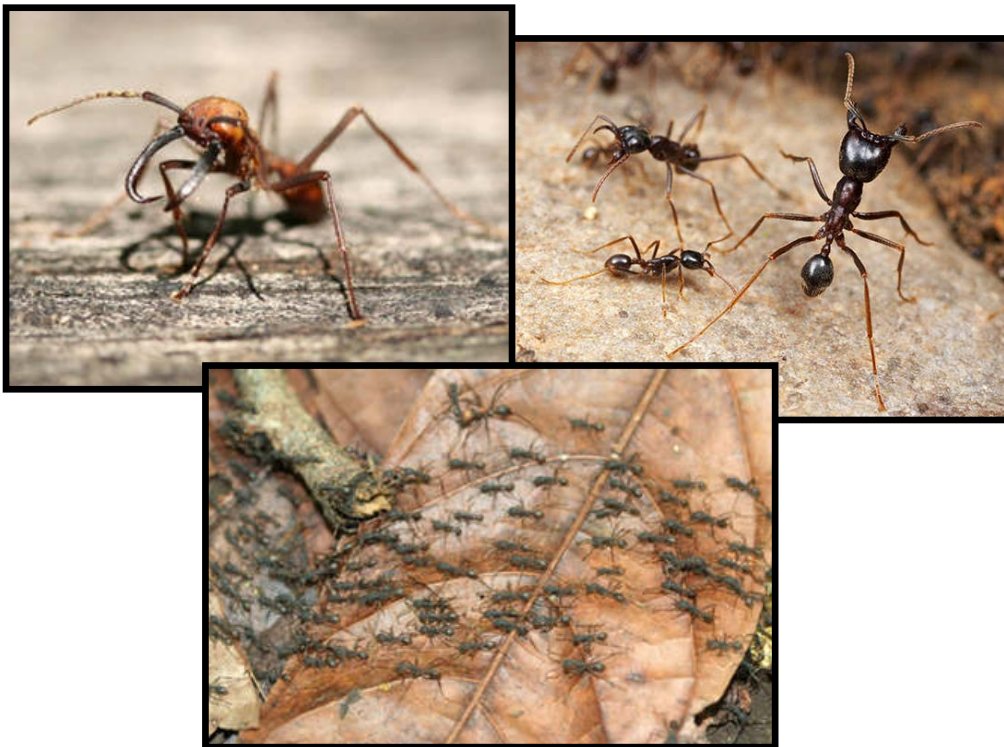**D i s s e r t a t i o n**

Causes and Consequences of extreme Polyandry in Army Ants

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Causes and Consequences of extreme Polyandry in Army Ants

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Contents

"Adapted from archaic times to a life of organized group predation and carnivorous diet, all army ants take their booty in mass forays into areas around their temporary nest. Their pillaging expedition or raids are the largest organized operations carried out regularly away from home by any animal except humans."

(Schneirla 1971, p. 67)

General introduction

"Why do females mate multiply?" This question (Jennions & Petrie 2000) has caused long standing debates among biologists since Bateman (1948) famously discovered in fruit flies "an undiscriminating eagerness in the males and a discriminating passivity in the females". This concept of generally "coy and choosy" females but promiscuous males, as already proposed by Darwin (1871), remained a basic principle in theories of sexual selection and animal mating system evolution until modern times (Thornhill & Alcock 1983; Andersson 1994; Arnqvist & Rowe 2005). Thus, evolutionary circumstances favoring female multiple mating within a single reproductive cycle, i.e., polyandry (Thornhill & Alcock 1983), are controversial, especially as it comes with a considerable set of costs. These include, e.g., time/energy expenditure, increased predation risks, pathogen transmission or harmful ejaculate accessory substances (Sherman *et al.* 1988; Moritz & Southwick 1992; Keller & Reeve 1994; Chapman *et al.* 1995, 2003; Crozier & Fjerdingstad 2001; Schlüns *et al.* 2005b).

However, over time polyandry has turned out to be so ubiquitous among animals, and potential benefits outweighing its costs so manifold (extensively reviewed in Keller & Reeve 1995; Birkhead & Møller 1998; Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Tregenza & Wedell 2000; Zeh & Zeh 2003; Simmons 2005; Slatyer *et al.* 2012) that some authors have argued to more appropriately ask why monandry exists in the first place (Pizzari & Wedell 2013, and references therein). A very special and promising case for studying the evolution of polyandry, are the eusocial insect societies of the Hymenoptera (all ants, some bees, some wasps) for two reasons: (1) Polyandry is rare among them (Strassmann 2001) and most likely derived from monandrous ancestors (Boomsma 2007, 2009; Hughes *et al.* 2008). (2) The eusocial life history poses exceptional conditions for polyandrous mating systems, hardly met in other animals. Thus, it offers opportunities not only to study the evolution of polyandry but also its consequences on social evolution and vice versa.

Polyandry in eusocial Hymenoptera

Eusociality is characterized by obligate reproductive division of labor within colonies into reproductive queen and altruistic worker castes (Wilson 1971; Crespi & Yanega 1995; Bourke 2011). According to Hamilton's (1964) inclusive fitness theory, a key to eusocial evolution is intracolonial relatedness, so that genes for altruism can be selected indirectly in the worker's

relatives, as long as the benefits of helping (e.g., additionally raised siblings), weighted by relatedness, exceed the costs (Hamilton's rule) (Bourke & Franks 1995; Crozier & Pamilo 1996; Queller & Strassmann 1998; Bourke 2011). Polyandry has two important consequences in the eusocial Hymenoptera: (1) It weakens the fundament of inclusive fitness by diminishing intracolonial relatedness as genotypic variance rises with additional sires. (2) It increases the potential for sexual conflict, which may lead to (often post-copulatory) sexual selection expressed as male/sperm competition or (cryptic) female choice (Parker 1970; Thornhill 1983; Eberhard 1996, 2009; Simmons 2001, 2003; Chapman *et al.* 2003; Arnqvist & Rowe 2005). Nevertheless, polyandry secondarily evolved several times in eusocial ants, bees and wasps (Crozier & Fjerdingstad 2001; Hughes *et al.* 2008; Boomsma *et al.* 2009), but despite many hypotheses, circumstances favoring it are still incompletely understood (Crozier & Page 1985; Palmer & Oldroyd 2000; Crozier & Fjerdingstad 2001; Strassmann 2001; Brown & Schmid-Hempel 2003; Kraus & Moritz 2010).

