Unusual modes of reproduction in social insects: Shedding light on the evolutionary paradox of sex

Tom Wenseleers and Annette Van Oystaeyen*

The study of alternative genetic systems and mixed modes of reproduction, whereby sexual and asexual reproduction is combined within the same lifecycle, is of fundamental importance as they may shed light on classical evolutionary issues, such as the paradox of sex. Recently, several such cases were discovered in social insects. A closer examination of these systems has revealed many amazing facts, including the mixed use of asexual and sexual reproduction for the production of new queens and workers, males that can clone themselves and the routine use of incest without deleterious genetic consequences. In addition, in several species, remarkable cases of asexually reproducing socially parasitic worker lineages have been discovered. The study of these unusual systems promises to provide insight into many basic evolutionary questions, including the maintenance of sex, the expression of sexual conflict and kin conflict and the evolution of cheating in asexual lineages.

Keywords:

- evolution of genetic systems; paradox of sex; sexual conflict; social evolution; social insects

DOI 10.1002/bies.201100096

Laboratory of Entomology, Department of Biology, University of Leuven, Leuven, Belgium

Both authors contributed equally to this work.

***Corresponding author:**
Tom Wenseleers
E-mail: tom.wenseleers@bio.kuleuven.be

Abbreviations:
AQS, asexual queen succession; **GCD,** genetic caste determination.

Introduction

The majority of all animals and plants reproduce sexually. Social insects, including ants, bees, wasps and termites, are no exception to this. In both the haplodiploid social Hymenoptera (ants, bees and wasps), as well as the diplodiploid termites, sexual reproduction is the rule. Only in a few species have females been unambiguously shown to have the capacity to produce female offspring asexually via a process known as thelytokous parthenogenesis (Table 1, [1]). The rarity of asexual reproduction in social insects, as well as in other multicellular organisms, represents a major evolutionary paradox [2–4]. This is because asexually reproducing lineages of females would be expected to gain a twofold advantage relative to their sexual counterparts, being able to produce twice as many offspring on a per capita basis and requiring no males for reproduction, and gaining a further advantage from the fact that they can transmit their genetic material to each offspring undiluted [2–5]. However, it is thought that this 'twofold cost of sex' [2] is counterbalanced by the fact that sex and recombination promote genetic variation among progeny, which can confer an advantage in rapidly changing environments, in arms races with co-evolving parasites or by helping to purge deleterious mutations [2–5]. Indeed, in various species of social insects, it has been shown that greater intra-colonial genetic diversity leads to better colony homeostasis, a more efficient division of labour [6, 7] and better disease resistance (reviewed in [8, 9]).

Interestingly, several recent studies have found that some ants and termites have evolved the perfect solution to the dilemma of whether to reproduce sexually or asexually: they use both modes of reproduction simultaneously in different contexts, thereby enabling them to experience the advantages of both. The system in question has been dubbed 'asexual queen succession' (AQS) [1] (see Glossary), and consists of a mixed mode of reproduction, whereby queens produce workers sexually but new queens asexually [1, 10]. In this way, the species' colonies are thought to benefit both from a genetically diverse workforce, whilst queens can benefit from transmitting their genetic material undiluted to the next generation of

Table 1. Well documented cases of thelytokous parthenogenesis in social insects (ants, bees and termites), with details on the type of thelytoky observed (FW = facultative worker thelytoky; OW = obligate worker thelytoky; OW + QL = obligate worker thelytoky associated with the loss of the queen caste; FQ = facultative queen thelytoky; OQ = obligate queen thelytoky; AQS = asexual queen succession, i.e. asexual queen production combined with sexual worker production; AQS + MC = asexual queen succession combined with male cloning, in which case next generation males are clones of their father), the presumed mode by which ploidy is restored (CF or TF = automixis with central or terminal fusion; CF + RMR = automixis with central fusion combined with a reduced mutation rate; AP = apomixis; ? = mechanism unknown; cf. Figs. 1 and 2), whether or not thelytoky is associated with genetic caste determination (GCD) and whether or not worker 'cheater' lineages are known that parasitise other colonies. (Modified after [1])

Taxon	Species	Type	Mode of ploidy restoration	Associated with GCD?	Parasitic worker lineages known?	References
Ants (Hymenoptera, Formicidae)						
Cerapachyinae	*Cerapachys biroi*	OW + QL[a]	CF		no	[54–56]
Dacetinae	*Pyramica membranifera*	OQ?	?			[53]
Formicinae	*Anoplolepis gracilipes*	AQS[b]	?	?		[25]
	Cataglyphis cursor	FW, AQS	CF	no	perhaps	[11, 15, 16, 18, 21, 28, 29]
	Cataglyphis sabulosa	FW	?			[49]
	Paratrechina longicornis	AQS + MC	CF + RMR or AP?[c]			[31]
Myrmicinae	*Messor capitatus*	FW	?			[48]
	Mycocepurus smithii	OQ[d]	CF + RMR or AP			[50–52]
	Pristomyrmex punctatus	OW + QL	CF		yes	[34, 41, 43, 45, 57, 58, 59]
	Vollenhovia emeryi	AQS + MC	CF	yes		[14, 19, 26, 27, 30]
	Wasmannia auropunctata	AQS + MC[e]	CF + RMR	yes		[12, 13, 17, 20, 22–24, 32]
Ponerinae	*Platythyrea punctata*	OW + QL[f]	CF + RMR		yes	[42, 60–67]
Honeybees (Apidae)						
	Apis mellifera capensis	OW or OW + QL[g]	CF + RMR		yes	[37, 46, 74, 103, 104, 105]; [40] and refs. therein
Termites (Isoptera)						[1] and refs. therein
Termopsidae	*Zootermopsis angusticollis*	FQ	?			
	Z. nevadensis	FQ	?			
Kalotermitidae	*Kalotermes flavicollis*	FQ	?			
	Bifiditermis beesoni	FQ	?			
Rhinotermitidae	*Reticulitermis virginicus*	FQ, AQS	TF	perhaps		[1]
	R. speratus	FQ, AQS	TF	perhaps[h]		[10]
Termitidae	*Velocitermes* sp.	FQ	?			

[a] Reproduction is divided among workers and a small percentage of unmated individuals with greater fecundity [55, 56], which could be considered either as a discrete worker caste [55] or atypical, unmated queens [56]. The production of these unmated individuals with larger fecundity is tuned in response to colony needs [56].

[b] AQS is present only in some populations, others are characterised by normal sexual reproduction. The exact details of the system, particularly the way in which males are produced, awaits further study [25].

[c] The observed lack of recombination (i.e. transition to homozygosity) [31] suggests that parthenogenesis is apomictic (ameiotic), but automixis combined with an extremely reduced recombination rate, as inferred for the little fire ant [32] and the Cape honeybee [37], remains possible (M. Pearcy, personal communication).

[d] Obligate queen thelytoky evolved repeatedly in several geographically isolated populations from sexually reproducing ancestors [52].

[e] AQS + MC is present only in some populations. Others, particularly in the native range in Central and South America, are characterised by normal sexual reproduction [20, 24].

[f] In Puerto Rico, worker thelytoky was found to be the dominant mode of reproduction, whereas in Costa Rica, colonies were found to be headed by mated workers ('gamergates') and workers were found to be incapable of reproducing by thelytoky [65]. A morphologically differentiated queen caste was shown to be absent in colonies collected from Puerto Rico, Barbados and Costa Rica [62, 65], but not in colonies collected from Florida, where colonies also occasionally contained queens or intercaste individuals, even though most of these were not laying eggs in the colonies examined [62].

[g] *Apis mellifera capensis* workers have been shown to contribute up to 10.5% of the workers and 0.5% of the drones in queenright colonies [92]. Most of the workers' contribution towards the production of new workers coincided with the colonies producing new queens during reproductive swarming [92]. There is also one known monophyletic parasitic lineage of *capensis* workers that specializes in reproductively parasitising colonies of the congeneric African honeybee *Apis mellifera scutellata* [37, 39, 40, 44]. Normal *capensis* colonies are headed by a single, sexually reproducing queen mated to ca. 10 males; in the parasitic *capensis* lineage, however, no queen has ever been recorded.

