

Thelytokous Parthenogenesis in Eusocial Hymenoptera

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Annu. Rev. Entomol. 2013. 58:273–92

First published online as a Review in Advance on October 15, 2012

The *Annual Review of Entomology* is online at ento.annualreviews.org

This article's doi:
10.1146/annurev-ento-120811-153710

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Keywords

apomixis, arrhenotoky, automixis, clonality, sex determination, thelytoky

Abstract

Female parthenogenesis, or thelytoky, is particularly common in solitary Hymenoptera. Only more recently has it become clear that many eusocial species also regularly reproduce thelytokously, and here we provide a comprehensive overview. Especially in ants, thelytoky underlies a variety of idiosyncratic life histories with unique evolutionary and ecological consequences. In all eusocial species studied, thelytoky probably has a nuclear genetic basis and the underlying cytological mechanism retains high levels of heterozygosity. This is in striking contrast to many solitary wasps, in which thelytoky is often induced by cytoplasmic bacteria and results in an immediate loss of heterozygosity. These differences are likely related to differences in haplodiploid sex determination mechanisms, which in eusocial species usually require heterozygosity for female development. At the same time, haplodiploidy might account for important preadaptations that can help explain the apparent ease with which Hymenoptera transition between sexual and asexual reproduction.

INTRODUCTION

The insect order Hymenoptera, comprising the bees, wasps, and ants, originated some 250 Ma in the late Permian or early Triassic and evolved into one of the most diverse insect groups in the world. Currently, about 125,000 species have been described and conservative estimates suggest that at least 5 to 10 times as many species remain to be discovered (32). In addition to their remarkable diversity, the Hymenoptera are also a cradle for the evolution of social behavior. Eusociality, which is characterized by reproductive division of labor, cooperative brood care, and overlapping generations (74, 120), has evolved at least 10 to 11 times independently in the bees, wasps, and ants (42).

In all hymenopterans, social and solitary alike, parthenogenesis is part of the regular life cycle, in which males develop from unfertilized eggs, i.e., via arrhenotokous parthenogenesis, and females develop from fertilized eggs. Arrhenotoky and haplodiploidy (males are haploid and inherit a single set of chromosomes from their mother, whereas females are diploid and inherit one set of chromosomes from each parent) usually coincide in Hymenoptera and are often regarded as synonymous (13). In contrast to arrhenotoky, thelytokous parthenogenesis, in which females develop from unfertilized diploid eggs, occurs less frequently. When the first thelytokous animals were discovered, they were thought of as mere developmental irregularities (13). Recent research efforts, however, have made it apparent that Hymenoptera, and ants in particular, are a diversity hot spot for the study of reproductive systems, especially those involving different forms of thelytokous parthenogenesis (39, 50, 117).

The evolutionary and ecological consequences of parthenogenetic reproduction are diverse and include the repeatedly cited advantages and disadvantages asexual species experience across the tree of life (3, 7, 73, 119). To summarize briefly, probably the most important selective advantage of sexually reproducing organisms is the continuous creation of novel genetic variation by recombination, which allows efficient purging of deleterious mutations from the genome and rapid evolutionary responses to changing selective pressures from the biotic and abiotic environment. Of course, genetic novelty does not arise without a cost, and having two sexes, expending energy during courtship, and falling victim to sexually transmitted diseases are just a few of the detriments associated with sexuality.

With the advent of highly informative molecular genetic markers for nonmodel organisms, it has now become clear that thelytokous parthenogenesis, in combination with haplodiploidy and eusociality, has resulted in several idiosyncratic evolutionary outcomes specific to the eusocial Hymenoptera. For example, the queens of some species can simultaneously harvest the benefits of clonality and sexual reproduction by producing daughter queens parthenogenetically and workers sexually (e.g., 23, 67, 77, 82, 83). Because only the young queens mate and reproduce, this maximizes the fitness of mother queens while maintaining the genetic variation within the worker force, i.e., those members of the colony that mostly interact with the external environment. Such systems can potentially provide novel insights into the costs and benefits of sex.

Furthermore, although female caste determination in most social insects is thought to be mediated exclusively via environmental factors, a strong genetic component is involved in caste determination in some species (22, 77, 83, 97). These species would therefore also be unique model systems to study the genetic and epigenetic underpinnings of caste determination and phenotypic plasticity in social insects. Several of the systems in which queens switch back and forth between asexual and sexual reproduction to produce young queens and workers, respectively, are associated with an exceedingly rare reproductive strategy, namely male clonality (23, 77, 83). How male clonality is achieved is currently unknown, but it probably involves either the exclusion of the maternal genome from fertilized eggs or the fertilization of anucleate ova

Genetic caste determination: an individual's genotype, rather than environmental factors, determines whether a female develops into a queen or a worker

Parthenogenesis: reproduction by development of an unfertilized egg; offspring can be either male or female

Arrhenotoky: males develop from unfertilized, haploid eggs

Haplodiploidy: males develop from unfertilized, haploid eggs and females develop from fertilized, diploid eggs

Clones: all individuals that have descended asexually from the same ancestor

(23, 24, 54, 83). As a consequence, males and queens maintain entirely separate gene pools that combine only to produce workers (24, 54, 83). These systems are suggested to represent the end point of a molecular arms race between males and females (23), which would make them unique model systems for the study of sexual conflict.

Finally, thelytokous eusocial insects have the potential to provide novel tests of inclusive fitness theory. Colonies of obligately parthenogenetic species, which are composed of genetically identical individuals, should be especially void of any selfish individual interests and function as the ultimate “superorganism.” The first studies, however, suggest that even in predominantly thelytokous species social conflicts and worker-policing behavior prevail, possibly because colonies often contain individuals from more than one clonal line (35, 51).

