Review

Lifelong commitment to the wrong partner: hybridization in ants

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The extraordinary lifelong partner commitment in social insects is expected to increase choosiness in both sexes and therefore to be associated with particularly low hybridization frequencies. Yet, more and more studies reveal that in many ant taxa hybrids are surprisingly common, with up to half of all female sexuals receiving sperm from allospecific males in extreme cases. In a few ant species, hybridization has led to the evolution of reproductively isolated new lineages with a bizarre system of genetic caste differentiation: colonies produce hybrid workers and pure-lineage female sexuals. This requires that colonies either contain multiple queens or that queens mate multiple times. In most other cases, hybridization appears to be an evolutionary dead end and fertile hybrid queens are rarely found. In such cases, haplodiploid sex determination appears to decrease the costs of mating with an allospecific male. As long as hybrid workers are viable, a cross-mated queen can partially rescue its fitness by producing males from unfertilized eggs. Mating with an allospecific partner may thus be an option for queens when conspecific mates are not available. The morphological similarity of most ant males, perhaps resulting from the lack of sexual conflict, may similarly contribute to the commonness of hybridization.

Keywords: levels of selection; caste determination; sperm theft; mating biology; evolutionary arms races

1. INTRODUCTION

Evolution in eusocial insects (ants, bees, wasps and termites) simultaneously acts on different levels of selection (e.g. Keller 1999). Consequently, research on hybridization in social insects may yield particularly exciting results, because hybridization may affect both the phenotype of the individual and the social phenotype of the whole society. This is nicely exemplified by recent findings of hybridization leading to such striking phenomena as genetic division of reproductive labour, changed patterns of colony-level reproductive allocation and 'sperm theft'. In this review, we examine the causes and consequences of hybridization in social insects, particularly highlighting the colony-level effects arising from social life and, in social Hymenoptera, peculiarities resulting from haplodiploid sex determination. As previous research on hybridization in social insects has concentrated on ants, we similarly focus on this extremely successful and important group.

2. SEEMINGLY INDISCRIMINATE MATING AND THE COMMONNESS OF HYBRIDIZATION IN ANTS

Social insects with perennial societies are characterized by partner commitment for life, which in the case of some ants or termites may be more than 25 years (Boomsma *et al.* 2005). In bees and ants, male and female sexuals usually mate once or repeatedly during a short period of frenzied activity soon after adult eclosion. Males die shortly after copulation and females store the sperm they received from their mates in a special receptacle in the reproductive tract and use it for the fertilization of eggs throughout the rest of their lives without ever mating again. In termites, females and males engage in similar lifelong pair-bonding, only that here the males survive and continue to mate with the same female (e.g. Wilson 1971). Partner commitment for life is expected to select for particularly increased choosiness of both mates, because the fitness costs resulting from mating or consorting with the wrong partner(s) early in life cannot be compensated by future reproductive episodes. One might therefore expect sexuals of social insects to be extremely careful about whom they mate with and hybridization to be a rare event.

Surprisingly, the opposite is the case: evidence for pre-mating sexual selection in social insects is as yet scarce, and recent studies suggest that in some taxa of

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ants hybridization abounds. Though large body size appears to be advantageous for males and probably also females in some ant species (e.g. Davidson 1982; Wiernasz *et al.* 1995; Abell *et al.* 1999), in others, sexuals seem to mate indiscriminately with the first available partner (Woyciechowski 1987, 1990). Multiple mating might potentially enable queens to increase their fitness through cryptic female choice and sperm competition (Boomsma *et al.* 2005), but most queens end up with a load of genetically identical sperm from a single, haploid mating partner, occasionally from another species.