Hypotheses for the evolution of polyandry

Most polyandry hypotheses for eusocial Hymenoptera are based on assuming an adaptive advantage of increasing the intracolonial genotypic variance (IGV, Tab. 1) with a higher paternity frequency[1]. Assumed benefits are enhanced colony efficiency and stability resulting from a half-sib work force composed of sub-families from different siring males (patrilines), on the expense of relatedness and potentially social coherence (Starr 1984; Keller & Reeve 1994; Boomsma & Ratnieks 1996; Yasui 1998; Palmer & Oldroyd 2000; Crozier & Fjerdingstad 2001; Boomsma *et al.* 2009). Other plausible hypotheses (Tab. 1) invoke the avoidance of sperm limitation in big, long-lived colonies (Cole 1983; Boomsma & Ratnieks 1996; Kraus *et al.* 2004; Schlüns *et al.* 2005b; Kraus & Moritz 2010) or an intercolonial variance reduction of the sterile diploid male load of the Hymenopteran single locus sex determination system (Page 1980, 1986; Page & Metcalf 1982; Crozier & Page 1985; Crozier & Fjerdingstad 2001). As the strength of this load depends on the population wide number of alleles at the sex locus, selection for polyandry is also related to the effective population size (N_e)[2] and has been suggested to compensate for low N_e and the associated risk of inbreeding (Antolin *et al.* 2003; Zayed 2004; van Wilgenburg *et al.* 2006; Hartl & Clark 2007; Jaffé *et al.* 2009).

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[1] The term paternity frequency will be used hereafter, representing the number of queen matings that actually resulted in a sire, i.e., a patriline present among offspring.

[2] Number of reproductive individuals in an ideal population that would show the same amount of inbreeding as the population under consideration (Wright 1938).

The problem of extreme polyandry

While most IGV hypotheses (Tab. 1) reasonably explain the evolution of low to intermediate paternity frequencies (six to ten matings) in eusocial Hymenoptera, they are insufficient in explaining more extreme polyandry as it occurs in *Apis* honeybees and the army ants[3] (see below). This is because of a non-linear increase in IGV, gaining only marginally from additional matings as the intracolonial relatedness reduction asymptotes that of half-sibs (0.25) (Fig. 1) (Crozier & Page 1985; Fuchs & Moritz 1998; Palmer & Oldroyd 2000; Kraus & Moritz 2010). Thus, IGV only explains extreme polyandry if non-linear fitness gains are predicted. Fuchs & Moritz (1998) did so with a modified worker task specialization model assuming overdominance of rare patrilines (Fig. 1) (see also Crozier & Page 1985), which has later been incorporated as social heterosis hypothesis explaining non-linear fitness gains from synergistic interactions across genotypically diverse individuals to a common mutual benefit (Tab. 1) (Nonacs & Kapheim 2007). The sperm limitation hypothesis may in principle explain even extreme polyandry but has been criticized as there is no obvious reason for males to not evolve higher sperm numbers (e.g., Boomsma *et al.* 2009), but see *Bauplan* restriction and optimal male size arguments in Kraus & Moritz (2010). Queen fitness gains from additional matings with respect to the diploid male load depend on N_e and the risk of inbreeding (Antolin *et al.* 2003; Zayed 2004; van Wilgenburg *et al.* 2006). So far extreme polyandry has relatively rarely (and only in *Apis*) been explicitly empirically addressed in eusocial Hymenoptera (e.g., Moritz *et al.* 1995; Franck *et al.* 2000; Palmer & Oldroyd 2000; Tarpy & Page 2000; Kraus *et al.* 2004; Schlüns *et al.* 2005a, 2005b). However, army ants are another excellent test case for extreme polyandry and its consequences on social and population structure, as they comprise a broad spectrum of different species with a morphologically diverse workforce (in contrast to *Apis*) and an extraordinary mating system.

Army ants as model system

Army ants are exceptional among eusocial Hymenoptera. They exhibit about the largest colonies (up to 20,000,000 workers) and the most sophisticated division of labor with highly advanced worker caste polymorphism and task specialization (Fig. 2) (Schneirla 1971; Franks 1985, 1989; Franks *et al.* 2001; Schöning *et al.* 2005; Jaffé *et al.* 2007). Further, the 'army ant adaptive syndrome' (Gotwald 1995; Brady 2003; Kronauer 2009) is characterized by a unique

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[3] Monophyletic group of (sub-)tropical New and Old World Aenictinae, Ecitoninae and Dorylinae (Brady 2003).

evolutionary related trait combination, which includes group predation, nomadism[4] and a wingless queen mating inside the colony with incoming males (Raignier & Van Boven 1955; Wilson 1958; Schneirla 1971; Gotwald 1995). Extreme paternity frequencies (about 20) are common and ancestral in army ants and have been linked to those army ant specific traits (Brady 2003; Denny *et al.* 2004; Kronauer *et al.* 2004, 2006, 2007; Jaffé *et al.* 2007, 2009).

Surely, the emerging organizational complexity of such huge, marauding colonies, which made naturalists wonder that "no spectacle of the tropical world is more exciting and mystifying than that of a colony of army ants on the march" (Hölldobler & Wilson 1990, p. 573), makes it highly promising to test IGV hypotheses invoking non-linear fitness gains of extreme polyandry. Further, it is appealing to test for sperm limitation by correlating paternity frequency and colony size (Cole 1983). Moreover, army ants are predestined to test for the significance of inbreeding risks and restricted N_e in shaping mating system evolution, due to queen winglessness and an unusual mode of colony reproduction via fission. This is probably a corollary of a minimal necessary colony size for group predation (Schneirla 1971; Gotwald 1995; Kronauer 2009), which severely limits dispersal abilities, making army ants particularly prone to low N_e, genetic sub-structuring and inbreeding (Bulmer 1983; Hartl & Clark 2007).

Objectives of this study

CHAPTER I – Testing hypotheses

This is the first study to phylogenetically correlate empirical paternity frequency data across ancestrally extremely polyandrous species to explicitly test polyandry hypotheses. If IGV is important, paternity skew[5] should fall with rising paternity frequency, indicating that queens use the full advantage of ever higher paternity frequencies (Haapaniemi & Pamilo 2012; Jaffé *et al.* 2012). Otherwise a high skew should be selected if intracolonial relatedness is of higher importance. Alternatively, if sperm limitation drives polyandry, paternity frequency should be correlated with colony size (Cole 1983; Boomsma & Ratnieks 1996). Furthermore, considering the particularly strong N_e reduction and associated inbreeding risks in army ants, high paternity frequencies may lower the average queen fitness loss from diploid males (Page 1980, 1986; Antolin *et al.* 2003; van Wilgenburg *et al.* 2006). For example, Jaffé *et al.* (2009) showed that *Eciton burchellii* queens mated preferably with unrelated males from surrounding

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[4] "relatively frequent colony emigration ... accomplished in such an orderly fashion" (Wilson 1958, p. 24)

[5] Distribution of proportional paternity among siring males (Nonacs & Hager 2011).

colonies, thereby even boosting N_e. Finding this trend in several army ant species, would confirm the potential of extreme polyandry in counteracting inbreeding and increasing N_e.