These interesting evolutionary implications of unusual reproductive modes in social insects have been expertly reviewed elsewhere (117). In this review we therefore focus mainly on other, equally fascinating aspects of parthenogenetic reproduction in eusocial Hymenoptera. We begin by discussing the different cytological mechanisms and the proximate causes of thelytoky in eusocial Hymenoptera, as well as the interplay between thelytoky and the different molecular sex-determining mechanisms. We then provide the first comprehensive overview of species that are known, or have been suggested, to reproduce thelytokously. Given the large number of species, detailed information on each species can be found in the **supplemental material** (follow the **Supplemental Material link** from the Annual Reviews home page at <http://www.annualreviews.org>). Finally, we discuss whether the recurrent emergence of thelytoky in Hymenoptera might be explained by preadaptations specific to that group, how the capacity to reproduce parthenogenetically contributes to the invasive potential of ants, and how thelytoky can act as an evolutionary route to social parasitism.

CYTOLOGICAL MECHANISMS AND THEIR GENETIC CONSEQUENCES

Thelytokous parthenogenesis can have a variety of underlying cytological mechanisms. Below we review those known to occur in Hymenoptera. A general overview of both arrhenotokous and different forms of thelytokous parthenogenesis is given in **Figure 1**. A more comprehensive list and detailed descriptions of the possible cytological mechanisms can be found in Reference 98.

Apomictic Parthenogenesis

Apomixis is mitotic, i.e., it does not involve full meiosis and fusion of meiotic products. As a consequence, the offspring is usually clonally identical to the mother. Over time, mutations are thought to accumulate independently in the two alleles at any given locus, leading to genetic divergence and high levels of heterozygosity in ancient apomictic lineages, the so-called Meselson effect (114). However, ameiotic recombination in apomictic lineages can lead to the loss of heterozygosity, which can have a substantially stronger effect on genome evolution than the accumulation of mutations can (79). The utility of allelic divergence as an indicator of ancient asexuality has been called into question on the basis of this finding (79).

Although apomixis is common among insects (e.g., 13, 98, 100), it is rare within the Hymenoptera. As far as we know, the only well-studied example is the chalcidoid wasp *Trichogramma cacoeciae*, and even here gamete formation is not strictly ameiotic (108). In this system, cells undergo a single meiotic equational division, followed by the expulsion of a single polar body, with a lack of meiotic recombination and reduction (108). Among social Hymenoptera, apomixis had initially been inferred for the little fire ant, *Wasmannia auropunctata* (23).

Thelytoky:

females develop from unfertilized, diploid eggs

Apomixis (or mitotic parthenogenesis):

formation of gametes from unfertilized eggs without meiosis, generally lacking genetic recombination

 Supplemental Material

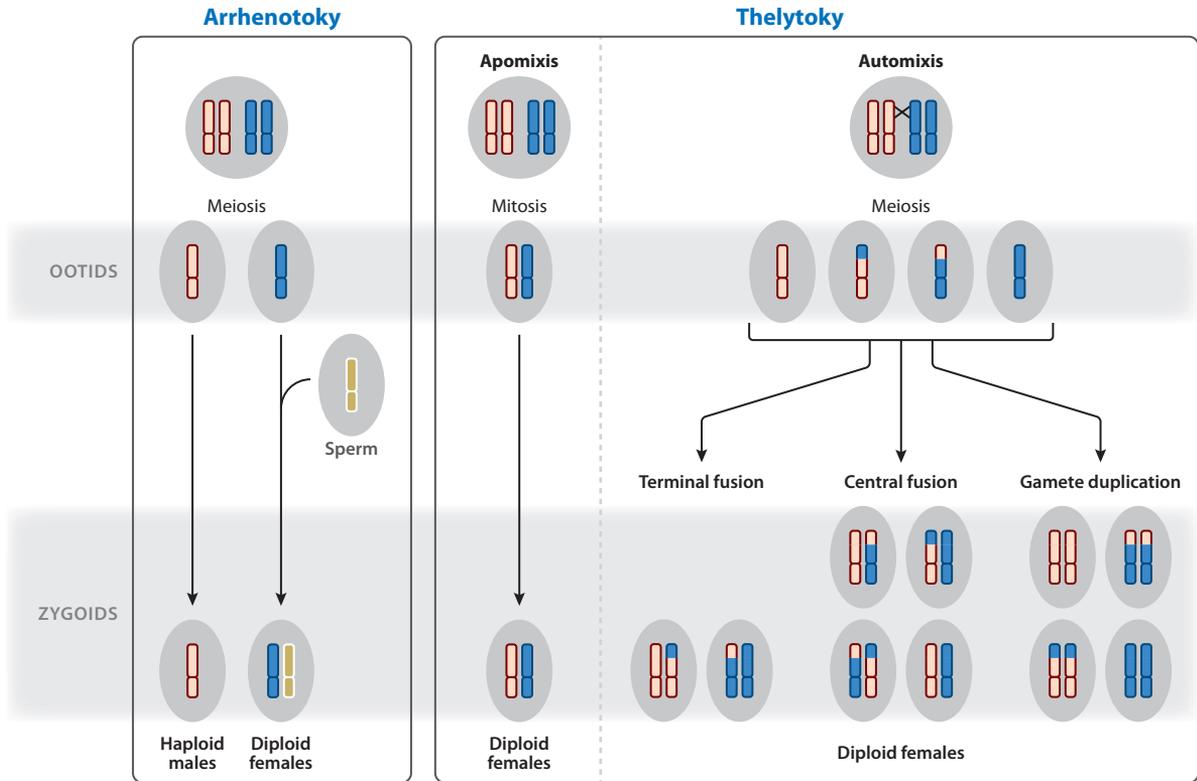


Figure 1

Modes of reproduction known from Hymenoptera. For automictic thelytoky, a case of crossing-over with recombination is represented to illustrate the effects on heterozygosity levels in the progeny.

However, it has since been shown that thelytoky in *W. auropunctata* most likely involves some form of automixis with central fusion and reduced recombination rates (90). In *Cerapachys biroi* (61), *Mycocetopus smithii* (87), and *Paratrechina longicornis* (83) recombination rates are very low, and it remains to be definitively determined whether this is due to apomixis or automixis.