[h] In other populations, a different reproductive system involving an X-chromosome-linked system of genetic caste determination has been observed [7].

Glossary

Thelytokous parthenogenesis: the process whereby diploid daughters are produced from unfertilised eggs.

Apomictic parthenogenesis: an ameiotic form of thelytokous parthenogenesis in which there is no recombination of alleles, so that offspring end up being clones of their mother.

Automictic parthenogenesis: a form of thelytokous parthenogenesis whereby the first stages of meiosis are similar to the ones in sexual reproduction, but fusion occurs between two nuclei originating from the same individual, so that gene recombination can occur; ploidy restoration can occur by several different mechanisms, including central and terminal fusion.

Asexual queen succession: a mode of reproduction now known from some ants and termites whereby one or more asexually produced queens replace the old one(s) if they happen to die.

Genetic caste determination: a system of queen and worker differentiation where an individual's genotype determines caste development, as opposed to environmental factors such as worker feeding.

Incest: mating between relatives.

Inbreeding depression: deleterious genetic consequences of incestuous mating.

Relatedness: the above-average probability of sharing the same allele at a particular locus.

Worker policing: the process whereby social insect workers prevent each other from successfully reproducing, for example by cannibalising each others' eggs.

queens. Significantly, this system does not appear to be an isolated oddity, but has now been shown to have evolved independently in at least five phylogenetically distant ant species [11–32] and two termites [1, 10]. Even more peculiar is that, in some species, AQS has been shown to be combined with other unusual traits, such as the ability of males to clone themselves [12, 17, 19, 30, 31], leading to a complete genetic separation of the male and female lineages [19, 31, 33], and the routine use of incest without leading to inbreeding depression [1, 21, 31]. Below, we give a brief review of these highly unusual systems that are unique in the animal kingdom. Before we do so, however, we first give a short overview of previously documented cases of thelytokous parthenogenesis in social insects – the study of which has resulted in the discovery of equally bizarre phenomena, such as lineages of asexually reproducing workers that evolved into dedicated socially parasitic 'cheaters' [34–45] or asexually reproducing honeybee workers that are capable of laying eggs directly into queen cells, thereby enabling them to reincarnate themselves as queens [46, 47].

Facultative worker thelytoky in social insects

In social insects, some of the earliest discovered examples of thelytokous parthenogenesis were cases where asexual reproduction was believed to play only a minor part in the species' life cycle; only being used very occasionally, for example as a way to produce a new queen in case the old one happened to die. In fact, this was true both for some termites (*Zootermopsis angusticollis*, *Z. nevadensis* and *Kalotermes flavicollis*, Table 1) and ants (*Messor capitatus*, [48] *Cataglyphis sabulosa* [49]). In the thelytokous termite species, parthenogenesis was thought to be used by queens merely as part of a strategy of 'making the best of a bad job' when they happened to fail to find a mate in the colony founding stage ('facultative queen thelytoky', Table 1, reviewed in [1]). Similarly, facultative thelytoky in ants, where workers reproduce parthenogenetically only under queenless conditions, was seen as a way for them to rear a new queen parthenogenetically and 're-queen' their colony after the accidental death of the old mother queen ('facultative worker thelytoky', Table 1).

Obligate worker thelytoky and asexual cheater lineages

Obligate asexuals in ants

That parthenogenesis could play a much more important role in the life cycle of social insects than initially suspected was shown by the discovery of several ant species with obligate asexual reproduction. One of the most striking examples is the fungus-gardening ant *Mycocepurus smithii*, where it was recently shown that in some populations, queens have abandoned sex altogether [50–52]. In these populations, all members of the colony are genetically identical to the queen [50] and males are completely absent [50, 51]. Furthermore, the females have a degenerated mussel organ, indicating that queens in some populations might even have lost the very capacity to mate [50]. A similar case of obligate queen thelytoky is also suspected in the tramp ant *Pyramica membranifera*, where males also appear to be absent, and queens produce new queens and workers asexually [53]. In these species, the twofold cost of sex presumably outweighs the short-term advantages of asexual reproduction.

Aside from these ants where queens reproduce asexually, earlier research also revealed several remarkable cases of obligate or near-obligate worker thelytoky. In the ants *Cerapachys biroi* [54–56] and *Pristomyrmex punctatus* [34, 41, 43, 45, 57–59] and in some populations of the Neotropical ant *Platythyrea punctata* [42, 60–67], for example, workers produce female offspring parthenogenetically (Table 1). In these species, there is a trend for the queen caste to be lost (Table 1), and reproductive division of labour is divided among the workers relatively equally, with all or most of the workers laying eggs when they are young and switching to foraging later in life (in *C. biroi* and *P. punctatus* [54, 55, 57, 58]; *P. punctata* has a more unequal reproductive division of labour, see below). Asexual reproduction in these cases possibly evolved as a way for the workers to overcome and revolt against the reproductive dominion of the queen [68]. In fact, some have argued that they might even represent early stages in the eventual loss of sociality [69, 70].

Asexual cheater lineages

Recent studies have found that worker thelytoky provides scope for the evolution of intraspecific worker parasitism, whereby workers end up reproductively parasitising other genetically unrelated colonies in the population, in which they then produce pseudoclones of themselves [40, 42, 45]. In *P. punctatus*, for example about half of all colonies have been shown to contain large-bodied workers that carry out no useful work inside the colony, but instead specialise in reproduction [34]. Recent genetic studies confirm that these large-bodied workers form a single monophyletic 'cheater' lineage [41, 43], which takes advantage of the other resident workers and persists by frequent horizontal transmission to other colonies. This is akin to some rare types of contagious cancers known among mammals [71], as well as to the cheater lineages that have been documented in clonal gall aphids [72] and certain social microorganisms, such as slime moulds and myxobacteria [71, 73]. Similarly, in *P. punctata*, natural colonies have also been frequently shown to be composed of several distinct genetic lineages [42], again showing that some lineages reproductively parasitise genetically unrelated colonies present in the larger population. Given that there is a great cost for colonies to be parasitised by genetically unrelated worker lineages, one would expect the evolution of specific adaptations to prevent such parasitism. Indeed, two alternative cheater-control strategies can be observed in *P. punctatus* and *P. punctata*. First, in *P. punctata*, conflict is limited by the fact that behavioural interactions normally cause only one, or very rarely two, workers to reproduce inside each colony [62, 64]. This mechanism results in the maintenance of very high, near-clonal levels of relatedness, even in the face of mutation; similar to the way in which many multicellular organisms maintain high relatedness via passage through a single-celled bottleneck or by sequestration of germ-line cells early in development [45, 71]. Second, in *P. punctatus*, where many workers reproduce inside the colony and so no strong genetic bottleneck exists, workers have been shown to reject unrelated intruders [59]. Such kin nepotism helps to actively exclude unrelated cheater lineages in a similar way to the allorecognition systems known from many clonal colonial marine invertebrates [71].

In a single subspecies of the honeybee, the Cape bee *Apis mellifera capensis*, workers have also convergently evolved the capacity to reproduce thelytokously (Table 1, [37, 74, 75], reviewed in [40]). Remarkably, here too evidence has been found for the repeated evolution of queenless lineages of cheater workers that specialise in infiltrating and reproductively parasitising colonies of both their own subspecies [76] as well as those of the congeneric African honeybee, *A. mellifera scutellata* [37, 39, 40, 44]. For beekeepers this causes great losses, since the *capensis* workers reproduce in infested colonies, but do no work, and so eventually kill any host colonies that they infest [77]. Traits that accompany this parasitic lifestyle include an increased propensity to drift to other colonies [35, 36], a stronger than usual ability to activate the ovaries in the presence of the queen [38, 39], and a reduced rate of genetic recombination during thelytokous meiosis [37], which preserves heterozygosity and prevents the production of inviable diploid males, which can arise due to homozygosity at the honeybee sex determining locus [44].