This astonishing lack of choosiness might be explained by the time limitation for mating activities. In particular in temperate ant species, mating is often restricted to a few hours on one or a few days per year (McCluskey 1992; Dunn *et al.* 2007), during which ten thousands of sexuals simultaneously search for mating chances. This is particularly the case in the first of the two most common types of mating behaviour, the 'male aggregation syndrome' (Hölldobler & Bartz 1985; Boomsma *et al.* 2005). Here, males from hundreds of colonies aggregate around a landmark, which may be marked with pheromones (Hölldobler & Bartz 1985). Females join these aggregations, mate and then fly off to found their own colonies. Such large-scale 'nuptial flights' tend to be highly synchronized in space and time and leave little opportunities for female or male scrutiny, owing to the intensity of competition and also predation. Mating in the second type, the 'female-calling syndrome', is considerably less synchronized. Single females try to attract males with pheromones over a period of several days (Hölldobler & Bartz 1985), and both female and male sexuals might therefore have much more time to be picky when choosing their mating partners. Observations indeed suggest that in such species potential mates are occasionally rejected (Oberstadt & Heinze 2003).

The chronology of mating throughout the course of the year is strongly influenced by the timing of larval development, which again appears to be rather conserved in evolution. For example, sexuals of many *Camponotus* species eclose in autumn, hibernate in the maternal nest and engage in nuptial flights already in late spring (e.g. Hölldobler 1966). By contrast, *Formica* and several other formicine ants hibernate without brood, and sexuals develop from eggs laid in late winter or early spring (Eidmann 1942). Their mating period is restricted to June and July, which gives young queens ample time for successfully founding their own societies before the onset of winter. As a consequence, congeneric species in temperate regions often do not differ strongly in the timing of their mating activities, resulting in the frequent co-occurrence of sexuals of several closely related species (see Baldrige *et al.* (1980) for tropical ants).

Hybridization resulting from this concurrence might in part be avoided by species-specific sex pheromones or species-specific day times of sexual activity, as documented in *Temnothorax* (Plateaux 1978), *Pogonomyrmex* and other ant species (Hölldobler 1976; McCluskey 1992). However, at least in the laboratory, males can be lured into mating with an allospecific partner by disorienting them with

pheromones released by a conspecific mate nearby (Heinze & Buschinger 1989), and similar phenomena might frequently occur in nature.

Recent morphological and genetic studies revealed a striking commonness of hybrids (table 1) in North American fire ants (e.g. Hung & Vinson 1977; Ross *et al.* 1987; Vander Meer & Lofgren 1989; Helms Cahan & Vinson 2003; Shoemaker *et al.* 2006) and several other ant genera (*Lasius*: Pearson 1983b, Seifert 1999, Umphrey 2006; *Formica*: Seifert 1999, Seifert & Goropashnaya 2004; *Temnothorax*: Douwes & Stille 1991, Seifert 1999, Pusch *et al.* 2006a,b; *Pogonomyrmex*: Helms Cahan *et al.* 2002, Julian *et al.* 2002, Anderson *et al.* 2006, Schwander *et al.* 2007b; *Crematogaster*: Feldhaar *et al.* 2003, H. Feldhaar 2006, unpublished data). It appears that in some areas of Central Europe, almost half of all female sexuals of the well-studied and highly diverse ant genus *Temnothorax* mate with allospecific males (Douwes & Stille 1991; Seifert 1999). Morphological studies and the narrowness of hybrid zones suggest that most cases of natural hybridization do not result in either the breakdown of species borders or the evolution of highly viable, permanent hybrid species with the exception of some lineages of *Pogonomyrmex* harvester ants (see below). Irrespective of occasional introgression (e.g. Goropashnaya *et al.* 2004; Ross & Shoemaker 2005; Shoemaker *et al.* 2006), hybridization in ants might therefore be seen as an evolutionary dead end, of interest only to a number of specialists, had it not been for the recent discovery of stable hybrid populations, in which mating nevertheless usually involve only pure-lineage sexuals. Given that detailed genetic or morphological analyses of closely related groups of species are needed to reveal such cases of evolutionarily stable hybridization, it is potentially a much more common phenomenon that should generally be taken into consideration in research on ant evolution, ecology and population biology.

3. INDIVIDUAL- AND SPECIES-LEVEL CONSEQUENCES OF HYBRIDIZATION IN ANTS

In Hymenoptera and other haplodiploids, hybridization among two parental species affects exclusively the phenotype of females, because males normally develop from unfertilized pure-species eggs. Even in those cases in which hybridization appears to be a more permanent phenomenon (e.g. *Pogonomyrmex*, *Solenopsis* and see below), natural matings usually involve pure-lineage female sexuals. Hybrid males have therefore been rarely found and most data on individual-level effects of hybridization therefore refer to the two female castes, workers and queens.