Automixis (or meiotic parthenogenesis):

formation of gametes from unfertilized eggs via a process that involves meiosis and varying degrees of genetic recombination

Central fusion:

following meiosis diploidy is restored by fusion of second-division non-sister nuclei, preserving heterozygosity

Automictic Parthenogenesis

Automixis involves meiotic reduction, and diploidy is restored by one of several mechanisms. Most thelytokous Hymenoptera reproduce by some form of automixis, each with different consequences for the genetic composition of the offspring.

Automictic parthenogenesis with terminal fusion. During automixis with terminal fusion, diploidy is restored by fusion of the second polar nucleus with the egg nucleus. In the absence of crossing-over, this leads to complete homozygosity of the resulting diploid offspring. If a single crossing-over event occurs, heterozygosity is maintained distal to the crossing-over event relative to the centromere (Figure 1). Average rates of transition to homozygosity at a heterozygous locus will range from 1 (for a locus close to the centromere, assuming no recombination between the focal locus and the centromere) to 1/3 (for a locus distant from the centromere, assuming several crossing-over events that effectively unlink the focal locus from the centromere) (84). In any case,

THELYTOKY IN TERMITES

Eusociality has evolved repeatedly throughout the animal kingdom, and outside of the Hymenoptera, termites are the second largest insect group with highly sophisticated and ecologically dominant societies. Unlike hymenopterans, both sexes in termites are diploid and workers can be either male or female. Colonies are founded by a royal couple (a queen and a king), and the male is long-lived and mates repeatedly with the queen. In many species, the primary reproductive couple is eventually superseded by secondary reproductives that are recruited from within the colony. Although it was long believed that this system involves repeated cycles of inbreeding within colonies, at least in some species secondary queens are in fact parthenogenetically produced by the primary queen via automixis with terminal fusion (i.e., secondary queens are homozygous for one of the two alleles in a heterozygous primary queen; 72, 107). Because several secondary queens develop, both alleles of the primary queen persist in the colony. Workers, soldiers, and winged reproductives, on the other hand, are produced sexually. This demonstrates once again how eusocial species can evolve to combine the benefits of both sexual and asexual reproduction in novel and unexpected ways (71, 72, 107, 121).

heterozygosity is quickly lost under automixis with terminal fusion. This form of thelytoky occurs in a small number of solitary Hymenoptera (66), as well as in termites (71, 72) and other insects (98). No case of automixis with terminal fusion is known from eusocial Hymenoptera.

Automictic parthenogenesis with central fusion. During automixis with central fusion, diploidy is restored by fusion of the two central polar nuclei. In the absence of crossing-over, heterozygosity is maintained and the offspring is genetically identical to the mother. If a single crossing-over event occurs during meiosis, any locus distal to the site of crossing-over has a 50% chance of becoming homozygous (**Figure 1**). Average rates of transition to homozygosity will range from 0 for loci close to the centromere to 1/3 for loci distant and effectively decoupled from the centromere (84). Heterozygosity can be maintained during automixis with central fusion, especially if recombination rates are low. This mechanism underlies thelytoky in the honey bee, *Apis mellifera*, and is also the demonstrated or likely mechanism in all well-studied ants (**Table 1**). It also occurs in some parasitoid wasps and other insects (66).

Traditionally, it has been possible to distinguish between apomixis and automixis using cytological methods (e.g., 110), while recent studies have used mostly neutral genetic markers to infer the cytological mechanisms underlying parthenogenesis (e.g., 84, 90). However, on the basis of molecular markers alone, apomictic parthenogenesis, especially with rare gene conversions via ameiotic recombination, can be difficult to distinguish from automictic mechanisms that retain high levels of heterozygosity, such as central fusion (e.g., 61, 87). One promising approach using molecular markers is to study recombination events on the background of a linkage map. Using microsatellite markers, Rey et al. (90) inferred that thelytoky in *W. auropunctata* is achieved by automixis with central fusion and reduced recombination rates, rather than by apomixis. This conclusion was based on the observation that markers on the same linkage group frequently co-transitioned to homozygosity, which is expected under automixis with central fusion, whereas under apomixis markers should be affected independently.

Automictic parthenogenesis with gamete duplication. During automixis with gamete duplication, the haploid egg cell divides and the cleavage nuclei fuse to restore diploidy. Alternatively, the egg cell fails to divide following chromosome replication. The genetic consequence is that the entire diploid genome becomes homozygous in a single generation, and this is independent

Terminal fusion: following meiosis diploidy is restored by fusion of second-division sister nuclei, resulting in the loss of heterozygosity

Table 1 Well-documented cases of regularly occurring thelytokous parthenogenesis in the eusocial Hymenoptera

Subfamily	Species	Social system	Cytogenetic mechanism	Occurrence of males	References
Bees					
Apinae	<i>Apis mellifera capensis</i>	QQ, QW ^b , WQ, WW, FM	CF	Regularly	4, 5, 30, 64, 65, 78, 105, 110
Ants					
Cerapachyinae	<i>Cerapachys biroi</i>	WW, FM	CF or AP?	Rarely	61, 89, 104
Formicinae	<i>Cataglyphis cursor</i> ^a	QQ, QS, WQ, WW ^c , FM	CF	Regularly	10, 68, 82, 84
	<i>Cataglyphis hispanica</i>	QQ, QS, FM	?	Regularly	67
	<i>Paratrechina longicornis</i>	QQ, QS, MC	?	Regularly	84
Myrmicinae	<i>Monomorium triviale</i>	QQ, QW	?	Males are unknown	29
	<i>Myocepeurus smithii</i>	QQ, QW ^d	CF or AP?	Only in sexual populations	41, 87, 88
	<i>Pristomyrmex punctatus</i>	QQ, QW?, WQ, WW	CF?	Rarely	15, 16, 29, 36, 47, 102, 103
	<i>Pyramica membranifera</i>	QQ, QW	?	Males are unknown	29, 46
	<i>Vollenbovia emeryi</i>	QQ, QS, MC	?	Regularly	53, 54, 77
	<i>Wasmannia auropunctata</i>	QQ, QS, FM, MC ^e	CF?	Regularly	22–25, 90
Ponerinae	<i>Platythyrea punctata</i>	QS, WW, FM	CF?	Rarely	34, 35, 40, 51, 52, 94, 95

Social system: FM, females (queens or workers) produce males by standard arrhenotokous parthenogenesis; MC, male clonality, in which queens produce males that are genetically identical to the queen's mate; QQ, queens produce queens by thelytoky; QS, queens produce workers sexually; QW, queens produce workers by thelytoky; WQ, workers produce queens by thelytoky; WW, workers produce workers by thelytoky. Cytogenetic mechanism: AP, apomixis; CF, automixis with central fusion. The question mark indicates that the proposed mechanism requires confirmation. A detailed discussion of each species is given in the **supplemental material**.