The benefit of being a social parasite

Why do both ants and bees with worker thelytoky display such a clear tendency to evolve into social parasites? The answer may well be linked to the fact that if all workers are reproductively totipotent, there would inevitably be an increase in competition between kin over reproduction. Competition among relatives can select for dispersal, which allows individuals to instead compete with non-kin [78, 79]. Dispersion of parasitic workers to other nests may therefore be an adaptation to kin competition. In fact, the same phenomenon is thought to explain the occurrence of workers that reproduce in other genetically unrelated colonies by laying unfertilised eggs in non-thelytokous species, such as Asian honeybees, bumblebees and halictid bees [40, 80–82]. In the Asian dwarf honeybee *Apis florea*, for example it was found that upon the accidental loss of the mother queen, colonies tend to be invaded by non-natal workers and that these workers show disproportionally high rates of ovary activation [81]. The original explanation for this phenomenon was that in honeybees, the queen normally has a monopoly over reproduction because any eggs that are laid by workers are quickly removed by other workers, but that such worker policing ceases when the colony happens to lose its queen [83]. Hence, it was postulated that the workers could evade policing and gain reproductive benefits by leaving their natal colony and parasitising other queenless colonies nearby [81]. A subsequent study, however, found that the phenomenon was better explained by workers seeking to avoid competition with relatives, since experiments demonstrated that the drifter workers present in queenless *A. florea* colonies primarily came from other queenless colonies [82], and not from colonies with a queen, as originally postulated [81].

The impact of asexual reproduction on the expression of kin conflicts

The study of regular, non-clonal Cape bee colonies has also proved fruitful for testing various hypotheses about the evolution of kin conflicts in social insect colonies [84, 85]. The ability of workers to produce female offspring parthenogenetically, for example, results in strong competition among the Cape bee workers over the maternity of daughter queens [40]. This leads to two predictions. First, it is expected that workers should attempt to lay their female eggs preferentially into queen cells, as this would provide them with much greater fitness benefits, allowing them to be reincarnated as more long-lived and more fecund queens rather than as workers [46]. This prediction has been borne out by a series of genetic studies, which showed that approximately half of all new queens reared either prior to swarming [46, 47] or after experimental removal of the mother queen [86, 87] were the offspring of laying workers rather than of the resident or recently replaced mother queen. Furthermore, a significant proportion of the new queens, up to 65% in one study [46], turned out to be produced by non-natal Cape bee workers that had invaded the colonies from elsewhere [46, 86, 87], and some were even shown to result from parasitic takeovers by foreign queens [87]. A second prediction regarding the evolution of kin conflict in Cape bee colonies is that there should be no fitness benefit to prefer the sexual eggs laid by the queen over the asexual eggs laid by other workers, as they are equally related to each. Therefore, there should be no selection pressure for workers or queens to evolve mechanisms to police thelytokously produced worker-laid eggs [88]. This is unlike the situation in other honeybees, in which workers are genetically more related to the queen's sons (brothers, $r = 0.25$) than to the sons of other workers (a mix of full- and half nephews, $r = 0.15$, assuming that queens mate with ca. 10 males) due to queen polyandry, and hence strongly benefit from removing eggs laid by other workers (reviewed in [83]). This prediction is again borne out by the fact that worker policing is only weakly expressed in *capensis* [89–91], resulting in many more Cape bee workers successfully reproducing in queenright colonies than in any other race of honeybees [46, 89, 92]. In addition, the limited amount of policing of worker-laid eggs that continues in Cape bees is thought to act as a mechanism that can help to exclude eggs laid by genetically unrelated parasitic cheater workers [93, 94].

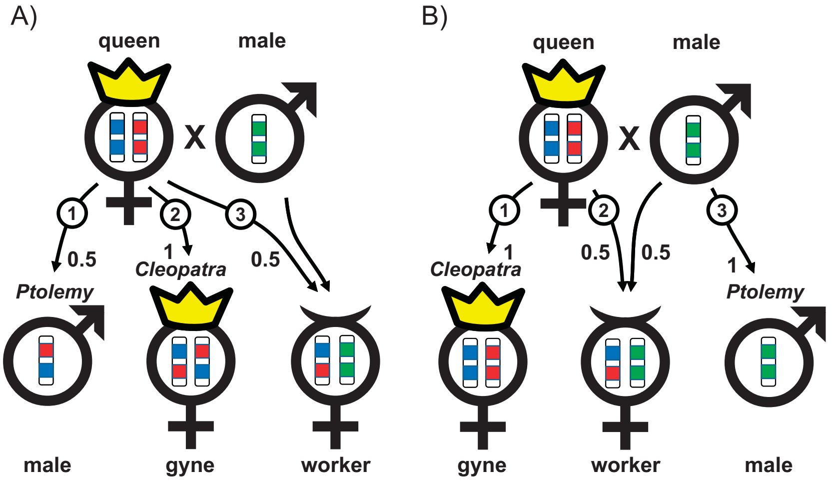

Figure 1. Newly discovered mixed modes of reproduction in ants. **A**: Clonal production of new queens and sexual production of workers, as observed in *Cataglyphis cursor* ants. Queens produce their sons and their worker daughters by the 'normal' method, i.e. males develop from unfertilised eggs and receive half the genes of their mother (1), leading to a genetic relatedness between the mother and her sons of $r = 0.5$, whereas workers are produced sexually, developing from fertilised eggs (3), leading to a relatedness to both parents of $r = 0.5$. New queens, however, are produced asexually (2, $r = 1$). In this case, the maternal cells divide in two, each of them bearing a set of chromosomes, which then recombine in a process known as automictic parthenogenesis with central fusion [16], to make a daughter cell. Note that, because of the chromosome recombinations that occur during parthenogenesis, the genomes of the mother and daughter are not identical. Further, note that colonies in this species are headed by a single queen mated with ca. four males [96], but that only one father is shown here. **B:** Cloning in both sexes combined with sexual production of new workers, as observed in the little fire ant *Wasmannia auropunctata* [12, 13, 17, 23, 32], the longhorn crazy ant *Paratrechina longicornis* [31] and the Japanese ant *Vollenhovia emeryi* [14, 19, 30] (note that colonies can have multiple queens but that only one is shown for illustrative purposes). Here, only workers are produced sexually (2, $r = 0.5$ to each parent). In contrast, new queens are produced by cloning (1) via a process known as automictic parthenogenesis with central fusion but with suppression of recombination [32]), which results in new queens being exact genetic copies of their mother ($r = 1$). Males also reproduce by cloning, and produce sons with the same genome as themselves (3, $r = 1$), so their degree of kinship is also equal to 1. Because of this mechanism of dual cloning, queens and males in this system are not genetically related [33]. In addition, there would be no decrease in heterozygosity if the original queen was replaced by a new queen that had incestuously mated with her brother, as can easily be checked by imagining that Cleopatra mated with Ptolemy. Hence, under system (B), but not that of (A), incest does not lead to inbreeding depression [31].

Mixed modes of reproduction and asexual queen succession in ants

Mixing sexual and asexual reproduction

Perhaps even more bizarre than the parasitic lifestyles documented in the ants and bees that reproduce by obligate worker thelytoky, several ants and termites have been recently shown to make use of a mixed mode of reproduction, whereby queens produce workers sexually but new queens asexually ('asexual queen succession') [1, 10]. The first species in which this peculiar mixed mode of reproduction was discovered was in the polyandrous ant *Cataglyphis cursor* [11] (Fig. 1A, Table 1). In this species, workers and males are both produced in the 'normal' way, developing from fertilised and unfertilised eggs laid by the queen. New queens, however, are produced asexually via queen thelytoky (Fig. 1A, [11]), after which colonies reproduce themselves via fissioning, i.e. by queens assisted by a group of workers [21]. The advantage of this system is thought to lie in the fact that by mixing asexual and sexual reproduction, queens increase the rate of transmission of their genes to their reproductive female offspring, while still maintaining genetic diversity in the worker force, where it is most needed [11, 21, 28]. In addition, it has been shown that *C. cursor* queens maximise the transmission rate of their genes by regulating the proportion of fertilised and parthenogenetic eggs laid over the course of the season, with queens laying many parthenogenetic eggs in spring when new queens are raised, but mostly sexual eggs the rest of the year when workers are raised [11, 15, 28]. Although the observed system evidently benefits the queens, the asexual production of new queens is also expected to greatly reduce the fitness of males, as they will be prevented from fathering reproductive female offspring [95]. In *C. cursor*, however, it appears that this cost is limited by the fact that males still contribute a small proportion of the reproductive queens, as approximately 4% of them arise from classical sexual reproduction [11], and the fact that workers can also produce new queens thelytokously following the accidental loss of the mother queen [21, 29]. For the males, the production of such worker-derived queens