Only a few studies have investigated whether closely related species yield viable offspring in enforced encounters in the laboratory, because sexuals of most ant species are reluctant to mate under experimental conditions. The results obtained with a few species in which mating takes place in or close to the nest and therefore can be elicited in the laboratory, range from complete failure either due to pre-mating isolation or abortion (several taxa in the *Cardiocondyla* 'kagutsuchi' group, K. Yamauchi 2004, unpublished data) to fully

viable and fertile hybrid queens (as in the workerless social parasites *Leptothorax pacis* and *Leptothorax kutteri*; Buschinger 1972), and various species of the slave-making genus *Myrmoxenus* (Jessen & Klinkicht 1990; Buschinger 2001). Such studies nicely demonstrate that hybridization in ants affects both the individual and social phenotypes of the society. For example, some species of *Myrmoxenus* are active slave-makers, which raid pupae from the nests of their host species, while other *Myrmoxenus* are 'degenerate' slave-makers, which almost exclusively produce sexual offspring and only a few workers or none at all. Cross-breeding active and degenerate slave-makers resulted in hybrid colonies producing intermediate worker numbers (Jessen & Klinkicht 1990; Buschinger 2001).

The morphology, anatomy and physiology of naturally occurring hybrids often reflect imbalances between paternal and maternal genes and/or heterosis (table 1). For example, a positive effect of hybridization was observed in naturally occurring *Solenopsis invicta* × *Solenopsis richteri* workers, which were more tolerant of low temperatures than pure-species workers of either *S. richteri* or *S. invicta* (James et al. 2002). In many other cases, however, hybridization appears to negatively affect offspring quality. For example, hybrids of *Leptothorax* (J. Heinze & R. Brandl 1993, unpublished data) and *S. invicta* × *S. richteri* (Ross & Robertson 1990) showed increased levels of fluctuating asymmetry, and hybrid female sexuals of *Temnothorax* were smaller and less successful during colony foundation (Pusch et al. 2006a) or had less ovarioles than those of the parental species (Plateaux 1979). Hybrid sterility or a generally lowered fitness of hybrid females might explain why hybrid female sexuals are rarely found despite the common occurrence of F₁ hybrid workers (Helms Cahan & Vinson 2003; Umphrey 2006).

Despite the reportedly low fitness of F₁ female sexuals, cross-mated queens do not completely lose their fitness (table 2). As long as hybrid workers are viable and capable of taking care of the brood, queens are still capable of producing non-hybrid males from unfertilized eggs (Nonacs 2006). Even the requirement of viable hybrid workers is not essential in the case of species which are either inter- or intraspecific social parasites. They can found their own nests by usurping established colonies of an allospecific host species or conspecific nests and exploit its work force for rearing their own young (e.g. Brandt et al. 2005). As yet, however, there is no evidence that hybridization is more common among social parasites than among their free-living relatives. The ease of experimentally mating sexuals of socially parasitic species with allospecific partners in the laboratory (see above) more likely reflects their readiness to mate on the ground, and recent and perhaps incomplete speciation, as in *Myrmoxenus*. In Southern Germany, *Temnothorax* hybrid workers were frequently found residing in the nests together with pure-species *Temnothorax nylanderi* or *Temnothorax crassispinus* workers, despite obligate monogyny and monandry in both species (Pusch et al. 2006a), suggesting that cross-mated queens are able to take over nests of the parental species.