^aThe taxonomic ambiguity regarding *Cataglyphis cursor* and *C. piliscapa* is discussed in the **supplemental material**.

^bVirgin queens reproduce thelytokously, but mated queens reproduce mainly sexually (5).

^cWorkers produce workers and queens by thelytokous parthenogenesis in orphaned colonies (10, 82).

^dSome populations reproduce sexually (87).

^eSome populations reproduce sexually (24, 25).

Supplemental Material

of crossing-over events during meiosis (**Figure 1**). Automixis with gamete duplication in Hymenoptera is found in all cases where thelytoky is induced by *Wolbachia* infections (see below). However, this mode is not known from eusocial Hymenoptera. A variety of other cytological mechanisms can underlie thelytokous parthenogenesis in other organisms (13, 63, 98, 100), but to the best of our knowledge these are not known to occur in Hymenoptera.

CAUSES OF THELYTOKOUS PARTHENOGENESIS IN HYMENOPTERA

In Hymenoptera, thelytoky is usually induced by infection with symbiotic microbes or it has a nuclear allelic basis (66). In vertebrates, on the other hand, thelytokous parthenogenesis almost always arises as a consequence of hybridization (3, 76), which seems to be the rare exception in Hymenoptera.

Infectious Origin of Thelytoky (Microbe-Induced)

From the perspective of cytoplasmically inherited endosymbiotic bacteria, which are common in arthropods, the production of male offspring by the host is wasteful in the sense that the

bacteria are not transmitted via males. Accordingly, these endosymbionts have evolved a wide range of mechanisms, such as male killing, cytoplasmic incompatibility, phenotypic feminization of genetic males, and female parthenogenesis, to manipulate host reproduction (reviewed in References 11, 49, 75). The induction of thelytokous parthenogenesis in the host is a particularly effective way for the bacteria to increase their own fitness (99). Although cytoplasmic *Wolbachia* are common in ants and potentially honey bees (e.g., 43, 116), no case of microorganism-induced thelytoky is currently known from eusocial Hymenoptera (31, 41, 61, 115). However, thelytoky is commonly induced by bacteria in parasitoid wasps and other arthropods (11, 18, 38, 49, 55, 66, 75, 106, 118), and it remains possible that at least in some cases thelytoky in eusocial Hymenoptera might also have an infectious origin. Three genera of parthenogenesis-inducing (PI) bacteria occur in Hymenoptera: *Wolbachia*, *Cardinium*, and *Rickettsia*. These bacteria also occur in other arthropods, but parthenogenesis induction seems to be generally restricted to species with haplodiploid sex determination (11, 80, 118). We briefly discuss each genus of PI bacteria in turn.

PI *Wolbachia* bacteria frequently occur in a wide range of hymenopteran families and are particularly common in the superfamilies Chalcidoidea and Cynipoidea (38, 55, 99). In the studied cases of Hymenoptera, *Wolbachia* bacteria induce thelytoky via gamete duplication, either by segregation failure in the first mitotic anaphase or through fusion of mitotic nuclei in the second prophase (80). In either case, the diploid offspring of a thelytokous female becomes fully homozygous within a single generation. However, across arthropods as a whole, *Wolbachia* bacteria do not necessarily induce thelytoky via gamete duplication. In the haplodiploid mite genus *Bryobia*, for example, *Wolbachia* infections lead to parthenogenesis in which the progeny is heterozygous and genetically identical to the mother, possibly via apomixis (113).

Among Hymenoptera, PI *Cardinium* bacteria are known to occur only in chalcidoid wasps (124–126). Although the precise cytogenetic mechanism of *Cardinium*-induced thelytoky in Hymenoptera is currently not known, in the parasitoid wasp *Encarsia hispida* the bacteria are required to feminize diploid embryos, which develop into diploid males in the absence of *Cardinium* (28). This shows that in some cases diploidy restoration alone is not sufficient to induce female development, and that the main effect of the bacteria in this system could be feminization, rather than the induction of thelytoky per se. However, the extent to which *Cardinium* is also involved in diploidy restoration in *E. hispida* and other chalcidoids remains unclear (28). The case of *Cardinium* in *E. hispida* is a good example of how inherited bacterial endosymbionts can interact with arthropod sex determination mechanisms (11, 28).

Similar to PI *Cardinium*, PI *Rickettsia* bacteria currently are known only from chalcidoid wasps (1, 27, 33, 85). One strain of *Rickettsia* induces thelytoky in *Neochrysocharis formosa* (1, 33), and a second, separate strain induces thelytoky in *Pnigalio soemius* (27). In both cases, the bacteria are required for the production of diploid female offspring (1, 27). However, at least in *N. formosa*, the underlying cytological mechanism is entirely different from those known from PI *Wolbachia* in Hymenoptera. Only a single equational division occurs during meiosis, without meiotic recombination and reduction. As a consequence, the progeny is genetically identical to the mother and heterozygosity is maintained (1).

Although cytoplasmic endosymbionts are commonly thought to be nearly exclusively transmitted vertically from mother to offspring, this is not always the case. In fact, we now know of several cases in which PI *Wolbachia* are regularly transmitted horizontally between hosts, both within and between species, for example, via shared food sources (44, 45, 57). The extent of horizontal transmission is an important factor in shaping the population biology of *Wolbachia* and the coevolutionary dynamics with its hosts. The precise molecular mechanisms by which cytoplasmic bacteria manipulate host reproduction are still largely unknown, but our understanding of this

matter is likely to improve as molecular biology resources for studying both hosts and symbionts become available (e.g., 59).