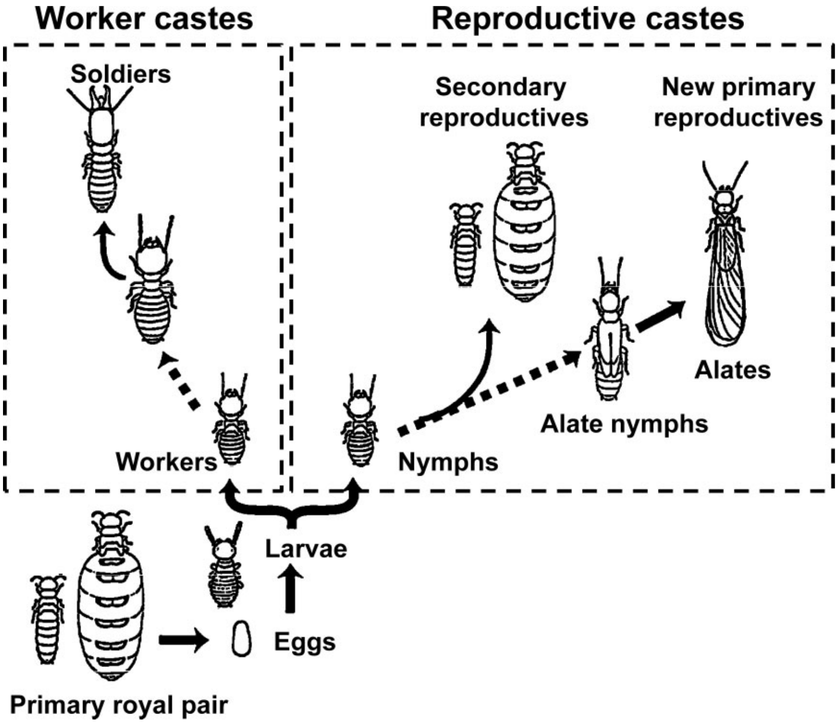

Figure 2. Caste differentiation in termites of the genus *Reticulitermes*. Colonies are normally founded by a winged pair of reproductives (a king and queen, bottom left) and their diploid offspring, which comprise various immature instars, act as workers. After the accidental death of either the primary queen or king, however, one or more offspring are stimulated to develop into wingless secondary reproductives. In *R. speratus*, only individuals that develop along the nymph track (right box) have this capacity. In addition, since nymphs can grow wingpads, they also have the potential to ultimately transform into new winged primary reproductives (alates), which are the dispersing forms. Individuals that develop along the worker track (left box), in contrast, will typically act as workers in the colony, or occasionally develop into soldiers. (Modified from [1]).

provides a way to indirectly transmit copies of their own genes, since the workers are produced sexually from fertilised eggs. In fact, one study estimated that as much as 60% of all colonies might be headed by such worker-produced queens [16]. Intriguingly, as in the examples cited above, a recent study also found evidence for the occasional presence of parasitic cheater workers in *C. cursor*, with about half of all colonies containing foreign, genetically unrelated workers that in queenless colonies were shown to sometimes produce a disproportionally high number of replacement queens [29].

Effects on the expression of intra-colony kin conflicts

The unusual mode of reproduction of *C. cursor*, combined with the fact that queens are known to mate with an average of approximately four males [96], leads to some specific predictions about the expression of kin conflicts in this species. In particular, multiple mating by queens results in workers of *C. cursor* being more closely related to parthenogenetically produced sister queens (by 0.5) than to either sexually produced sister queens or new queens that are produced parthenogenetically by the workers (both by 0.4, which is equal to the average relatedness amongst workers) [11, 15, 28]. This leads to two predictions. The first is that, as in the Western honeybee, workers should benefit genetically from policing the reproduction of other workers [11]. Consistent with this idea, workers in *C. cursor* do not reproduce in the presence of the queen [11], perhaps because such worker reproduction is made unprofitable by the presence of worker policing [97], although the latter remains to be demonstrated. Second, it is expected that workers should benefit from preferentially rearing the clones of their mother as new queens rather than a mix of sexually produced sisters and half-sisters. In support of this prediction, it has been found that even though about half of all queen-laid eggs were fertilised in early spring, all new queens that were genotyped were found to be produced asexually [28]. This suggests that workers favour rearing parthenogenetic sisters into reproductive queens. Alternatively, it might also be possible that thelytokous eggs have a genetic predisposition to develop into new queens, i.e. that caste is partly genetically determined [6, 7]. Further study is needed to resolve which of these two mechanisms is actually at play.

Mixed modes of reproduction in ants: Males strike back

A sexual arms race

The *C. cursor* case might easily be dismissed as an isolated oddity. However, similar mixed modes of reproduction have been demonstrated to occur in three other phylogenetically distant ants, namely the invasive little fire ant *Wasmannia auropunctata* [12, 13, 17, 23, 32], the longhorn crazy ant *Paratrechina longicornis* [31], and the Japanese queen polymorphic ant *Vollenhovia emeryi* [14, 19, 30], all of which form nests headed by multiple queens (Table 1). In addition, a mixed mode of reproduction is suspected to occur in another invasive species, the yellow crazy ant *Anoplolepis gracilipes*; although the exact details of that system still await further study [98, 25]. The most remarkable aspect of these cases is that, unlike *C. cursor*, these ants not only produce queens asexually and workers sexually, but males also reproduce clonally by a process that either involves the elimination of the maternal genome in fertilised eggs or the fertilisation of anucleate ovules [12, 17, 19, 30, 31] (Fig. 1B). This highly unusual genetic system has been suggested to be a derivative of a mixed mode of reproduction, whereby workers were produced sexually and queens asexually, but where males use the queens as surrogate mothers to clone themselves, thereby optimising fitness in a totally different way than in *C. cursor* [12, 33] (unlike *C. cursor*, *Wasmannia, Vollenhovia* and probably also *Paratrechina* workers are obligately sterile, [14]). In other words, this genetic system might represent an expression of a sexual arms race [99], whereby both females and males each try to obtain maximal fitness by the successive evolution of various adaptations and counter-adaptations [12, 33] (but see [17] for some alternative hypotheses). Interestingly, the fact that in these systems males and females each reproduce asexually leads to a separation of male and female gene pools, and this prompted one commentator to liken the males in these species to a separate species to the females, 'perhaps from Mars' [33]. Two recent genetic studies confirmed this prediction by showing that in *Vollenhovia* and *Paratrechina*, the male and female lineages were indeed completely genetically separated [19, 31]. In *Wasmannia*, however, separation is not complete, due to the fact that sexual reproduction may also rarely be used [13, 23], which results in the continuous production of novel clonal lineages [13]. In fact, particularly in its native range in Central and South America, some *Wasmannia* populations have been shown to use sexual reproduction, even though virtually all introduced populations worldwide have been shown to be clonal and derived from dominant clonally reproducing populations within the native range [20, 24]. Furthermore, introduced populations were found to be derived from clonal couples, which associated male and female genotypes that produced workers with unusually high heterozygosity [24].

Incest without deleterious genetic consequences

A recent study on the longhorn crazy ant *P. longicornis* showed that a system whereby both males and females each reproduce asexually can have unexpected benefits [31]. In laboratory experiments, they showed that in this species, queens readily mate with their brothers. Surprisingly, however, the genetic system of double cloning (Fig. 1B) allows such incestuous matings to occur without any consequent increase in the level of homozygosity. In fact, under the observed genetic system, the heterozygosity of new queens is completely independent of the genealogical link between the mother queen and her mate, as there is no mixing of the paternal and maternal lineages. In contrast, worker heterozygosity depends on the genetic similarity between the maternal and paternal lineages, because workers are produced sexually. In the population that was studied, however, all queens and males had the same genotype, which meant that queens were no more genetically similar to their brothers than to any other male in the population, and that the level of worker heterozygosity was therefore not affected by queens mating with their brothers or with any other male in the population [31]. The fact that the observed reproductive system allows incestuous mating without leading to an increased level of homozygosity may well apply to the other known cases of AQS combined with male cloning, and may be an important pre-adaptation that allows these species to quickly colonise new habitats [24, 31]. Indeed, both the little fire ant and the longhorn crazy ant are highly invasive pest species.