Decreased viability, fecundity and colony founding success of F₁ hybrid queens (Pusch et al. 2006a) affect

reproductive decisions on the colony level, because both hybrid workers and cross-mated queens should favour the production of males over that of low-quality female sexuals. When male offspring are produced by the queen, as is normally the case in ants (e.g. Hammond & Keller 2004), the sex allocation optima in colonies with cross-mated queens would be selected to be more male biased in comparison with colonies with queens which copulated with a conspecific male. Such effects on sex allocation may be obscured either by multiple mating of individual queens, like in *Pogonomyrmex* (see below), or by the presence of multiple queens (polygyny), of which some queens carry sperm of a conspecific and others of an allospecific male, as in *Solenopsis* (see below). Obviously, such sex ratio adjustment is only feasible when queens and workers can recognize the species of the queen's mate. Furthermore, sex allocation ratios in addition depend on resource availability, nest size, etc. and it may therefore be difficult to identify an effect of hybridization on sex allocation in the field. It is suggestive that *T. nylanderi* × *T. crassispinus* hybrid female sexuals were lighter than female sexuals of the parental species, but more data are needed to determine whether this is a direct consequence of their hybrid genotype, sex ratio manipulation or decreased efficiency of hybrid workers on the colony level (e.g. Pusch et al. 2006a). The latter explanation is corroborated by the observation that, similar to hybrid female sexuals, pure-species female sexuals reared by hybrid workers in natural *T. nylanderi* × *T. crassispinus* colonies usurped by a *T. crassispinus* queen were smaller than those from nests of the parental species (Pusch et al. 2006a). Colonies of *T. crassispinus*, *T. nylanderi* and their hybrids are often taken over by young founding queens or fuse with established colonies when nest sites are limited or decay (Foitzik & Heinze 2000). Though the lighter weight of female sexuals in hybrid colonies suggests a lower efficiency of hybrid workers, usurpation rates, albeit generally high, did not differ between the three categories of colonies (S. Träger 2007, unpublished data).

Negative effects of hybridization on the fitness of female sexuals appear to be mostly avoided in an unusual way through genetic caste determination in *Pogonomyrmex* harvester ants and *Solenopsis* fire ants. Though the origins of this unique phenomenon in *Pogonomyrmex* are as yet controversially discussed (Helms Cahan et al. 2002, 2006; Helms Cahan & Keller 2003; Anderson et al. 2006; Linksvayer et al. 2006), there is general agreement that several distinct lineages of *Pogonomyrmex rugosus* and *Pogonomyrmex barbatus* co-occur where the ranges of the two species overlap in the south western USA. These lineages are reproductively isolated from the pure parental species and obligately hybridize. Sterile workers are produced from interlineage zygotes, while female sexuals are raised from eggs fertilized by sperm from the same lineage (Helms Cahan et al. 2002, 2004; Julian et al. 2002; Volny & Gordon 2002; Helms Cahan & Keller 2003; Anderson et al. 2006), resulting in a reproductive division of labour between hybrid workers and pure-lineage female sexual. This mechanism of caste determination appears to be genetically fixed, as

Table 1. Natural occurrence of hybridization in ants (genetic caste determination, GCD).