Allelic Origin of Thelytoky

Thelytoky with a genetic basis also occurs in a wide range of hymenopteran taxa, including Apoidea, Ichneumonoidea, Tenthredinoidea, and Vespoidea. Given that no microbial infections have been implicated in thelytokous reproduction of eusocial Hymenoptera, it currently seems plausible that these cases have a genetic basis. The allelic basis of thelytoky has so far only been investigated in the Cape honey bee, *Apis mellifera capensis* (48, 64, 65, 91), and the ichneumonoid wasp *Lysiphlebus fabarum* (92). In both cases, thelytoky is controlled by a single recessive locus (65, 92). This locus has been termed *thelytoky* (*th*) in the Cape honey bee (65), and a recent study showed that within this locus the transcription factor *CP2*, a homolog of the *Drosophila* transcription factor *gemini*, is the main candidate gene causing the thelytoky phenotype (48). *CP2* shows differential splicing between arrhenotokous and thelytokous workers, which may be mediated by the lack of a short splice enhancer motif in the Cape honey bee allele. In fact, laying Cape honey bee workers produce different splice forms in ratios similar to those of arrhenotokous queens. Using RNAi, Jarosch et al. (48) showed that the knockdown of the splice variant containing the candidate exon in arrhenotokous worker bees indeed resulted in rapid ovary activation, one of the characteristic traits of Cape honey bee workers. In the case of an allelic basis, thelytoky can be “contagious” in the sense that the trait can introgress into sexual populations if thelytokous lines continue to produce functional males at some frequency or if females have retained the capacity to mate and thelytoky is facultative to some extent (e.g., 65, 92). This could explain, for example, the occurrence of distinct unrelated clonal lines that most likely arose independently in sexual or partly sexual source populations in several ant species (22, 61, 87). At least in Cape honey bees, however, parthenogenetically produced queens mate and reproduce sexually, which suggests that thelytoky might not be entirely under simple genetic control as is currently believed (5).

Hybrid Origin of Thelytoky

During hybridization, two distinct genomes join in a cell, which may explain why chromosomes often do not pair correctly and normal meiosis becomes dysfunctional. The resulting parthenogens are highly heterozygous and carry one allele from each parent species at each locus (3, 76). In Hymenoptera, this mechanism of creating thelytokous lineages seems rare but has been implicated in a number of *Trichogramma* species (108). Although several cases of between-species hybridization are known from social Hymenoptera (20), none is known to have given rise to thelytokous parthenogenesis (e.g., 87).

THELYTOKY AND SEX DETERMINATION

Although a large number of cytological mechanisms can underlie thelytokous parthenogenesis, in each case the mechanism has to be compatible with the genetic sex determination mechanism of the respective species. All Hymenoptera are haplodiploid, and males normally develop from unfertilized haploid eggs via arrhenotokous parthenogenesis, whereas females develop from fertilized diploid eggs. A few exceptional cases exist in chalcidoid wasps in which males develop from initially diploid eggs after paternal genome elimination, however (38). At least two alternative genetic mechanisms underlie haplodiploidy in Hymenoptera. First, under complementary sex

determination (CSD), one or more loci have to be heterozygous to elicit female development, whereas hemizygous (haploid) and homozygous individuals develop into males. CSD is currently thought to be the predominant mechanism of sex determination in the eusocial Hymenoptera, although reliable data are limited to a few well-studied species (2, 106). Second, sex determination can be mediated via maternal imprinting, in which the presence of a paternally derived genome determines the expression and splicing patterns of genes in the sex determination cascade. The best-understood examples of these two forms of sex determination are the eusocial honey bee, *Apis mellifera* (9, 26, 37), and the parasitoid jewel wasp *Nasonia vitripennis* (8, 109), respectively. According to a recent phylogenetic reconstruction, CSD is the ancestral mechanism of sex determination in the Hymenoptera (2).

In honey bees, sex is determined by a single locus, termed *complementary sex determiner*, or *csd* (single locus complementary sex determination, or sl-CSD). Heterozygosity at this locus and the resulting different versions of the *csd* protein lead to female-specific splicing of *feminizer* (*fem*) mRNA, which in turn mediates female-specific splicing of *doublesex* (*dsx*), the next downstream gene in the sex determination cascade, and sustains female-specific splicing of *fem* in an autoregulatory feedback loop (9, 26, 37). In the homozygous or hemizygous state, the *csd* protein is inactive, which results in the male-specific splicing of *fem* mRNA. An important consequence of this system is that individuals homozygous for *csd* develop into diploid males, which are sterile in honey bees and probably many other hymenopteran species (106). In other cases, diploid males sire triploid female offspring, which in turn are most likely sterile (e.g., 69). This effect becomes most severe under inbreeding in species with sl-CSD and therefore has important consequences for the conservation biology of Hymenoptera (e.g., 123). On the other hand, we can expect to see specific adaptations in species for which regular inbreeding is part of the normal life cycle. The two most obvious routes are selection for diploid male fertility or, alternatively, selection against sl-CSD. For example, in the solitary vespid wasp *Euodynerus foraminatus*, which has sl-CSD, males mate with their emerging sisters in the natal nest. As a consequence, diploid males occur at high frequencies. However, these males are fertile and sire normal diploid female offspring (12). Inbreeding is also common in the ant *Cardiocondyla obscurior*. Diploid males are nevertheless rare in this species, suggesting a system of CSD that involves two or more unlinked loci (96).