CHAPTER II – Consequences at population level

Mating system evolution is inevitably interconnected with dispersal biology and population wide gene flow (Thornhill & Alcock 1983; Nunney 1993; Bourke & Franks 1995; Clobert 2001). This chapter is to explore the consequences of the army ant mating system on genetic population structuring. Colony fission with restricted queen dispersal is linked to a strongly male-biased sex-ratio in army ants (Schneirla 1971; Macevicz 1979; Bulmer 1983). Thus, male-biased dispersal is expected to shape the population structure by compensating restricted maternal gene flow (e.g., Berghoff *et al.* 2008; Jaffé *et al.* 2009) and counteracting risks of inbreeding and small N_e. This study is the first to test this on geographically large scale in case of a widely distributed, but almost exclusively subterranean African army ant, *Dorylus fulvus*, for which particularly strong female philopatry is expected.

CHAPTER III – Insights from species diversity

To test hypotheses on trait (e.g., polyandry) evolution it is of great importance to compare related species, accounting for phylogeny (Keller & Reeve 1994; Boomsma & Ratnieks 1996; Palmer & Oldroyd 2000; Brown & Schmid-Hempel 2003; Schlüns *et al.* 2005a; Boomsma *et al.* 2009; Jaffé *et al.* 2012). However, army ant polyandry data comes so far mainly from the few very conspicuous *Eciton* and *Dorylus* species (Kronauer *et al.* 2004, 2006; Jaffé *et al.* 2007, 2009; Berghoff *et al.* 2008; but see Kronauer *et al.* 2007). This chapter aims to disclose the potential of exploring more of the almost 400 listed species (Bolton 2014), plus several preliminarily described taxa (e.g., Longino 2005; Smith *et al.* 2014), for deeper insights into army ant biology and evolution. It further shows in a case study in the genus *Labidus* that micro-allopatry may have led to cryptic speciation and that phylogenetic relationships among army ants are not always clear. This emphasizes the importance of gene flow preventing spatiotemporal barriers in army ant evolution (Roberts *et al.* 2000; Meisel 2006; Kumar & O'Donnell 2009).

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Tab. 1 Summary of the main hypotheses for the evolution of polyandry in the eusocial Hymenoptera, marking those particularly related to IGV[1] (given in arbitrary order).

Hypothesis	Description and references
IGV Worker task specialization	Based on genetically determined differences in response thresholds to given task stimuli among workers (Robinson & Page 1989), colonies of mixed worker genotypes may have an increased efficiency of task specialization and performance (Page *et al.* 1989; Jaffé *et al.* 2007; Kraus & Moritz 2010).
IGV Colony homeostasis	A mixture of worker genotypes with varying inherent response thresholds to environmental stimuli may improve colony homeostasis in unpredictable environments (Cole & Wiernasz 1999; Mattila & Seeley 2007; Oldroyd & Fewell 2007).
IGV Pathogen resistance	Varying immune properties among multiple worker genotypes may improve the herd immunity and thus disease/parasite resistance of the whole colony (Sherman *et al.* 1988; Schmid-Hempel 1998; Baer & Schmid-Hempel 2001; Hughes & Boomsma 2004).
IGV Queen/worker conflict	Due to Hymenopteran haplodiploidy (haploid males, diploid females) intracolonial conflicts may arise in monandrous single queen colonies: (1) Conflict over sex allocation among reproductive brood: Full-sib workers are closer related to sisters (75%) than to brothers (25%) and prefer a more female biased sex ratio (3:1) than the queen (1:1) (Trivers & Hare 1976). (2) Conflict over male production: Workers prefer worker derived nephews (37.5% relatedness) over queen derived brothers (Ratnieks 1988; Ratnieks & Reeve 1992). Polyandry, by lowering intracolonial relatedness (half- instead of full-sibs), may bring worker interests closer to queen interests (Starr 1984; Moritz 1985; Bourke & Franks 1995).
IGV Social heterosis	Polyandry is selected for the synergistic fitness effect of mutualistically beneficial interactions between genotypically diverse workers of a colony (Fuchs & Moritz 1998; Palmer & Oldroyd 2000; Nonacs & Kapheim 2007).
Sperm limitation	Since queens mate only during a short time to store sperm for their sometimes years-long life (Hölldobler & Wilson 1990), polyandry may overcome sperm limitation to establish bigger, more long-lived colonies (Cole 1983; Kraus & Moritz 2010).
Diploid male load[2]	Hymenoptera usually have single locus sex determination with diploid individuals heterozygous at the sex locus becoming females and hemizygous ones (from haploid eggs) becoming males (Beye *et al.* 1996). Depending on the population wide number of sex alleles this leads to a specific load of usually sterile diploid homozygouse males. Depending on the relationship between queen fitness loss and proportion of diploid male load polyandry may increase individual queen fitness (Page 1980, 1986; Crozier & Page 1985).

[1] IGV refers to genotypic variance (the probability of randomly drawing two different genotypes from a colony), which differs from genetic variance in that the latter can also arise in monogamy from heterozygous offspring (Crozier & Page 1985; Yasui 1998).