Genetic caste determination

Another unusual consequence of the genetic system of double cloning is that in *W. auropunctata*, and perhaps also in *V. emeryi*, caste determination has become to some extent genetically hardwired [6, 7]. This is highly unusual for social insects, where caste is normally determined environmentally by the amount or quality of food that is given to developing larvae [84, 85] (but see [6, 7]). Genetic caste determination (GCD) has been inferred from the finding that there was a perfect phenotype-genotype match in *W. auropunctata*, with all workers being sexually produced and all queens being clonally produced, implying that caste may be determined by levels of heterozygosity [12, 23]. In addition, unlike in *C. cursor* [28], queens and workers have been shown to be reared simultaneously so that the genotype-caste association could not be explained by a seasonal production of parthenogenetic and sexual eggs [28]. In *V. emeryi*, less information is available, but the genotype-phenotype association appears somewhat weaker, since up to 5% of the workers are produced parthenogenetically rather than sexually [14]. Genetically hardwired caste determination ('strong' GCD) has long been considered puzzling and evolutionarily unstable, since worker-specific alleles would be expected to be lost from the population [7]. In addition, GCD would constrain the allocation to queens versus workers, and so could potentially upset the optimal colony allocation to the production of both castes [6, 7]. In the cases discussed, however, this problem might be averted in two ways. First, by the introduction of worker-specific alleles via hybridisation between differentiated genomes [7]. Second, by the fact that in other recent cases where caste has been shown to be genetically determined (reviewed in [6, 7, 100]), workers have still been shown to have final control over what proportion of the queen-destined eggs they actually rear into adults [6], and that this is also likely to be true for *W. auropunctata* and *V. emeryi*i. For example, in the best documented case of strong GCD discovered so far, in a harvester ant hybrid zone, queens mate both with males of their own species as well as with those of a sibling species, and inter-lineage crosses have been shown to develop into workers, whereas intra-lineage crosses become queens (reviewed in [6, 100]). Nevertheless, queen-destined eggs that are laid at a time when queens are not required, such as in the colony founding stage, are usually not reared into adulthood [6]. This means that even though caste is genetically determined, the workers still have final control over the ratio in which new queens and workers are reared. In fact, it has been argued that the inference of caste being genetically determined may simply be an artefact of the fact that all cases of strong GCD discovered so far are associated with unusual breeding systems, and that these invariably have the effect of preventing gene flow between the male and female gene pools. This results in all female genes, including the one(s) responsible for GCD, being linked to the genetic markers used in any one study, thereby resulting in a 'strong' signal of GCD [23]. According to this view, the signal of GCD that is being detected may merely represent a signature of an unusual breeding system, rather than provide evidence for a fundamentally different mechanism of caste determination [6, 23]. Hence, the exact extent to which the allocation to new queens and workers is determined by individual genotype, as opposed to by worker feeding or even maternal control, remains to be studied. In fact, suggestive evidence for maternally controlled caste ratios was recently provided for *V. emeryi* by the fact that colonies invested more heavily in the production of queen-destined eggs than either male- or worker-destined ones, and that this would be predicted based on queen-controlled, but not worker-controlled, caste allocation ratios [26]. This is because in the *Vollenhovia* system, in which both sexes are produced clonally, the queen is clonally related to new queens but unrelated to new males [26], and so benefits from investing mainly in the production of new queens (Fig. 1B). The sexually produced workers, in contrast, are more symmetrically related to sister queens and brothers, and should benefit from producing relatively more males [14]. An alternative explanation, however, is that since all new queens mate and remain in the nest, and that only one queen lineage (or very few at most) is expected to be found in each colony, daughter queens would likely compete with clones, and that this should predispose the queens to merely produce a ratio of new males and queens that ensures adequate fertilisation of all daughter queens.

Comparison to mixed modes of reproduction in multicellular organisms

A striking aspect of the mixed modes of reproduction in ants that combine sexual reproduction for worker production or ‘somatic’ growth and asexual reproduction for the production of new queens (or males), i.e. for ‘germ line’ production, is that the observed pattern is exactly the opposite to the situation in other multicellular organisms, where the soma is usually clonally derived from a single cell, and germ cells are produced sexually [71]. Why the difference? One possible answer [45] may lie in the fact that in primitive multicellular organisms, such as slime moulds, a genetically polymorphic soma has been shown to decrease individual performance, either due to the increased expression of conflict or the mere fact that genetically different cells do not manage to work together as well [71, 73]. The reverse, however, appears to be the case for most social insects, where a genetically diverse work force generally appears to be beneficial (reviewed in [8, 9]). Second, an important factor is that the sexually produced gametes in multicellular organisms usually contribute to the production of offspring that disperse to unpredictable environments, in which case sexual reproduction would indeed be expected to be beneficial [2–4, 5]. In contrast, in all the examples discussed above, the asexually produced queens remain in or close to their natal colony, buffered from the environment by a group of workers. This means that the locally adapted queen genotype is likely to do well, and in which case asexual reproduction indeed has been shown to be the best strategy [2–4, 5]. This explanation is supported by the fact that in *C. cursor*, which has non-flying, non-dispersing queens, queens are produced asexually, whereas in a related species, *C. sabulosa*, queens disperse on the wing and are mainly produced sexually, despite the fact that they too have the capacity to reproduce thelytokously [49]. Another theoretical possibility [101] is that when both widely and locally dispersing propagules are formed, a mixed strategy can evolve, whereby locally dispersing propagules, which will tend to encounter predictable environments, are produced asexually, but widely dispersing propagules that will land in more variable environments, are produced sexually. Recently, exactly such a mixed mode of reproduction – akin to strawberries and many other herbaceous plants that produce runners locally but sexually produced dispersing seeds [5, 2, 3] – has also been discovered in social insects, this time in termites [1, 10].

Mixed modes of reproduction and asexual queen succession in termites

The two species in which yet another amazing mixed mode of reproduction was discovered are the two lower termites, *Reticulitermes speratus* [10] and *Reticulitermis virginicus* [1] (Table 1). In termites, colony organisation is quite distinct from the social Hymenoptera discussed above [1] (Fig. 2), and colonies are normally founded by a winged pair of reproductives, i.e. a queen and king. After the accidental death of the primary queen, however, wingless secondary reproductives can replace the original queen. In most termites, such replacement reproductives are produced sexually. In *R. speratus* and *R. virginicus*, however, genetic studies have shown that the

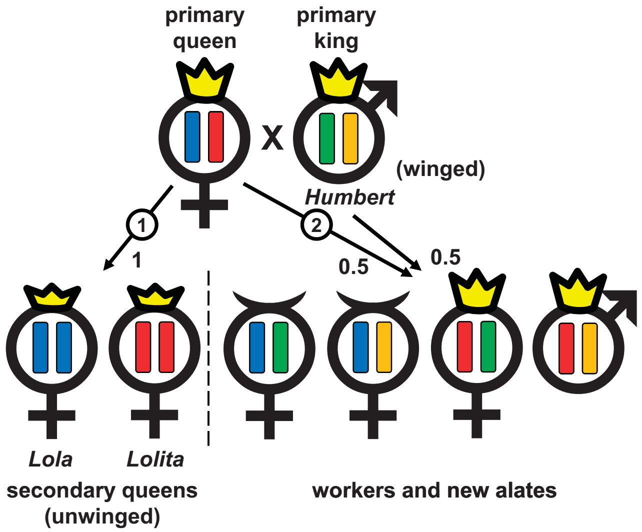

Figure 3. The mixed mode of reproduction as observed in the termites *R. speratus* and *R. virginicus* [1, 10], in which new queens are produced clonally (1, $r = 1$ to their mother), via automictic parthenogenesis and terminal fusion [1], but workers and new alates are produced sexually (2, $r = 0.5$ to their mother and father). Note that even with incestuous mating, common in termites, this system does not result in a significant decrease in heterozygosity. Imagine, for example that Lola and Lolita each mate with their father Humbert after the primary queen dies, and that both replace her (in these species, many secondary queens can co-occur within the same colony, [1]). In that case, the new worker and alate offspring that Lola and Lolita produce would have roughly the same heterozygosity as those produced by the primary queen and king [1]. This is because the secondary queens are produced asexually, and so do not share any genes with their father.

secondary replacement reproductives are produced asexually, whereas new workers and dispersing alates are both produced in the normal sexual way [1, 10] (Fig. 3). Such a system is analogous to the mixed vegetative and sexual reproduction observed in many plants [5, 2, 3], but with the difference that they also buffer themselves against the environment via a genetically diverse, sexually produced workforce [1]. Furthermore, as in the case of the longhorn crazy ant [31], the observed system enables the replacement queens to mate incestuously, without experiencing a drastic decrease in heterozygosity [1, 10]. This can be seen from the fact that if newly produced replacement queens mate incestuously with their father (with whom they do not share any genes, they themselves having been asexually produced by their mother), their worker or alate offspring would roughly have the same heterozygosity as that in the previous generation (Fig. 3). The exact genetic system that underlies this peculiar system remains as yet unknown, but selfish meiotic drive elements and GCD have both been implicated [1].

