non-gel-based approaches. Gel-based approaches run proteinaceous samples on one- or two-dimensional SDS Page gels. This provides a visual impression of a proteome and the size, pH and abundance of proteins of interest. Gel-based proteomics also allows quantifying differences in protein abundance between samples. The DIGE (differential in-gel electrophoresis) technique is currently the state-of-the-art technology used to do this. It is a special type of a two-dimensional polyacrylamide gel electrophoresis using defined fluorescent dyes. Several protein samples are loaded onto the same gel, and differences in protein abundances among the samples are then visualized with a laser scanner measuring individual protein spots for each sample. The change of intensity of a protein spot can then be compared and statistical analysis of protein profiles from multiple DIGE gels is possible because one of the dyes is used as a standard for biological replication. Proteins are finally identified using mass spectrometry and peptides are matched to genes within the organism studied using publicly available sequence databases. Non-gel based proteomics uses the latest technological advances where entire pre-digested samples are analyzed using mass spectrometry. The opportunities for proteomic studies of ant sperm, seminal fluid and female contributions to sperm will arise over the coming years as a number of ant genomes are currently being sequenced (SMITH & al. 2010). Proteomics was recently used to characterize the main protein components in seminal fluid, spermathecal fluid and sperm of honeybees (BAER & al. 2009b, c), confirming that the necessary technologies can be adapted for the use in social insects.

Conclusions

As pointed out, the copulation biology of ants remains poorly investigated. This is somewhat surprising given the general interest in this insect group, its ecological importance and the opportunities available (Tab. 1). A useful conceptual evolutionary framework to study ant-mating biology has been developed over recent years and generated a coherent set of questions to investigate, and a large number of ant species can potentially be used to answer these questions. New technologies from the molecular sciences further offer the necessary opportunities to approach the questions at a deeper level than was possible so far.

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Zusammenfassung

Während der Kopulation transferieren Männchen Ejakulate in den weiblichen Geschlechtstrakt, die aus zwei Komponenten bestehen: den Spermien und der Seminalflüssigkeit. Letztere besteht meist aus einem wasserlöslichen Teil, der die Spermien umgibt, und einem wasserunlöslichen Teil, der als Spermatophore oder Paarungspfropf bezeichnet wird. Wissenschaftliche Arbeiten der letzten Jahre haben gezeigt, dass Spermien und Seminalflüssigkeit weitaus komplexer sind als bisher angenommen und den Fortpflanzungserfolg grundlegend beeinflussen.

Unser Wissen über die Paarungsbiologie von Ameisen ist erstaunlich gering, da viele Ameisen während des Paarungsfluges in der Luft kopulieren. Dieser Umstand macht es schwierig, Ameisenkopulationen zu beobachten oder experimentell zu manipulieren. Erschwerend kommt die geringe Körpergröße vieler Ameisenarten hinzu. Allerdings zeigt ein Blick in die existierende Literatur, dass das Kopulationsverhalten für etwa 100 verschiedene Ameisenarten beschrieben worden ist, die dementsprechend alle als mögliche zukünftige Modellorganismen benutzt werden könnten.

Hier fasse ich unser bestehendes Wissen über die Kopulationsbiologie von Ameisen zusammen. Ich zeige auf, dass wir mittlerweile über genügend Methoden verfügen, um die Paarungsbiologie dieser Insekten zu untersuchen. Spermien und Seminalflüssigkeit von Ameisen scheinen eine ganze Reihe von Möglichkeiten zu haben, den Kampf um Vaterschaft zu beeinflussen. Neue bahnbrechende Methoden der Molekularbiologie sind mittlerweile so weit entwickelt worden, dass sie auch von Nicht-Spezialisten benutzt werden können und Möglichkeiten eröffnen, evolutionäre Prozesse auf der molekularen Stufe zu verstehen. Ameisen bieten eine einmalige Gelegenheit, Fragen im Zusammenhang mit der sexuellen Reproduktion zu untersuchen, die von generellem Interesse in der Evolutionsbiologie, der Reproduktionsbiologie und der Systembiologie sind.

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