[2] Diploid male load is a somewhat special kind of IGV hypothesis, referring to genetic rather than genotypic variance at a specific locus, with strong links to population structure, inbreeding and demography.
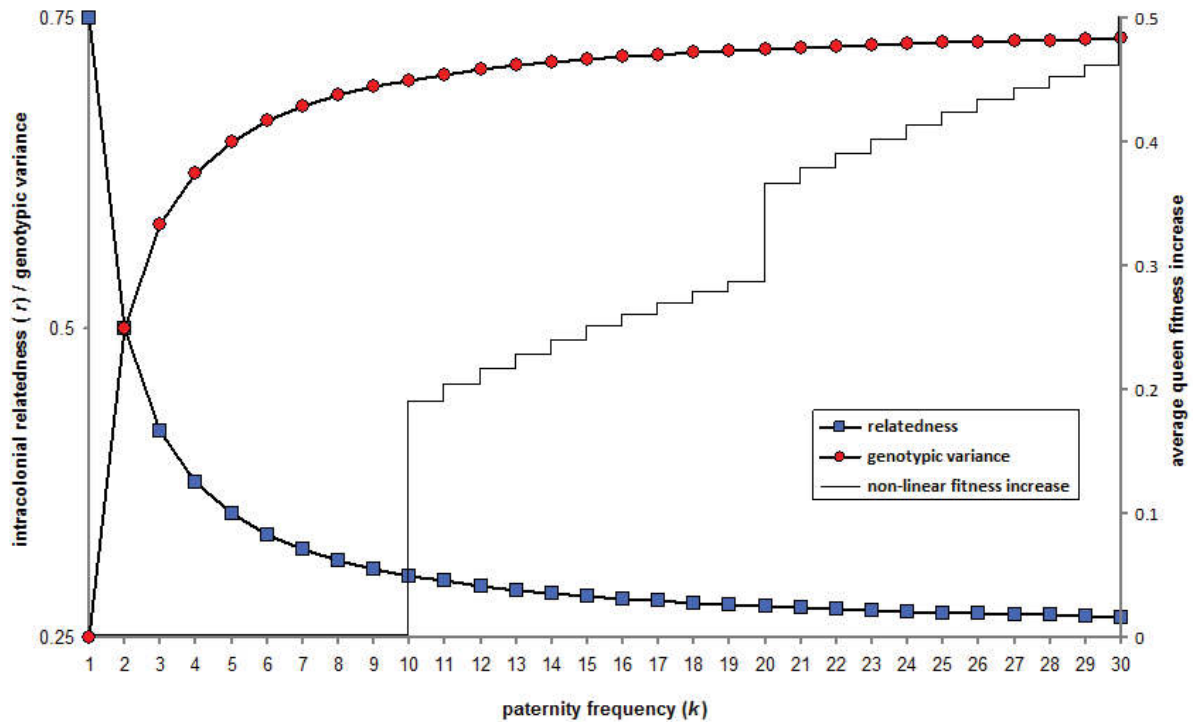
Fig. 1 Average intracolonial relatedness (r) and the respective genotypic variance (IGV) in dependency of the paternity frequency (k) (Kraus & Moritz 2010), with $r = 0.5 \times (0.5 + 1/k)$ (Hamilton 1964), assuming a single queen, and k to represent equally distributed patrilines, i.e., the effective paternity frequency (Starr 1984; Nielsen *et al.* 2003). After an initial steep decrease until a k of six to ten, r asymptotes 0.25 so that only little linear fitness gain from IGV is expected with higher k. In contrast, the thin line represents non-linear fitness gains assuming overdominance of rare specialized patrilines that occur with a frequency of not more than 0.05 among the colony work force, following the stepwise fitness function of Fuchs & Moritz (1998). In this case, IGV fitness gains are expected from extreme polyandry with a minimum k of ten.

Figures

A: *Eciton mexicanum* (small image: *E. burchellii*)
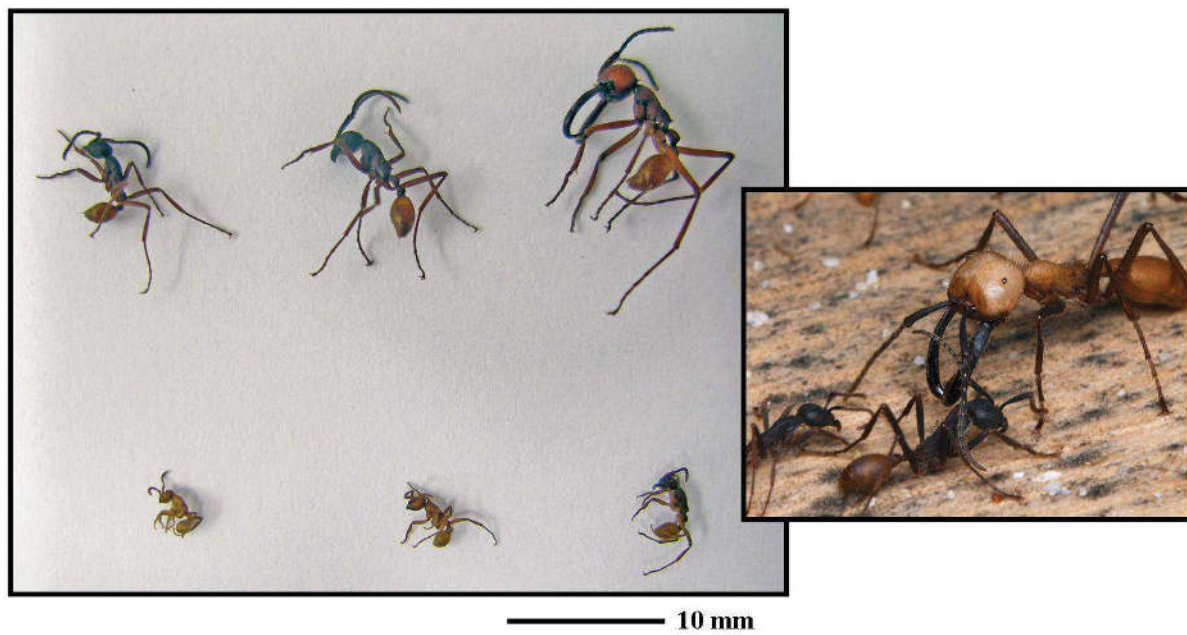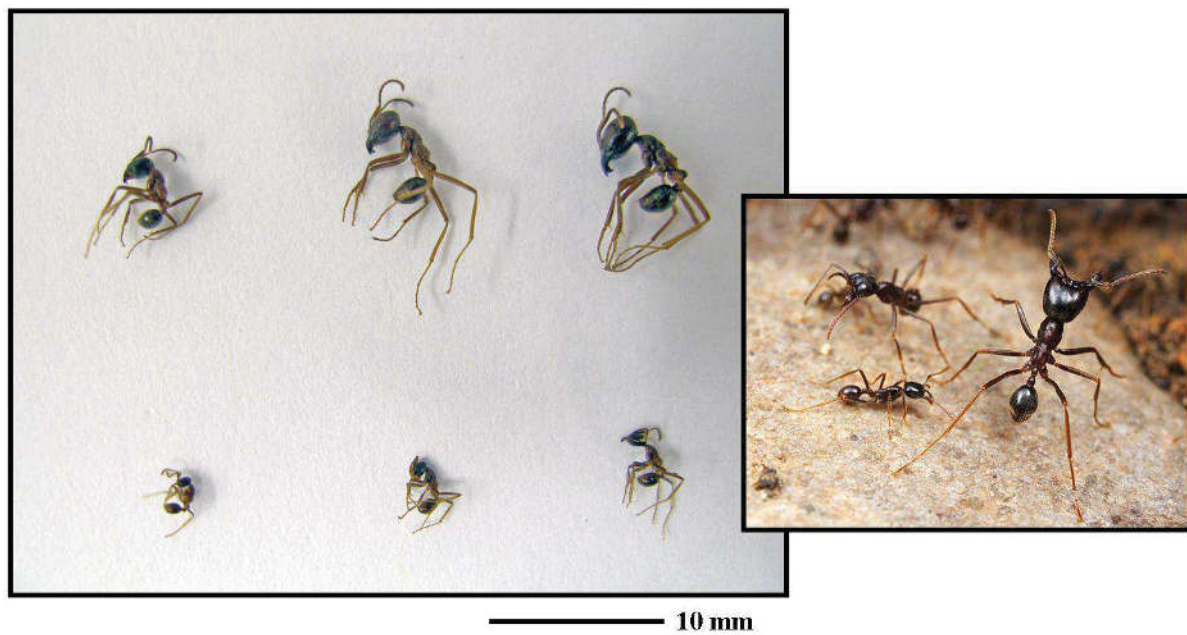

B: *Labidus praedator*

Fig. 2 Examples of extreme intracolonial worker caste polymorphism in army ants of the two genera **(A)** *Eciton* and **(B)** *Labidus*, which have been investigated in this study. Small image courtesy: © Alexander Wild (www.alexanderwild.com).