Conclusion

From the obligate asexual *Mycocepurus* leafcutter ants to the clonal lineages of parasitic Cape bee workers or the mixed modes of reproduction of the little fire ant and *Reticulitermes* termites, it is clear that the mode of reproduction and genetic system used by social insects is infinitely more complex than the simple, stereotyped mechanisms of haplodiploidy or diplodiploidy to which they were earlier thought to conform [84]. The further study of these newly discovered systems now offers to provide insights into a vast array of questions, ranging from the maintenance of sex [2–4] and the advantage of mixed modes of reproduction [2, 3, 101], the expression of sexual conflict [33, 99] and kin conflict [84, 85, 102] to the evolution of cheating and cheater-control mechanisms in asexual lineages [45, 71, 73]. In addition, the convergent evolution of systems with remarkably similar features, in groups as diverse as ants and termites, clearly shows that these systems are not mere curiosities, but likely illustrate important, basic evolutionary forces.

Acknowledgments

We would like to thank Morgan Pearcy and Jochen Drescher for useful comments and discussion, and the Research Foundation Flanders for funding.

References

1. **Matsuura K.** 2011. Sexual and asexual reproduction in termites. In Bignell DE, Roisin Y, Lo N, eds; *Biology of Termites: A Modern Synthesis*. New York: Springer. p 255–77.
2. **Maynard Smith J.** 1978. *The Evolution of Sex*. Cambridge: Cambridge University Press.
3. **Bell G.** 1982. *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. Berkeley: University of California Press. p. 635.
4. **West SA**, **Lively CM**, **Read AF.** 1999. A pluralist approach to sex and recombination. *J Evol Biol* **12**: 1003–12.
5. **Williams GC.** 1975. *Sex and Evolution*. Princeton, New Jersey: Princeton University Press.
6. **Anderson KE**, **Linksvayer TA**, **Smith CR.** 2008. The causes and consequences of genetic caste determination in ants (Hymenoptera: Formicidae). *Myrmecol News* **11**: 119–32.
7. **Schwander T**, **Lo N**, **Beekman M**, **Oldroyd BP**, et al. 2010. Nature versus nurture in social insect caste differentiation. *Trends Ecol Evol* **25**: 275–82.
8. **Oldroyd BP**, **Fewell JH.** 2007. Genetic diversity promotes homeostasis in insect colonies. *Trends Ecol Evol* **22**: 408–13.
9. **Hughes WOH**, **Ratnieks FLW**, **Oldroyd BP.** 2008. Multiple paternity or multiple queens: two routes to greater intracolonial genetic diversity in the eusocial Hymenoptera. *J Evol Biol* **21**: 1090–5.
10. **Matsuura K**, **Vargo EL**, **Kawatsu K**, **Labadie PE**, et al. 2009. Queen succession through asexual reproduction in termites. *Science* **323**: 1687.
11. **Pearcy M**, **Aron S**, **Doums C**, **Keller L.** 2004. Conditional use of sex and parthenogenesis for worker and queen production in ants. *Science* **306**: 1780–3.
12. **Fournier D**, **Estoup A**, **Orivel J**, **Foucaud J**, et al. 2005. Clonal reproduction by males and females in the little fire ant. *Nature* **435**: 1230–4.
13. **Foucaud J**, **Jourdan H**, **Le Breton J**, **Loiseau A**, et al. 2006. Rare sexual reproduction events in the clonal reproduction system of introduced populations of the little fire ant. *Evolution* **60**: 1646–57.
14. **Ohkawara K**, **Nakayama M**, **Satoh A**, **Trindl A**, et al. 2006. Clonal reproduction and genetic caste differences in a queen-polymorphic ant, *Vollenhovia emeryi*. *Biol Lett* **2**: 359–63.
15. **Pearcy M**, **Aron S.** 2006. Local resource competition and sex ratio in the ant *Cataglyphis cursor*. *Behav Ecol* **17**: 569–74.
16. **Pearcy M**, **Hardy O**, **Aron S.** 2006. Thelytokous parthenogenesis and its consequences on inbreeding in an ant. *Heredity* **96**: 377–82.
17. **Foucaud J**, **Fournier D**, **Orivel J**, **Delabie JHC**, et al. 2007. Sex and clonality in the little fire ant. *Mol Biol Evol* **24**: 2465–73.
18. **Clemencet J**, **Rome Q**, **Federici P**, **Doums C.** 2008. Aggressions and size-related fecundity of queenless workers in the ant *Cataglyphis cursor*. *Naturwissenschaften* **95**: 133–9.
19. **Kobayashi K**, **Hasegawa E**, **Ohkawara K.** 2008. Clonal reproduction by males of the ant *Vollenhovia emeryi* (Wheeler). *Entomol Sci* **11**: 167–72.
20. **Foucaud J**, **Orivel J**, **Fournier D**, **Delabie JHC**, et al. 2009. Reproductive system, social organization, human disturbance and ecological dominance in native populations of the little fire ant, *Wasmannia auropunctata*. *Mol Ecol* **18**: 5059–73.
21. **Lenoir A**, **Aron S**, **Cerdá X**, **Hefetz A.** 2009. *Cataglyphis* desert ants: a good model for evolutionary biology in Darwin's anniversary year – A review. *Isr J Entomol* **39**: 1–32.
22. **Vonshak M**, **Dayan T**, **Foucaud J**, **Estoup A**, et al. 2009. The interplay between genetic and environmental effects on colony insularity in the clonal invasive little fire ant *Wasmannia auropunctata*. *Behav Ecol Sociobiol* **63**: 1667–77.
23. **Foucaud J**, **Estoup A**, **Loiseau A**, **Rey O**, et al. 2010. Thelytokous parthenogenesis, male clonality and genetic caste determination in the little fire ant: new evidence and insights from the lab. *Heredity* **105**: 205–12.
24. **Foucaud J**, **Orivel J**, **Loiseau A**, **Delabie JHC**, et al. 2010. Worldwide invasion by the little fire ant: routes of introduction and eco-evolutionary pathways. *Evol Appl* **3**: 363–74.
25. **Grüber M**, **Hoffmann B**, **Ritchie P**, **Lester P.** 2010. Crazy ant sex: genetic caste determination, clonality and inbreeding in a population of invasive yellow crazy ants. *Proc 16th Congr IUSSI*. p 93.
26. **Okamoto M**, **Ohkawara K.** 2010. Egg production and caste allocation in the clonally reproductive ant *Vollenhovia emeryi*. *Behav Ecol* **21**: 1005–10.
27. **Okamoto M**, **Ohkawara K.** 2010. Genetic structures in colonies at a new site of *Vollenhovia emeryi* (Hymenoptera: Formicidae) with clonal reproduction. *Sociobiology* **55**: 191–6.
28. **Aron S**, **Timmermans I**, **Pearcy M.** 2011. Ant queens adjust egg fertilization to benefit from both sexual and asexual reproduction. *Biol Lett* **7**: 571–3.
29. **Cheron B**, **Monnin T**, **Federici P**, **Doums C.** 2011. Variation in patriline reproductive success during queen production in orphaned colonies of the thelytokous ant *Cataglyphis cursor*. *Mol Ecol* **20**: 2011–22.
30. **Kobayashi K**, **Hasegawa E**, **Ohkawara K.** 2011. No gene flow between wing forms and clonal reproduction by males in the long-winged form of the ant *Vollenhovia emeryi*. *Insectes Soc* **58**: 163–8.
31. **Pearcy M**, **Goodisman MAD**, **Keller L.** 2011. Sib mating without inbreeding in the longhorn crazy ant. *Proc R Soc Lond B Biol Sci* **278**: 2677–81.