species	evidence ^a	caste	directionality	consequences	incidence ^b (%)	reference
<i>Crematogaster captiosa</i> × <i>C. sp. 10</i>	M; mtDNA; nDNA	workers, queens	reciprocal	fertile F ₁ hybrid queens	2.5	Feldhaar <i>et al.</i> (2003) and H. Feldhaar (2006, unpublished data)
<i>Formica bruni</i> × <i>F. pressilabris</i>	M	workers	?		2.5	Seifert (1999)
<i>Formica pratensis</i> × <i>F. lugubris</i>	M; mtDNA	workers	unidirectional (<i>F.p.</i> male × <i>F.l.</i> queen)		14.5	Seifert & Goropashnaya (2004)
<i>Formica aquilonia</i> × <i>F. lugubris</i>	M; mtDNA	workers	?		11.1	Seifert & Goropashnaya (2004)
<i>Formica polycтена</i> × <i>F. aquilonia</i>	M; mtDNA	workers	?		12.5	Seifert & Goropashnaya (2004)
<i>Formica lugubris</i> × <i>F. rufa</i>	M; mtDNA	workers	?		?	Seifert & Goropashnaya (2004)
<i>Formica polycтена</i> × <i>F. rufa</i>	M; mtDNA	workers	?		26	Seifert (1991) and Goropashnaya <i>et al.</i> (2004)
<i>Lasius alienus</i> × <i>L. niger</i>	A; M	workers	unidirectional (<i>L.a.</i> queen × <i>L.n.</i> male)	F ₁ queens produced but not able to found colonies	16	Pearson (1983a,b)
<i>Lasius jensi</i> × <i>L. umbratus</i>	M	workers	unidirectional (<i>L.j.</i> queen × <i>L.u.</i> male)	F ₁ queens do not seem to be produced	19	Seifert (1999)
<i>Lasius (Acanthomyops) latipes</i> × <i>L. claviger</i>	A; M	workers, queens	unidirectional (<i>L.l.</i> queen × <i>L.c.</i> male)	F ₁ queens produced but not able to found colonies	57	Umphrey & Danzman (1998) and Umphrey (2006)
<i>Pogonomyrmex barbatus</i> × <i>P. rugosus</i>	A; M; mtDNA; nDNA	workers, queens	reciprocal	obligate multiple mating of queens needed for species persistence; F ₁ queens rare and with lower fertility	100 for lineages with GCD; 0 in pure species	Julian <i>et al.</i> (2002), Anderson <i>et al.</i> (2006) and Schwander <i>et al.</i> (2007a,b)
<i>Solenopsis xyloni</i> × <i>S. geminata</i>	A; M; nDNA	workers, queens	unidirectional (<i>S.x.</i> queen × <i>S.g.</i> male)	possibly, higher survival of polygynous colonies with hybrid workers; F ₁ queens?	100	Hung & Vinson (1977) and Helms Cahan & Vinson (2003)
<i>Solenopsis invicta</i> × <i>S. richteri</i>	A; M	workers, queens	reciprocal	hybrid zone only in introduced range; higher fluctuating asymmetry in F ₁ queens	62	Ross <i>et al.</i> (1987) and Ross & Robertson (1990)
<i>Solenopsis invicta</i> × <i>S. quinquecupis</i>	A; M; mtDNA	workers	biased introgression from <i>S.i.</i> towards <i>S.q.</i>	?	?	Ross & Shoemaker (2005) and Shoemaker <i>et al.</i> (2006)

(Continued.)

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species	evidence ^a	caste	directionality	consequences	incidence ^b (%)	reference
<i>Solenopsis richteri</i> × <i>S. quinquecupis</i>	A; M; mtDNA	workers	biased introgression from <i>S.r.</i> towards <i>S.q.</i>		?	Ross & Shoemaker (2005) and Shoemaker <i>et al.</i> (2006)
<i>Tennothorax unifasciatus</i> × <i>T. nigriceps</i>	A; M	workers, queens	?	fertility of F ₁ queens not known;	approximately 1	Douwes & Stille (1991) and Seifert (1999)
<i>Tennothorax nigriceps</i> × <i>T. albipennis</i>	A; M	workers	unidirectional (<i>T.a.</i> queen × <i>T.n.</i> male)		7	Douwes & Stille (1991) and Seifert (1999)
<i>Tennothorax unifasciatus</i> × <i>T. albipennis</i>	A; M	workers, queens	?	F ₁ queens seem to be fertile and backcrosses to <i>T. unifasciatus</i> probably	4	Douwes & Stille (1991) and Seifert (1999)
<i>Tennothorax tuberum</i> × <i>T. albipennis</i>	A; M	workers	?		~6	Douwes & Stille (1991) and Seifert (1999)
<i>Tennothorax parvulus</i> × <i>T. lichensteini</i>	M; laboratory experiment	queens	?	F ₁ queens have fewer ovarioles and eggs do not develop	?	Plateaux (1979)
<i>Tennothorax nylanderii</i> × <i>T. crassispinus</i>	A; M; mtDNA	workers, queens	unidirectional (<i>T.n.</i> male × <i>T.c.</i> queen)	hybrid colonies produced more workers; F ₁ queens have lower weight than pure-species queens	more than 10	Pusch <i>et al.</i> (2006a,b)

^a Hybridization between the respective species was detected with: A, allozyme data; mtDNA, mitochondrial DNA; nDNA, nuclear DNA including microsatellite data; M, morphological data.