CHAPTER I

The evolution of extreme polyandry in social insects: insights from army ants

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Abstract

The unique nomadic life-history pattern of army ants (army ant adaptive syndrome), including obligate colony fission and strongly male-biased sex-ratios, makes army ants prone to heavily reduced effective population sizes (N_e). Excessive multiple mating by queens (polyandry) has been suggested to compensate these negative effects by increasing genetic variance in colonies and populations. However, the combined effects and evolutionary consequences of polyandry and army ant life history on genetic colony and population structure have only been studied in a few selected species. Here we provide new genetic data on paternity frequencies, colony structure and paternity skew for the five Neotropical army ants *Eciton mexicanum*, *E. vagans*, *Labidus coecus*, *L. praedator* and *Nomamyrmex esenbeckii*; and compare those data among a total of nine army ant species (including literature data). The number of effective matings per queen ranged from about 6 up to 25 in our tested species, and we show that such extreme polyandry is in two ways highly adaptive. First, given the detected low intracolonial relatedness and population differentiation extreme polyandry may counteract inbreeding and low N_e. Second, as indicated by a negative correlation of paternity frequency and paternity skew, queens maximize intracolonial genotypic variance by increasingly equalizing paternity shares with higher numbers of sires. Thus, extreme polyandry is not only an integral part of the army ant syndrome, but generally adaptive in social insects by improving genetic variance, even at the high end spectrum of mating frequencies.

Keywords

Running title

Evolution of extreme polyandry in social insects

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CHAPTER II

Male-biased dispersal promotes large scale gene flow in a subterranean army ant, *Dorylus* (*Typhlopone*) *fulvus*

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Abstract

Sex-biased dispersal is a widespread phenomenon in the animal kingdom, which strongly influences gene flow and population structure. Particularly army ants, important keystone predators in tropical ecosystems, are prone to population fragmentation and isolation due to their extraordinary mating system: Queens are permanently wingless, propagate via colony fission, and only the males disperse in mating flights. Here we report on sex-biased dispersal and the genetic population structure of an African subterranean army ant, *Dorylus* (*Typhlopone*) *fulvus*. Using maternally inherited mtDNA markers and bi-parentally inherited nuclear microsatellites we found strong geographical structuring of mtDNA haplotypes, whereas the nuclear genetic population structure was less pronounced. Strong mtDNA ($\Phi_{ST} = 0.85$), but significantly lower nuclear ($F_{ST} = 0.23$) genetic differentiation translated into a more than an order of magnitude larger male migration rate compared to that of queens, reflecting the low motility of queens and strong, promiscuous dispersal by males. Thus, the well flying *D. fulvus* males appear to be the sex to promote large scale gene flow, and *D. fulvus* is indeed a species in which sex specific dispersal patterns and the mating system profoundly affect the population structure and phylogeography.

Running title

Male dispersal promotes gene flow

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CHAPTER III

Genetic differentiation at species level in the Neotropical army ant *Labidus praedator* (Ecitoninae)

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Abstract

The nomadic, swarm-raiding army ant *Labidus praedator* (Smith, 1858) is an important arthropod predator in the Neotropics with a strong ecological impact on invertebrate communities. However, despite its high abundance and ubiquity over a large distribution range it received relatively little scientific attention. Moreover, the taxonomic status is confusing because some morphological descriptions point towards the co-occurrence of several distinct taxa which are lumped together as *L. praedator* in most classical keys. Yet, clarifying genetic studies are lacking. Here we show strong genetic differentiation within an *L. praedator* population in Mexico. Both microsatellite genotype patterns and phylogenetic analyses (concatenated nuclear and mtDNA sequences, including the *coxI* genetic barcoding region) reveal the occurrence of two strongly isolated lineages. Colonies from the very same location, clearly identified as the same species (*L. praedator*) according to classical morphological keys, exhibit an extremely high average sequence divergence (9.7% to 12.8%), which was well in the range of divergence among GenBank sequences from other *Labidus* species. Thus, our data very likely show genetic differentiation at species level or cryptic speciation within *L. praedator*, which should be recognized when investigating biodiversity and ecological importance of army ants (or other arthropods) in the Neotropics.

Keywords

Running title

Genetic differentiation in *Labidus praedator*

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General discussion

"In colonies of army ants, perhaps to a greater extent than in any
other animal except man, we see the emergence of flexible problem
solving far exceeding the capacity of the individual."

(Franks 1989, p. 139)

Hitherto, the most plausible hypotheses explaining polyandry in social Hymenoptera are those related to intracolonial genotypic variance (IGV), sperm limitation and the diploid male load (Tab. 1), though problems remain to apply them to paternity frequencies beyond six to ten (Crozier & Page 1985; Crozier & Fjerdingstad 2001; Kraus & Moritz 2010).

CHAPTER I of this thesis provides the first empirical evidence that IGV is a potential proximate driver of such extreme polyandry in army ants, contrary to earlier studies stating that higher paternity frequencies might be selectively neutral (Tarpy & Page 2000, 2002; Kronauer *et al.* 2007). A lowered paternity skew with increasing paternity frequency, as shown in *CHAPTER I*, is against paternity monopolizing male interests and can only be explained by IGV benefits for queens by using all patrilines equally (Boomsma & Sundström 1998; Strassmann 2001; Jaffé *et al.* 2012). IGV benefits beyond the lower intracolonial relatedness limit of 0.25 are most plausibly explained by social heterosis and overdominance of rare patrilines (Fig. 1) (Fuchs & Moritz 1998; Nonacs & Kapheim 2007; Jaffé *et al.* 2012). Future studies may focus on the relative importance of the different IGV hypotheses as ultimate drivers of extreme polyandry in huge and complex colonies, like the need for more efficient colony homeostasis, pathogen resistance and worker task specialization (Tab. 1). The latter is particularly prevalent in army ants with their polymorphic work force (Fig. 2), and worker caste determination has already been shown to have a genetic basis (Jaffé *et al.* 2007) as also found in other ants (e.g., Fraser *et al.* 2000; Hughes *et al.* 2003; Rheindt *et al.* 2005).