32. **Rey O**, **Loiseau A**, **Facon B**, **Foucaud J**, et al. 2011. Meiotic recombination dramatically decreased in thelytokous queens of the little fire ant and their sexually produced workers. *Mol Biol Evol* **28**: 2591–601.
33. **Queller D.** 2005. Males from Mars. *Nature* **435**: 1167–8.
34. **Tsuji K.** 1995. Reproductive conflicts and levels of selection in the ant *Pristomyrmex pungens*: contextual analysis and partitioning of covariance. *Am Nat* **146**: 586–607.
35. **Neumann P**, **Radloff SE**, **Moritz RFA**, **Hepburn HR**, et al. 2001. Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): host finding and resistance of hybrid host colonies. *Behav Ecol* **12**: 419–28.
36. **Neumann P**, **Radloff SE**, **Pirk CWW**, **Hepburn R.** 2003. The behaviour of drifted Cape honeybee workers (*Apis mellifera capensis*): predisposition for social parasitism? *Apidologie* **34**: 585–90.
37. **Baudry E**, **Kryger P**, **Allsopp M**, **Koeniger N**, et al. 2004. Whole-genome scan in thelytokous-laying workers of the cape honeybee (*Apis mellifera capensis*): central fusion, reduced recombination rates and centromere mapping using half-tetrad analysis. *Genetics* **167**: 243–52.
38. **Dietemann V**, **Pflugfelder J**, **Härtel S**, **Neumann P**, et al. 2006. Social parasitism by honeybee workers (*Apis mellifera capensis* Esch.): evidence for pheromonal resistance to host queen's signals. *Behav Ecol Sociobiol* **60**: 785–93.
39. **Dietemann V**, **Neumann P**, **Hartel S**, **Pirk CWW**, et al. 2007. Pheromonal dominance and the selection of a socially parasitic honeybee worker lineage (*Apis mellifera capensis* Esch.). *J Evol Biol* **20**: 997–1007.
40. **Beekman M**, **Oldroyd BP.** 2008. When workers disunite: intraspecific parasitism by eusocial bees. *Annu Rev Ent* **53**: 19–37.
41. **Dobata S**, **Sasaki T**, **Mori H**, **Hasegawa E**, et al. 2009. Cheater genotypes in the parthenogenetic ant *Pristomyrmex punctatus*. *Proc R Soc Lond B Biol Sci* **276**: 567–74.
42. **Kellner K**, **Barth B**, **Heinze J.** 2010. Colony fusion causes within-colony variation in a parthenogenetic ant. *Behav Ecol Sociobiol* **64**: 737–46.
43. **Dobata S**, **Sasaki T**, **Mori H**, **Hasegawa E**, et al. 2011. Persistence of the single lineage of transmissible 'social cancer' in an asexual ant. *Mol Ecol* **20**: 441–55.
44. **Oldroyd BP**, **Allsopp MH**, **Lim J**, **Beekman M.** 2011. A thelytokous lineage of socially parasitic honey bees has retained heterozygosity despite at least 10 years of inbreeding. *Evolution* **65**: 860–8.
45. **Tsuji K**, **Dobata S.** 2011. Social cancer and the biology of the clonal ant *Pristomyrmex punctatus* (Hymenoptera: Formicidae). *Myrmecol News* **15**: 91–9.
46. **Jordan LA**, **Allsopp MH**, **Oldroyd BP**, **Wossler TC**, et al. 2008. Cheating honeybee workers produce royal offspring. *Proc R Soc Lond B Biol Sci* **275**: 345–51.
47. **Allsopp MH**, **Beekman M**, **Gloag RS**, **Oldroyd BP.** 2010. Maternity of replacement queens in the thelytokous Cape honey bee *Apis mellifera capensis*. *Behav Ecol Sociobiol* **64**: 567–74.
48. **Grasso DA**, **Wenseleers T**, **Mori A**, **Le Moli F**, et al. 2000. Thelytokous worker reproduction and lack of *Wolbachia* infection in the harvesting ant *Messor capitatus*. *Ethol Ecol Evol* **12**: 309–14.
49. **Timmermans I**, **Hefetz A**, **Fournier D**, **Aron S.** 2008. Population genetic structure, worker reproduction and thelytokous parthenogenesis in the desert ant *Cataglyphis sabulosa*. *Heredity* **101**: 490–8.
50. **Himler AG**, **Caldera EJ**, **Baer BC**, **Fernandez-Marin H**, et al. 2009. No sex in fungus-farming ants or their crops. *Proc R Soc Lond B Biol Sci* **276**: 2611–6.
51. **Rabeling C**, **Lino-Neto J**, **Cappellari SC**, **Dos-Santos IA**, et al. 2009. Thelytokous parthenogenesis in the fungus-gardening ant *Mycocepurus smithii* (Hymenoptera: Formicidae). *Plos One* **4**: e6781.
52. **Rabeling C**, **Gonzales O**, **Schultz TR**, **Bacci M**, et al. 2011. Cryptic sexual populations account for genetic diversity and ecological success in a widely distributed, asexual fungus-growing ant. *Proc Natl Acad Sci USA* **108**: 12366–71.
53. **Ito F**, **Touyama Y**, **Gotoh A**, **Kitahiro S**, et al. 2010. Thelytokous parthenogenesis by queens in the dacetine ant *Pyramica membranifera* (Hymenoptera: Formicidae). *Naturwissenschaften* **97**: 725–8.
54. **Tsuji K**, **Yamauchi K.** 1995. Production of females by parthenogenesis in the ant, *Cerapachys biroi*. *Insectes Soc* **41**: 333–6.
55. **Ravary F**, **Jaisson P.** 2004. Absence of individual sterility in thelytokous colonies of the ant *Cerapachys biroi* Forel (Formicidae, Cerapachyinae). *Insectes Soc* **51**: 67–73.
56. **Lecoutey E**, **Chaline N**, **Jaisson P.** 2011. Clonal ant societies exhibit fertility-dependent shifts in caste ratios. *Behav Ecol* **22**: 108–13.
57. **Tsuji K.** 1988. Obligate parthenogenesis and reproductive division of labor in the Japanese queenless ant *Pristomyrmex pungens* - Comparison of intranidal and extranidal workers. *Behav Ecol Sociobiol* **23**: 247–55.
58. **Tsuji K.** 1990. Reproductive division of labor related to age in the Japanese queenless ant, *Pristomyrmex pungens*. *Anim Behav* **39**: 843–9.
59. **Nishide Y**, **Satoh T**, **Hiraoka T**, **Obara Y**, et al. 2007. Clonal structure affects the assembling behavior in the Japanese queenless ant *Pristomyrmex punctatus*. *Naturwissenschaften* **94**: 865–9.
60. **Heinze J**, **Hölldobler B.** 1995. Thelytokous parthenogenesis and dominance hierarchies in the ponerine ant, *Platythyrea punctata*. *Naturwissenschaften* **82**: 40–51.
61. **Schilder K**, **Heinze J**, **Gross R**, **Hölldobler B.** 1999. Microsatellites reveal clonal structure of populations of the thelytokous ant *Platythyrea punctata* (F. Smith) (Hymenoptera; Formicidae). *Mol Ecol* **8**: 1497–507.
62. **Schilder K**, **Heinze J**, **Hölldobler B.** 1999. Colony structure and reproduction in the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera, Formicidae). *Insectes Soc* **46**: 150–8.
63. **Hartmann A**, **Heinze J.** 2003. Lay eggs, live longer: division of labor and life span in a clonal ant species. *Evolution* **57**: 2424–9.
64. **Hartmann A**, **Wantia J**, **Torres JA**, **Heinze J.** 2003. Worker policing without genetic conflicts in a clonal ant. *Proc Natl Acad Sci USA* **100**: 12836–40.
65. **Hartmann A**, **Wantia J**, **Heinze J.** 2005. Facultative sexual reproduction in the parthenogenetic ant *Platythyrea punctata*. *Insectes Soc* **52**: 155–62.
66. **Kellner K**, **Heinze J.** 2011. Mechanism of facultative parthenogenesis in the ant *Platythyrea punctata*. *Evol Ecol* **25**: 77–89.
67. **Kellner K**, **Heinze J.** 2011. Absence of nepotism in genetically heterogeneous colonies of a clonal ant. *Ethology* **117**: 556–64.
68. **Hamilton WD.** 1964. The genetical evolution of social behaviour. I & II. *J Theor Biol* **7**: 1–52.
69. **Crespi BJ**, **Yanega D.** 1995. The definition of eusociality. *Behav Ecol* **6**: 109–15.
70. **Gadagkar R.** 1997. Social evolution – has nature ever rewound the tape? *Curr Sci* **72**: 950–6.
71. **Grosberg RK**, **Strathmann RR.** 2007. The evolution of multicellularity: a minor major transition? *Annu Rev Ecol Evol Syst* **38**: 621–54.
72. **Abbot P**, **Withgott JH**, **Moran NA.** 2001. Genetic conflict and conditional altruism in social aphid colonies. *Proc Natl Acad Sci USA* **98**: 12068–71.
73. **Foster KR.** 2010. Social behaviour in microorganisms. In Szekely T, Moore AJ, Komdeur J, eds; *Social Behaviour: Genes, Ecology and Evolution*. Cambridge: Cambridge University Press. p 331–56.
74. **Verma S Rüttner F.** 1983. Cytological analysis of the thelytokous parthenogenesis in the Cape honeybee (*Apis mellifera capensis* Escholtz) *Apidologie* **14**: 41–57.
75. **Oldroyd BP**, **Allsopp MH**, **Gloag RS**, **Lim J**, et al. 2008. Thelytokous parthenogenesis in unmated queen honeybees (*Apis mellifera capensis*): central fusion and high recombination rates. *Genetics* **180**: 359–66.
76. **Härtel S**, **Neumann P**, **Raassen FS**, **Moritz RFA**, et al. 2006. Social parasitism by Cape honeybee workers in colonies of their own subspecies (*Apis mellifera capensis* Esch.). *Insectes Soc* **53**: 183–93.
77. **Oldroyd BP.** 2002. The Cape honeybee: an example of a social cancer. *Trends Ecol Evol* **17**: 249–51.
78. **Hamilton WD**, **May RM.** 1978. Dispersal in stable habitats. *Nature* **269**: 578–81.
79. **Taylor PD.** 1988. An inclusive fitness model for dispersal of offspring. *J Theor Biol* **130**: 363–78.
80. **Lopez-Vaamonde C**, **Koning JW**, **Brown RM**, **Jordan WC**, et al. 2004. Social parasitism by male-producing reproductive workers in a eusocial insect. *Nature* **430**: 557–60.
81. **Nanork P**, **Paar J**, **Chapman NC**, **Wongsiri S**, et al. 2005. Asian honeybees parasitize the future dead. *Nature* **437**: 829.
82. **Chapman NC**, **Nanork P**, **Gloag RS**, **Wattanachaiyingcharoen W**, et al. 2009. Queenless colonies of the Asian red dwarf honey bee (*Apis florea*) are infiltrated by workers from other queenless colonies. *Behav Ecol* **20**: 817–820.
83. **Wenseleers T**, **Ratnieks FLW.** 2006. Comparative analysis of worker reproduction and policing in eusocial Hymenoptera supports relatedness theory. *Am Nat* **168**: E163–79.
84. **Crozier RH**, **Pamilo P.** 1996. *Evolution of Social Insect Colonies. Sex Allocation and Kin Selection*. Oxford: Oxford University Press.
85. **Ratnieks FLW**, **Foster KR**, **Wenseleers T.** 2006. Conflict resolution in insect societies. *Annu Rev Ent* **51**: 581–608.