Overall, this means that any form of thelytoky that results in loss of heterozygosity at the *csd* locus (or loci) will in most cases be incompatible with CSD. This could explain the apparent absence of thelytoky-inducing microorganisms in social Hymenoptera, because at least *Wolbachia* bacteria commonly induce gamete duplication, which results in completely homozygous progeny in a single generation (e.g., 80, 106). In fact, the majority of known cases of PI *Wolbachia* are found in the wasp superfamilies Chalcidoidea and Cynipoidea, which do not have CSD, whereas thelytoky in taxa with CSD, such as Apoidea, Vespoidea, and Tenthredinoidea, typically has a genetic basis (2, 38, 66). The superfamily Ichneumonoidea contains species both with and without CSD (2) and, accordingly, species in which thelytoky is *Wolbachia* induced and others in which thelytoky has a genetic basis (58, 66). On the other hand, heterozygosity is maintained during functional apomixis in a case of *Rickettsia*-induced thelytoky in the chalcidoid wasp *Neobrysocharis formosa* (1, 33). Furthermore, for most eusocial Hymenoptera, the genetic mechanism of sex determination is currently unknown. To conclude, it remains possible that thelytoky might indeed be microbe-induced in some eusocial Hymenoptera, and the possibility should be tested in future studies while considering the mechanism of sex determination.

The second well-studied case of sex determination in the Hymenoptera is that of the chalcidoid jewel wasp *Nasonia vitripennis*. In this species, the maternally provided *transformer* (*tra*, which is homologous to honey bee *fem*) transcript autoregulates female-specific splicing of *tra* in the

Complementary sex determination

(CSD): genetic sex determination mechanism in which homo- or hemizygous individuals develop into males, whereas heterozygous individuals develop into females

zygote. However, *tra* is only expressed in the zygote and leads to female development in the presence of a paternally derived genome, whereas it is silenced in haploid embryos, leading to male development. This suggests a maternal imprinting mechanism that prevents zygotic expression of the maternally derived *tra* allele in haploid, unfertilized eggs (8, 109). Because in thelytokous species female development must be induced in the absence of a paternally derived genome, additional mechanisms would have to be in place to make thelytoky compatible with sex determination via maternal imprinting. For example, the maternal imprint might not be copied during meiosis, resulting in fusion of one imprinted ootid and one unimprinted ootid during automixis (8). Alternatively, cytoplasmic symbionts could lead to feminization of diploid eggs that would otherwise develop into males (28). Until the precise molecular imprinting mechanism in *Nasonia* is known, it remains unclear how exactly it would interact with different forms of thelytoky.

With the advent of genomic resources for studying an ever-increasing number of social and solitary Hymenoptera, we hopefully will soon have a better understanding of the diversity and evolutionary history of sex-determining mechanisms in this group. This knowledge will in turn allow us to study the implications for the evolution of reproductive systems and different forms of thelytokous parthenogenesis in more detail.

PHYLOGENETIC DISTRIBUTION OF THELYTOKY IN EUSOCIAL HYMENOPTERA

How many species are affected by thelytokous parthenogenesis, and is thelytoky relevant to the evolution of eusocial Hymenoptera? To the first part of the question, we can give only an approximate answer, reflecting our patchy knowledge about the biology of most invertebrate species. Among eusocial bees, wasps, and ants, 51 species have been demonstrated or claimed to reproduce via thelytokous parthenogenesis. Among these, the best-studied species is *A. mellifera*, and most of the remaining 50 species are found in 4 subfamilies of ants (**Figure 2**; **Tables 1** and **2**). Curiously, to the best of our knowledge, not a single case of thelytoky has been reported from the eusocial wasps. In 11 ant species (22%), thelytoky clearly plays a significant role in the respective species' life cycle and sexual reproduction is rare, restricted to a geographic region or confined to worker production while queens are produced asexually (**Table 1**). In 20 ant species (40%), thelytoky occurs infrequently or is limited to worker reproduction in orphaned colonies. Thelytoky was initially proposed and subsequently refuted by more comprehensive studies in 9 cases (18%). The remaining 10 species of ants (20%) have been cited, often repeatedly, for reproducing thelytokously, even though this had not been proposed in the original study. The species in these latter three categories are listed in **Table 2**. A detailed account of each case is given in the **supplemental material**. All species currently known to be capable of worker thelytoky in orphaned colonies belong to the subfamilies Formicinae (70%) and Myrmicinae (30%) (**Table 2**). Whether the taxonomic distribution of asexual taxa represents the natural distribution of thelytoky, or whether it is mainly a reflection of the study focus of researchers in their respective geographic areas, is not known. Despite the risk of oversimplifying the emerging pattern, it seems that the genetic systems of eusocial Hymenoptera are exceptionally flexible and that thelytoky probably occurs in many species. Arguably, given the high diversity of the Formicidae, the greatest challenge will be to determine the number of species capable of thelytoky and the significance of thelytoky for a given species' life cycle and evolution. Nevertheless, the high frequency with which asexual species are discovered and the high diversity of genetic mechanisms responsible for thelytoky suggest that thelytoky plays an important role in the evolution of eusocial Hymenoptera, and that we have only just begun to scratch the surface of a world teeming with diverse genetic mechanisms.

 [Supplemental Material](#)