The sperm limitation hypothesis could not be supported in this study. No correlation between mean paternity frequency and colony size across army ant species, and no random fluctuation in paternity skew from natural between male sperm number differences was found (*CHAPTER I*), as would be expected if queens simply used all available sperm (Cole 1983; Boomsma & Ratnieks 1996; Strassmann 2001; Jaffé *et al.* 2012). In army ants four to six males could theoretically fully inseminate a queen (Kronauer & Boomsma 2007a; but see

Bauplan restrictions, Kraus & Moritz 2010), so that sperm limitation probably plays a minor role, at least for the evolution of higher paternity frequencies.

CHAPTER II of this thesis nicely shows how male-biased dispersal promotes gene flow in army ants, and how mating system evolution may in this way counteract genetic depletion and shape population structuring even at large geographical scale. In monogynous ants this is characteristic if colony founding queens depend on workers, as by fission (Peeters & Ito 2001; Doums *et al.* 2002; Sanetra & Crozier 2003; Clémencet *et al.* 2005), which often leads to strongly male-biased sex-ratios (Bulmer 1983). Interestingly, striking parallels to honeybees show a close interplay between life history and mating system evolution across taxa (Kronauer *et al.* 2007; Kraus & Moritz 2010). Like army ants, honeybees reproduce via colony fission and are extremely polyandrous, despite having elevated mating costs from predation risks during their nuptial flights (Ruttner 1980; Moritz & Southwick 1992; Moritz *et al.* 1995; Seeley 1995; Palmer & Oldroyd 2000; Tarpy *et al.* 2004; Schlüns *et al.* 2005). Colony fission is a minimizing risk strategy with high investment into new colonies, making a queen fitness function conducive to select for polyandry for decreasing intercolonial trait variance (while increasing intracolonial genetic variance). Such variance based selection has been shown to counteract the diploid male load (Page 1980, 1986; Crozier & Page 1985; Crozier & Fjerdingstad 2001), and may apply also for any other trait with a genetic basis, as e.g., task specialization (Fuchs & Moritz 1998; Kraus *et al.* 2005; Rueppell *et al.* 2008).

In army ants this is potentially relevant as demonstrated by the outbreeding tendencies of queens in CHAPTER I of this thesis and in Jaffé *et al.* (2009), as well as in Fig. 1 in Kronauer *et al.* (2007). As further shown in CHAPTER II, army ant queens, in contrast to winged honeybees, are probably more challenged by strong maternal population sub-structuring. Thus, counteracting low effective population sizes and inbreeding risks by polyandry with outbreeding seems essential for population wide genetic variance and responsiveness to selection (Antolin *et al.* 2003; Kraus *et al.* 2005; Neff & Pitcher 2008; Jaffé *et al.* 2009). For future studies it would be interesting to test for the prevalence of diploid males in army ants (see, e.g., Kronauer & Boomsma 2007a) and inter- and intra-population variance in polyandry indicating environmentally induced differential selection pressures on colony IGV needs, as found in other ants (Fjerdingstad *et al.* 2003; Corley & Fjerdingstad 2011) and honeybees (Franck *et al.* 2000; El-Niweiri & Moritz 2011).

In army ants the males "must fly to foreign colonies where they must first be accepted by the workers before they can gain access to the queens" (Franks & Hölldobler 1987, p. 234). Such male selection, probably for relatedness in order to outbreed, shows the prevalence of sexual

selection by means of female choice in army ants. This fits equalized sperm use, which conforms queens' IGV interests (Strassmann 2001), prevents costly male competition (Rankin *et al.* 2011; Holman & Kokko 2013) and is also known from other highly polyandrous ants (Den Boer *et al.* 2009; Holman *et al.* 2011) and honeybees (Moritz 1986; Page 1986; Schlüns *et al.* 2004). Thus, usually obligate high or extreme polyandry is probably a fundamentally different system than low polyandry (oligoandry), which is often facultative and most likely ancestral (Boomsma 2009, 2013). The former seems to be driven by IGV needs in large and complex colonies with queen control as outcome of arising sexual conflict, while oligoandry is mostly dominated by high paternity skews from male precedence and selection for relatedness (Hamilton's rule). The two systems may constitute extremes of a trade-off between high relatedness at the basis of sociality and IGV needs after an irreversible transition to complex sociality, when a loss of relatedness becomes less crucial for social cohesion (Bourke 2011; Korb & Heinze 2016).

CHAPTER III of this thesis reveals the potential to explore more of the impressive army ant diversity to further test the above drawn conclusions (see Fig. 3). Army ants are about the most specious group of almost always extremely polyandrous eusocial insects, though little is known about most species but the unity in their adaptive syndrome (Rettenmeyer 1963; Gotwald 1995; Brady 2003). However, exceptions exist, as e.g., the reversion from polyandry to polygyny in *Neivamyrmex carolinensis* (Kronauer & Boomsma 2007b), and similarly a loss of polyandry in *Acromyrmex insinuator*, a workerless social parasite of usually polyandrous leaf cutter ants (Sumner *et al.* 2004). Both cases support the assumption of polyandry to be driven by the need for a genotypically diverse work force. Finding more such exceptions among army ants will surely shed more light onto the connection between their life history and extreme polyandry.

CHAPTER III further shows that strong genetic sub-structuring may occur in army ants even despite male dispersal. In fact, army ants are vulnerable to dispersal barriers through habitat fragmentation (Patridge *et al.* 1996; Peters *et al.* 2011; Pérez-Espona *et al.* 2012); and male flight times are highly variable (Watkins 1982). Such spatiotemporal gene flow barriers may have contributed to speciation events, depending on ecological circumstances (Coyne & Orr 2004). For example, *Labidus* sp. JTL has been discovered in an ecologically very distinct mountain area (Longino 2005). Given these considerations it would be interesting to further analyze the phylogeography of *Dorylus fulvus* (*CHAPTER II*) across its whole African distribution range and compare its habitat structuring with that of Central American *Labidus*. In general it seems urgent to improve phylogenetic knowledge particularly of the less well investigated army ant taxa to foster cross-species studies on their exceptional (mating) biology and evolution.

Altogether, this thesis highlights in which complex manners evolutionary causes and consequences can be interconnected. Mating system evolution is influenced by and in turn

shapes life history, population structure and demography (Gowaty 2012, 2013; Holman & Kokko 2013; Taylor *et al.* 2014). This is strongly linked to sex-ratio and dispersal biology, which again affects social evolution and sexual conflict (Gardner 2010; Rankin *et al.* 2011), looping back to mating systems. Fig. 3 shows a possible evolutionary scenario in army ants.