86. **Holmes MJ**, **Oldroyd BP**, **Allsopp MH**, **Lim J**, et al. 2010. Maternity of emergency queens in the Cape honey bee, *Apis mellifera capensis*. *Mol Ecol* **19**: 2792–9.
87. **Moritz RFA**, **Lattorff HMG**, **Crous KL**, **Hepburn RH.** 2011. Social parasitism of queens and workers in the Cape honeybee (*Apis mellifera capensis*). *Behav Ecol Sociobiol* **65**: 735–40.
88. **Greeff JM.** 1996. Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee, *Apis mellifera capensis*. *Philos Trans R Soc Lond B Biol Sci* **351**: 617–25.
89. **Moritz RFA**, **Kryger P**, **Allsopp MH.** 1999. Lack of worker policing in the cape honeybee (*Apis mellifera capensis*). *Behaviour* **136**: 1079.
90. **Beekman M**, **Good G**, **Allsopp MH**, **Radloff S**, et al. 2002. A non-policing honey bee colony (*Apis mellifera capensis*). *Naturwissenschaften* **89**: 479–82.
91. **Pirk CWW**, **Neumann P**, **Ratnieks FLW.** 2003. Cape honeybees, *Apis mellifera capensis*, police worker-laid eggs despite the absence of relatedness benefits. *Behav Ecol* **14**: 347–52.
92. **Beekman M**, **Allsopp MH**, **Jordan LA**, **Lim J**, et al. 2009. A quantitative study of worker reproduction in queenright colonies of the Cape honey bee, *Apis mellifera capensis*. *Mol Ecol* **18**: 2722–7.
93. **Pirk CWW**, **Neumann P**, **Hepburn R.** 2007. Nestmate recognition for eggs in the honeybee (*Apis mellifera* L.). *Behav Ecol Sociobiol* **61**: 1685–93.
94. **Sumner S**, **Keller L.** 2008. Reincarnation, free-riding and inexplicable modes of reproduction. *Curr Biol* **18**: R206–7.
95. **Fournier D**, **Aron S.** 2009. No-male's land for an Amazonian ant. *Curr Biol* **19**: R738–40.
96. **Pearcy M**, **Timmermans I**, **Allard D**, **Aron S.** 2009. Multiple mating in the ant *Cataglyphis cursor*: testing the sperm limitation and the diploid male load hypotheses. *Insectes Soc* **56**: 94–102.
97. **Wenseleers T**, **Hart AG**, **Ratnieks FLW.** 2004. When resistance is useless: policing and the evolution of reproductive acquiescence in insect societies. *Am Nat* **164**: E154–67.
98. **Drescher J**, **Bluthgen N**, **Feldhaar H.** 2007. Population structure and intraspecific aggression in the invasive ant species *Anoplolepis gracilipes* in Malaysian Borneo. *Mol Ecol* **16**: 1453–65.
99. **Arnqvist G**, **Rowe L.** 2005. *Sexual Conflict*. Princeton: Princeton University Press. p. 337.
100. **Nonacs P.** 2006. Interspecific hybridization in ants: at the intersection of ecology, evolution, and behavior. *Ecology* **87**: 2143–7.
101. **Bengtsson B**, **Ceplitis A.** 2000. The balance between sexual and asexual reproduction in plants living in variable environments. *J Evol Biol* **13**: 415–22.
102. **Bourke AFG.** 2011. *Principles of Social Evolution*. Oxford: Oxford University Press.
103. **Neumann P**, **Moritz RFA.** 2002. The Cape honeybee phenomenon: the sympatric evolution of a social parasite in real time? *Behav Ecol Sociobiol* **52**: 271–81.
104. **Lattorff HMG**, **Moritz RFA**, **Fuchs S.** 2005. A single locus determines thelytokous parthenogenesis of laying honeybee workers (*Apis mellifera capensis*). *Heredity* **94**: 533–7.
105. **Lattorff HMG**, **Moritz RFA**, **Crewe RM**, **Solignac M.** 2007. Control of reproductive dominance by the thelytoky gene in honeybees. *Biol Lett* **3**: 292–5.