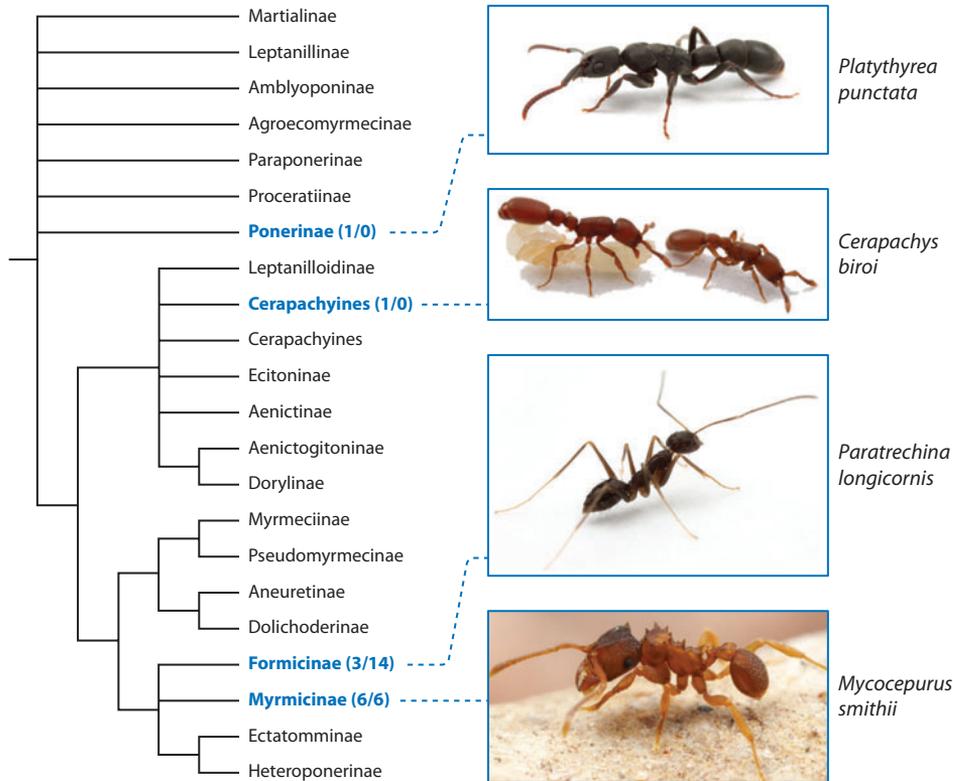


Figure 2

Phylogenetic distribution of thelytokous species among the ants. Subfamilies containing thelytokous species are highlighted in bold. The first number in parentheses gives the number of species in which thelytoky is known to be a major part of the reproductive cycle (listed in **Table 1**), and the second number gives the number of species in which thelytoky occurs sporadically (listed in **Table 2**). The photographs depict examples of well-studied thelytokous ants [from top to bottom: *Platythyrea punctata* (Ponerinae), *Cerapachys biroi* (Cerapachyinae), *Paratrechina longicornis* (Formicinae), and *Mycocepurus smithii* (Myrmicinae)]. The subfamily Cerapachyinae is depicted as nonmonophyletic and represented as two separate taxa labeled “cerapachyines” to illustrate the phylogenetic uncertainty (112). The phylogeny is modified with permission from Reference 112. Photographs are courtesy of Daniel Kronauer (*C. biroi*) and Alex Wild (all other photos).

ARE HYMENOPTERA PREADAPTED FOR THELYTOKY?

The evolution of thelytokous parthenogenesis can be constrained by a number of factors (19). For example, fertilization is often necessary for egg activation, and paternally inherited centrosomes and other factors might be important for normal embryonic development. Furthermore, as outlined above, the ancestral sex determination system can be incompatible with certain forms of thelytoky and, depending on the genetic consequences of thelytoky, deleterious recessive alleles might become fully expressed.

All Hymenoptera are haplodiploid and in the vast majority of cases males are produced by arrhenotokous parthenogenesis. Arrhenotokous parthenogenesis suffers from several of the constraints on thelytokous parthenogenesis mentioned above, which means that in Hymenoptera, many of the common constraints on thelytokous parthenogenesis must have already been

Table 2 Cases of thelytokous parthenogenesis in eusocial Hymenoptera in which thelytoky is restricted to orphaned colonies, or in which thelytoky has been suggested and either requires further validation or has since been refuted

Family	Subfamily	Species/subspecies	Evidence for thelytoky
Apidae	Apinae	<i>Apis mellifera ligustica</i>	B
		<i>Apis mellifera caucasica</i>	B
TOTAL BEES	1 subfamily	1 species with 2 subspecies	–
Formicidae	Dolichoderinae	<i>Tecbnoformica</i> sp.	X
	Formicinae	<i>Anoplolepis gracilipes</i>	M
		<i>Camponotus anderseni</i>	R
		<i>Camponotus</i> sp.	X
		<i>Cataglyphis bicolor</i>	B
		<i>Cataglyphis piliscapa</i> ^a	X
		<i>Cataglyphis sabulosa</i>	B
		<i>Cataglyphis savignyi</i>	B
		<i>Cataglyphis mauritanica</i>	?
		<i>Cataglyphis viatica</i>	?
		<i>Cataglyphis velox</i>	?
		<i>Cataglyphis bombycina</i>	?
		<i>Formica polyctena</i>	B
		<i>Formica pratensis</i>	X
		<i>Formica rufa</i>	X
		<i>Formica sanguinea</i>	R
		<i>Lasius alienus</i>	B
		<i>Lasius brunneus</i>	B
		<i>Lasius flavus</i>	B
		<i>Lasius niger</i>	B, D
		<i>Oecophylla longinoda</i>	R
		<i>Oecophylla smaragdina</i>	R
		<i>Polyrbachis lama</i>	D
		Myrmicinae	<i>Aphaenogaster lamellidens</i>
	<i>Aphaenogaster picea</i>		B
	<i>Aphaenogaster rudis</i>		X
	<i>Aphaenogaster senilis</i>		B
	<i>Atta cephalotes</i>		R
	<i>Crematogaster auberti</i>		X
	<i>Crematogaster scutellaris</i>		B
	<i>Crematogaster skounensis</i>		X
	<i>Crematogaster vandeli</i>		X
	<i>Crematogaster impressa</i>		X
<i>Messor capitatus</i>	B, D		
<i>Messor aciculatus</i>	C		

(Continued)

Table 2 (Continued)

Family	Subfamily	Species/subspecies	Evidence for thelytoky
		<i>Protomognathus americanus</i>	R
		<i>Temnothorax curvispinosus</i>	R
		<i>Temnothorax unifasciatus</i>	R
		<i>Solenopsis invicta</i>	R
TOTAL ANTS	3 subfamilies	39 species	–

Source of evidence supporting thelytoky: B, breeding experiment; C, chromosome study; D, dissection of reproductive females; M, genetic markers; R, thelytoky hypothesis refuted by an independent study; X, erroneously cited in the literature because thelytoky was not suggested in the original study. The question mark indicates thelytoky was mentioned as a personal observation or similar, but a published study is lacking. A detailed discussion of each species, including the references to the original studies, is given in the **supplemental material**.