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Fig. 3 Possible evolutionary scenario for the extremely polyandrous mating system in army ants as derived from this thesis on the basis of Gotwald (1995), Palmer & Oldroyd (2000), Brady (2003), Kronauer *et al.* (2007) and Kronauer (2009); including hypotheses of Tab. 1. Cause and consequence are often strongly interconnected. For example, the army ant syndrome as a life history consequence may in turn select for higher polyandry in a positive feet-back loop. Final limits are set by the costs of polyandry. Balancing selection between costs and colony benefits may maintain a high additive genetic variance of paternity frequencies (Kraus *et al.* 2005). Sexual conflict as a consequence may result in high paternity skew from male competition/precedence at low polyandry and in low skew due to female choice at high polyandry. The thickness of arrows symbolizes increasing or decreasing trait values. The colored images (all *Eciton burchellii*) exemplify the army ant syndrome: **(A)** Nomadic, group-raiding colonies with **(B)** specialized worker morphs and **(C)** temporary nest sites (bivouac). Image courtesy: © Bernhard Kraus, with friendly permission.
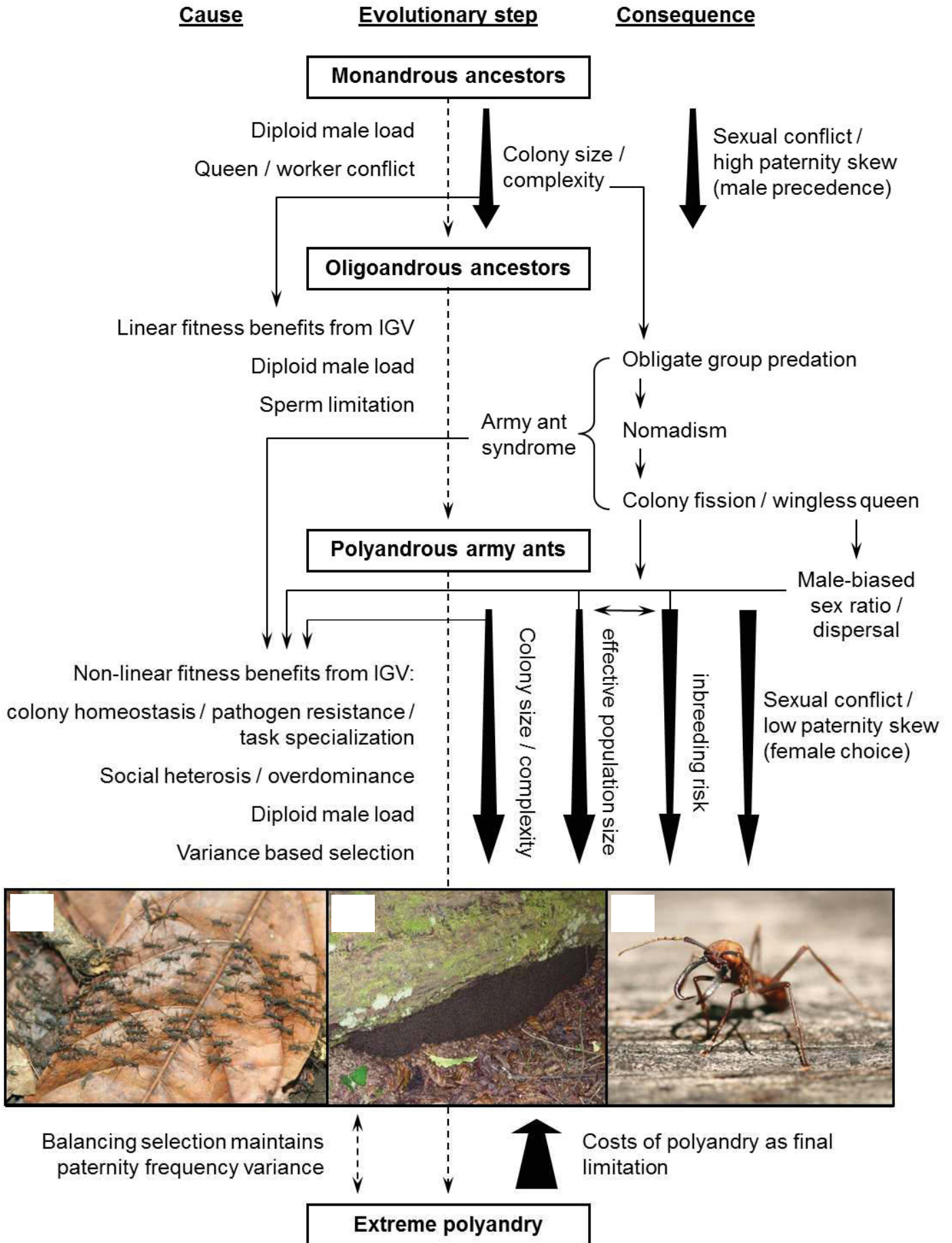
Summary

In evolutionary biology circumstances favoring female multiple mating (polyandry) despite considerable fitness costs are intensely debated, particularly in the eusocial Hymenoptera (ants, bees, wasps), in which ancestral monogamy is crucial for intracolonial relatedness and sociality. Leading hypotheses readily explain low to intermediate polyandry by intracolonial genotypic variance (IGV), sperm limitation or intercolonial variance reduction counteracting the Hymenopteran specific diploid male load. But more difficult to explain extreme polyandry (i.e., above six matings) regularly occurs, as in the diverse and organizationally highly advances army ants. Making use of such an ideal test case this thesis aims at investigating the significance of the above hypotheses in the evolution of extreme polyandry and its consequences.

IGV as a driving cause is confirmed in army ants despite meager variance gains above six matings. Paternity equalization by queens with increasing paternity frequency, contrary male monopolization interests, shows not only potentially over proportionally beneficial IGV fitness gains from diverse patrilines in a colony, but also female control over sexual conflict arising from polyandry. The sperm limitation hypothesis could not be supported. Outbreeding tendencies show the relevance of the diploid male load. Army ant life history, including wingless queens and colony fission, strengthens this load by increased risks of inbreeding, lowered effective population sizes and restricted dispersal, but is in the same time conducive to polyandry counteracting these effects. Consequences are extremely male-biased sex-ratios, dispersal and gene flow. Thus, evolutionary causes and consequences of extreme polyandry appear to be closely interconnected and manifested in the army ant adaptive syndrome.