^b Maximum reported incidence of nests containing hybrid individuals per population in a hybrid zone.

Table 2. Comparison of the costs and consequences of hybridization in ants and other non-social diploid–diploid animal species.

ants		non-social animals
costs	no re-mating possible later in life, if mated with heterospecific male	re-mating, to reduce the impact of inbreeding may be frequently possible depending on species
	queens can regain some fitness through parthenogenetic male production if hybrid workers are viable	complete fitness loss for both sexes, if hybrid offspring are unfertile
	fitness losses for males in species where queens use heterospecific sperms to produce sterile workers	complete fitness loss for both sexes, if hybrid offspring are unfertile
	can be reduced for queens with dependent nest foundation as the help of non-hybrid workers can allow production of fertile males (and queens)	no help from conspecifics, which are not affected by hybridization
consequences	hybridization can lead to genetic caste determination	n. a.
	hybridization can influence colony allocation strategies	if sexes are differentially affected by hybridization, skewed sex ratios can be selected
	sperm theft selects for choosy males	n. a.

pure-lineage eggs usually fail to develop into workers in the absence of interlineage brood (Helms Cahan *et al.* 2004; but see Schwander *et al.* 2007a).

As long as individual queens can ensure to receive sperm from both lineages or species through multiple mating, they are capable of producing a viable colony. Singly mated queens have a low reproductive success, because they either produce only workers and males, but no female sexuals, or they are incapable of starting their new colony, because they cannot produce any workers (Helms Cahan *et al.* 2004). Mating with two or more appropriate males is apparently facilitated by variation in the cuticular hydrocarbons between males of the two different lineages with which *P. barbatus* queens need to mate. This possibly provides the cues necessary for lineage discrimination and selection (Volny *et al.* 2006). Nevertheless, Schwander *et al.* (2006) showed that queens of lineages most commonly represented in a mating flight produce a higher proportion of pure-lineage eggs laid by queens, suggesting random mating among queens with the different types of males resulting in frequency-dependent selection on pairs of lineages in a population.

Even for multiple-mated queens, genetic caste determination is associated with considerable costs, since all eggs fertilized with conspecific sperm become female sexuals regardless of the size and age of the colony. In small experimental founding colonies, the larger female sexual brood was culled by workers, leading to a decrease in the proportion of this type of brood from egg to pupal stage (Clark *et al.* 2006; Schwander *et al.* 2006; Volny *et al.* 2006). This might eventually select for sperm clumping and cryptic female choice, that is, queens should preferentially use allopecific sperm to produce workers during the ergonomic phase of colony life until colonies have reached the size necessary to successfully rear female sexuals from eggs fertilized by conspecific sperm.

Genetic caste determination has also been demonstrated in colonies of fire ants, *Solenopsis xyloni*. As fire ant queens mate only with a single male, the system is evolutionarily stable only because colonies contain multiple queens. *Solenopsis xyloni* queens, which mated with *Solenopsis geminata* males, produce F₁ hybrid workers and *S. xyloni* queens, which mated with *S. xyloni* males, produce pure *S. xyloni* female sexuals (Helms Cahan & Vinson 2003). All colonies of *S. xyloni* in the studied population contained hybrid workers, suggesting that these are needed for colony survival (Helms Cahan & Vinson 2003). As in *Pogonomyrmex*, hybrid larvae appear to be shunted into developing into workers and pure *S. xyloni* larvae into female sexuals, possibly by genomic imprinting (Helms Cahan & Vinson 2003). Single mating and multiple queening in *S. xyloni* leads to an evolutionary conflict, because queens mated with allopecific males should favour a more male-biased sex ratio than queens mated with conspecific males. Nonetheless, the sex ratio in hybrid colonies was found to be strongly female biased (Helms Cahan & Vinson 2003). A third case of what might be genetic caste determination through hybridization has been described from the Japanese ant *Vollenhovia emeryi*, however, the details are not yet clear (Ohkawara *et al.* 2006).

From all these examples, it becomes clear that mating with the wrong partner bears considerably less costs for female sexuals than males, because they are capable of producing female sexuals through additional conspecific matings, as in *Pogonomyrmex*, or of producing males from unfertilized eggs, while the males' sperm leads to sterile hybrid workers or hybrid female sexuals with decreased viability. If no conspecific male is available, mating with an allopecific male might therefore be a better option than not mating at all, because hybrid workers can take care of the queen's male offspring (Nonacs 2006) and probably also because mating itself might have positive effects on queen fecundity and/or longevity (e.g. Schrempf *et al.* 2005). Female sexuals may therefore be less fastidious concerning the species of their mates than males and readily engage in what has been called sperm theft (or cleptogamy in Seifert 1999) or 'sperm parasitism' (Umphrey 2006). By contrast, males should be strongly selected to efficiently discriminate against allopecific female sexuals and to avoid cross-species copulations. Ants use cuticular hydrocarbons as recognition cues to determine species and colony membership (Lahav *et al.* 1999; Howard & Blomquist 2005) and, in consequence, female sexuals might exhibit less species-specific signals, while male sensory organs should focus on detecting species-specific differences. In any case, when hybridization is common or (as in *Pogonomyrmex*) has led to the evolution of new genetically isolated lineages, an arms race between female and male sexuals ensues that may lead to the evolution of ants with particularly unusual reproductive life histories (Umphrey 2006). For example, the extraordinary pattern of reproduction in *Wasmannia auropunctata*, where workers develop from fertilized eggs, female sexuals from unfertilized eggs, and males from fertilized eggs after exclusion of the maternal nucleus (Fournier *et al.* 2005), might represent an option for males to restore their fitness once a genetic caste determination system has evolved (Umphrey 2006).

As mentioned above, males produced by cross-mated queens are not hybrids. This is different if hybrid female sexuals reproduce or if hybrid workers lay eggs. In most species, workers are capable of laying haploid eggs at least in queenless colonies and occasionally also in queenright colonies. The sons of hybrid workers will show stronger effects from hybridization because they are haploid. According to Pearson (1983b), males are worker offspring in *Lasius niger*, but queen offspring in *Lasius alienus*. Where the two species co-occur in sympatry and hybridize, selection for pre-mating isolation should consequently be stronger in female and male sexuals of *L. niger* and in males of *L. alienus* than in females of *L. alienus*, because the latter can compensate fitness losses through hybridization with the production of males. In accordance with this expectation, hybridization was found to be mostly asymmetric, with female sexuals of *L. alienus* mating with *L. niger* males (Pearson 1983a,b). Alternatively, this asymmetry might simply reflect *L. alienus* reaching its species boundary and conspecific mating partners becoming rare.

4. CAUSES FOR THE COMMONNESS OF HYBRIDIZATION IN ANTS: THE MALE THAT NEVER EVOLVED

Several not mutually exclusive phenomena might contribute to the surprising commonness of hybridization in ants. First, as already outlined above, from the perspective of the queen allospecific matings may be better than remaining virgin. Hybridization may therefore particularly be an option in rare species, when conspecific males are unavailable, as in some social parasites or close to the margins of a species' distribution (Nonacs 2006). Furthermore, genes involved in post-zygotic isolation typically act as partial recessives in hybrids. Following Haldane's rule, this first leads to hybrid sterility in the heterogametic sex (Orr 1997; Orr & Presgraves 2000). In ants, the haploid (heterogametic) males are mostly produced by pure-lineage females.

Second, reproductive barriers might be lower than in other organisms. Speciation and radiation of many animal and plant taxa are driven by sexual selection and sexual conflict, which result in arms races leading to increased diversity of genital structures and compounds (Simmons 2001; Arnqvist & Rowe 2005). By contrast, lifelong partner commitment in ants and other social insects presumably leads to a lower evolutionary rate of genital morphology and physiology. It appears that ant males, whose roles are limited to locating a female sexual and mating, are much less diverse in morphology and behaviour than the female sexuals and workers, which are subject to multiple selection pressures. As observed by one of the founding fathers of myrmecology, Wheeler (1910), 'the male is far and away the most stable of the three typical phases... This is best shown in the general uniformity of structure and coloration, which characterize this sex in genera whose females are widely different'. Though natural selection should be stronger for males than for females to maintain, or reinforce, species differences as they will lose all their individual fitness when mating with an allospecific queen, male uniformity might proximately explain the frequent occurrence of hybrids.