Not least, this thesis emphasizes the importance of phylogenetically correlated cross-species analyses in studying trait evolution. It reveals the potential of still unexplored army ant diversity, but also a lack of knowledge on deep phylogeny and spatiotemporal processes shaping army ant differentiation and speciation. Future studies may explore this potential to shed more light on the interplay of causes and consequences in the evolution of extreme polyandry and other aspects of the extraordinary and fascinating army ant biology.

Zusammenfassung

Warum paaren sich Weibchen mehrfach (Polyandrie), trotz erheblicher damit verbundener Fitness Nachteile? Diese Frage ist in der Evolutionsbiologie höchst umstritten, vor allem bei eusozialen Hymenopteren (Ameisen, Bienen, Wespen), für welche Monogamie ursprünglich und essentiell für den sozialen Koloniezusammenhalt ist. Führende Hypothesen erklären mittelmäßig starke Polyandrie durch intra-koloniale genotypische Varianz (IGV), Spermien-limitierung oder Reduktion inter-kolonialer Varianz des Hymenopteren typischen Problems diploider Männchen. Jedoch kommt auch schwieriger zu erklärende extreme Polyandrie (über sechs Paarungen) in einigen Gruppen vor, wie bei den vielfältig und hoch entwickelten Treiberameisen. Diese Dissertation soll die Relevanz erwähnter Hypothesen für Evolution und Konsequenzen extremer Polyandrie an diesem idealen Modellsystem untersuchen.

Trotz geringen erwarteten Zugewinns über sechs Paarungen, wird IGV als treibende Kraft bestätigt. Dies zeigt sich in zunehmend gleich verteilten Vaterschaften durch Köni-ginnen mit höheren Paarungsfrequenzen, gegen Monopolisierungsinteressen der Männchen, was sowohl für potentiell überproportionalen Fitnessgewinn durch intra-koloniale Patrilinien-diversität spricht, als auch für Kontrolle der Königin über resultierende sexuelle Konflikte. Spermienlimitierung konnten nicht bestätigt werden. Anzeichen von Inzuchtvermeidung zeigen die Relevanz des Problems diploider Männchen. Der Lebenszyklus der Treiberameisen mit Kolonieteilung durch ungeflügelte Königinnen verstärkt dieses Problem durch reduzierte effektive Populationsgrößen und Verbreitungsfähigkeit, sowie erhöhte Inzuchtgefahr, ist aber gleichzeitig der Kompensation dieser Probleme durch Polyandrie zuträglich. Konsequenzen sind ein extrem männchenlastiges Geschlechterverhältniss, Verbreitungs- und Genfluss-muster. Das heißt, Evolution und Konsequenz extremer Polyandrie sind eng miteinander und mit der speziell angepassten Lebensweise der Treiberameisen verknüpft.

Nicht zuletzt hebt diese Dissertation die Wichtigkeit phylogenetisch korrelierter artübergreifender Studien zur Merkmalsevolution hervor. Sie deckt fernern Potential dafür in noch unerforschter Treiberameisendiversität auf, aber auch den Mangel an tiefgründigem Verständnis für stammesgeschichtliche Zusammenhänge und räumlich-zeitliche Prozesse, die zur Diversifizierung und Artbildung bei Treiberameisen führen. Zukünftige Studien mögen dieses Potential nutzen und mehr Klarheit in das komplexe Zusammenspiel von Ursachen und Konsequenzen der Evolution extremer Polyandrie und anderer Aspekte der Biologie dieser außergewöhnlichen und faszinierenden Tiere bringen.

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Appendix

Declaration on the author contributions

Chapter I: Barth MB, Moritz RFA, Kraus FB (2014) The evolution of extreme polyandry in social insects: insights from army ants. *PLoS One* **9**: e105621, DOI:10.1371/journal.pone.0105621

I participated in design of the project, genotyped the samples, extracted data from the literature and performed analyses and manuscript writing. I and F.B. Kraus collected samples around Tapachula, Mexico. R.F.A. Moritz provided laboratory facilities. F.B. Kraus and R.F.A. Moritz participated in project design, supervised it and provided helpful discussions on project and manuscript.

Chapter II: Barth MB, Moritz RFA, Pirk CWW, Kraus FB (2013) Male-biased dispersal promotes large scale gene flow in a subterranean army ant, *Dorylus* (*Typhlopone*) *fulvus*. *Popul. Ecol.* **55**: 523–533, DOI:10.1007/s10144-013-0383-4 – Editor's Choice Article

I participated in design of the project, genotyped the samples and performed analyses and manuscript writing. R.F.A. Moritz provided laboratory facilities. C.W.W. Pirk coordinated sampling in South Africa. F.B. Kraus and R.F.A. Moritz participated in project design, supervised it and provided helpful discussions on project and manuscript.

Chapter III: Barth MB, Moritz RFA, Kraus FB (2015) Genetic differentiation at species level in the Neotropical army ant *Labidus praedator* (Ecitoninae). *Insectes Soc.* **62**: 299–306, DOI:10.1007/s00040-015-0410-x

I participated in design of the project, photographed and genotyped the samples, extracted data from GenBank and performed analyses and manuscript writing. I and F.B. Kraus collected samples around Tapachula, Mexico. R.F.A. Moritz provided laboratory facilities. F.B. Kraus and R.F.A. Moritz participated in project design, supervised it and provided helpful discussions on project and manuscript.

Publication list

Peer-review publications

Buchwalder K, <u>Barth MB</u>, Kawahara AY, Zhou X, Liu S, Hundsdoerfer AK (in rev.) *De novo* assembly and functional annotation of the *Hyles euphorbiae* hawkmoth transcriptome: focus on phorbol ester detoxification and cold hardiness. *BMC Genomics* – Shared First Authorship Article

<u>Barth MB</u>, Moritz RFA, Kraus FB (2015) Genetic differentiation at species level in the Neotropical army ant *Labidus praedator* (Ecitoninae). *Insectes Soc.* **62**: 299–306, DOI:10.1007/s00040-015-0410-x

<u>Barth MB</u>, Moritz RFA, Kraus FB (2014) The evolution of extreme polyandry in social insects: insights from army ants. *PLoS One* **9**: e105621, DOI:10.1371/journal.pone.0105621

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Editorial work

<u>Barth MB</u>, Scharpenberg H, Moritz RFA (2012) 5th European Conference of Apidology (Eurbee 5), 2012. Conference proceedings.

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Erklärung

Hiermit erkläre ich, dass diese Arbeit von mir bisher weder bei der Naturwissenschaftlichen Fakultät I der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Ich erkläre, dass ich mich bisher noch nicht um den Doktorgrad beworben habe.

Ferner erkläre ich, dass ich diese Arbeit selbständig und nur unter Zuhilfenahme der angegebenen Hilfsmittel und Literatur angefertigt habe.

Benjamin Barth