Third, hybridization among ant species appears to often result from recent range expansions and secondary contact of parapatric or allopatric species pairs, where gene flow across species borders is not yet completely prevented by reinforcing pre-mating isolation. Such range expansions may be caused by manmade environmental changes (Seifert 1999) or post-glacial recolonization of previously uninhabitable areas (Pusch et al. 2006a), and both factors appear to contribute to the common occurrence of ant hybrids in Central Europe, such as *T. nylanderi* × *T. crassispinus*. In addition, features that prevent regular hybridization among two ant species in their native range might have been lost during bottlenecks associated with their accidental introduction across biogeographic barriers. This is nicely exemplified by the fact that *S. invicta* and *S. richteri* do not hybridize in their native habitat in South America, but form a relatively stable hybrid zone in their introduced range in North America (Ross & Robertson 1990; Shoemaker et al. 2006). That both species seem to have lost their *Wolbachia* endosymbionts during or after the introduction to North America (Shoemaker et al. 2006) might point towards endosymbiont-driven cytoplasmic

incompatibility acting as a post-zygotic reproductive barrier in the native habitat of these ants.

5. CONCLUSION AND FUTURE DIRECTIONS

Many recent studies on species complexes in ants using morphological and genetic data have revealed a certain degree of hybridization. This phenomenon may therefore be rather common, in particular as the opportunity for sexual selection is usually low (Boomsma et al. 2005), at least from the point of view of the queen. The true extent of hybridization across ant taxa still needs to be determined, but we predict that detailed research will reveal numerous additional cases of hybridization, in particular in those ant faunas that are characterized by post-glacial range expansion or the recent introduction of multiple invasive species. Though there is some evidence for introgression (e.g. Goropashnaya et al. 2004; Ross & Shoemaker 2005; Shoemaker et al. 2006), it is not clear whether hybridization eventually leads to a breakdown of species barriers or conversely to speciation, with the one exception of *P. barbatus* × *P. rugosus* where hybrid lineages persist that are reproductively isolated from both parental species (Helms Cahan & Keller 2003; Anderson et al. 2006; Schwander et al. 2007a,b). The species-rich genus *Temnothorax*, in which hybridization appears to be particularly common (Douwes & Stille 1991; Seifert 1999), might be a particularly promising candidate for searching for such hybrid species. The literature teems with large numbers of ill-defined 'varieties' or 'races', such as *Temnothorax tuberum* var. *tubero-interruptus* Forel, which are morphologically very similar to other species, but it needs to be shown whether some of these taxa in fact represent stable species with hybrid origin.

Future studies should also investigate the incidence of hybridization among species in relation to their mating system, since opportunity and strength for sexual selection may be uncovered indirectly by such studies. Owing to the obviously less fast-paced mating activity in species with female calling, sexuals might have more opportunities for scrutinizing and choosing their mating partner, and mechanisms of sexual communication may therefore be more elaborate and discriminative than in species with male aggregations. Pre-mating isolation mechanisms are predicted to be least developed in species in which mating occurs in the nest, where sexuals under natural conditions never encounter a partner from another species.

Furthermore, the recent discovery of ant species or lineages that require allospecific matings to produce a sterile work force raises questions about how the resulting strong conflict between males and females are resolved in hybrid colonies. It remains to be shown whether unusual patterns of caste and sex determination, such as in *Cataglyphis cursor* (Pearcy et al. 2004) or *Wasmannia* (Fournier et al. 2005), reflect different stages in the arms race between the sexes resulting from ancient hybridization and genetic caste differentiation.

Finally, more research needs to be done concerning the effects of hybridization on the colony phenotype. Molecular additions to the existing hybridization experiments between *Myrmoxenus* species with and without workers (Jessen & Klinkicht 1990; Buschinger

2001) might yield important insights into the genetic basis of workerlessness and caste bias in this genus and, more generally, colony-level allocation phenomena in other ants.

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