^aThe taxonomic ambiguity regarding *Cataglyphis cursor* and *C. piliscapa* is discussed in the **supplemental material**.

overcome in a common ancestor (19). For example, hymenopteran eggs can be activated by mechanical stimuli during oviposition (e.g., 93), and centrosomes in embryos can originate from maternal accessory nuclei (21). Furthermore, deleterious mutations are fully expressed in haploid males (e.g., 122). Consequently, hymenopterans (and other arrhenotokous organisms) should be preadapted to nascent thelytoky, and this might explain at least partially the large number of thelytokous species in this group (19).

Finally, genomic imprinting can disrupt normal development in uniparental offspring. Genomic imprinting occurs when paternally and maternally derived alleles at a given locus display parent-of-origin-specific epigenetic modifications (mostly differential DNA methylation) and expression. In fact, genomic imprinting is thought to be a major barrier to parthenogenesis in mammals (e.g., 19, 56). Hymenoptera have functional DNA methylation systems, possibly constituting a molecular mechanism for genomic imprinting (62, 70, 111). This prospect has been met with excitement, especially given the potential for novel tests of inclusive fitness theory, and a theoretical framework to test predictions for genomic imprinting conflicts in eusocial Hymenoptera has been developed (17, 60, 86). However, whereas several studies have shown potential functions of DNA methylation in Hymenoptera (70), genomic imprinting has yet to be demonstrated. The facts that hymenopteran males are generally uniparental and that thelytokous parthenogenesis has evolved repeatedly in distantly related groups indicate that Hymenoptera might indeed lack genomic imprinting, or that it plays a minor role, at least during embryonic development (60).

ECOLOGICAL CONSEQUENCES: INVASIVE POTENTIAL AND SOCIAL PARASITISM

Two ecological consequences of thelytokous parthenogenesis in eusocial Hymenoptera are especially noteworthy. First, thelytoky is particularly common among invasive species (81). For example, among the 11 ant species listed in **Table 1**, 6 are invasive (*Wasmannia auropunctata*, *Paratrechina longicornis*, *Pyramica membranifera*, *Vollenbovia emeryi*, *Monomorium triviale*, and *Cerapachys biroi*), and an additional 3 species, though currently not classified as invasive, are unusually widespread geographically and do well in anthropogenically modified habitats (*Mycocepurus smithii*, *Platythyrea punctata*, and *Pristomyrmex punctatus*). A likely reason for this ecological success of parthenogenetic ants is that thelytoky is an effective way of circumventing the challenges associated with low population densities in founding populations, such as inbreeding depression and

 Supplemental Material

the inability to find mates (101). For example, young queens of species such as *W. auropunctata*, *V. emeryi*, and *P. longicornis* readily mate with their brothers inside the nest. However, this does not lead to increased levels of homozygosity and inbreeding depression because the female and male gene pools are separated. As a consequence, a queen is equally related to her brothers as she is to any other male in the population, and sib-matings produce heterozygous diploid female offspring (23, 77, 83). The benefits of thelytokous reproduction during colonization are even more pronounced in species that forgo mating and sexual reproduction entirely, as is the case for *C. biroi*, *M. smithii*, and *P. punctatus*. In these species, a single queen (e.g., *M. smithii*) or any small colony fragment (e.g., *C. biroi* and *P. punctatus*) has the potential to colonize a new habitat without the necessity of mating and sexual reproduction (61, 87, 103).

Second, thelytokous parthenogenesis might provide an evolutionary route to social parasitism. In the Cape honey bee, for example, workers reproduce thelytokously and invade colonies of another African honey bee subspecies, *Apis mellifera scutellata* (6). Upon arrival in the host colonies, Cape honey bee workers activate their ovaries and start laying eggs. The eggs escape detection by *scutellata* workers and are raised to adulthood, and the newly emerged workers produce more parasitic daughters, causing the decline and ultimately the collapse of the host colony.

The Japanese queenless ant *Pristomyrmex punctatus* is a second example in which thelytoky is involved in social parasitism. Colonies of *P. punctatus* consist of workers and ergatoid queens and both castes are capable of thelytokous reproduction (103). A few genetically distinct lineages produce significantly larger ergatoid queens, so-called cheaters, which exclusively reproduce and do not contribute to colony maintenance. These cheater lineages presumably disperse to host colonies via horizontal transmission. The cheater lineages have been suggested to comprise an incipient parasite species, but a molecular phylogenetic analysis showed that the parasite lineage is actually nested inside the *P. punctatus* clade (16). In fact, tests of reproductive isolation are of little use in organisms that reproduce primarily via thelytoky, and the delimitation of asexual species therefore has to rely on other criteria (e.g., 14). It will remain interesting to ponder whether asexual eusocial insect lineages speciate and whether thelytokous intraspecific parasites are bound to evolve into inquiline parasites.

OUTLOOK

The surge of molecular genetic techniques in the past decades has facilitated the discovery and systematic study of the diversity of reproductive systems in the eusocial Hymenoptera. Judging from our literature survey, thelytoky is ubiquitous across the ants and it is entirely possible that it is also widespread among eusocial bees and wasps, but that it has so far gone largely undetected because of comparatively fewer studies on these groups. Hence, more species capable of thelytoky and potentially additional reproductive systems are expected to be discovered. Based on our current knowledge, a targeted approach to identifying additional thelytokous species should focus on invasive species, on polydomous and/or polygynous species in otherwise monodomous and/or monogynous clades, and on the many species for which males are not known. Furthermore, with the rise of genomic techniques for nonmodel organisms, it is now possible to study the molecular mechanisms underlying the high propensity of eusocial hymenopterans to switch between sexual and asexual reproduction, over both ecological and evolutionary timescales. That being said, not all is dependent on technology. Carefully executed natural history studies and behavioral observations will always be important tools for discovering novel phenomena in nature. Studying the evolutionary ecology of reproductive systems is a truly interdisciplinary endeavor, and groundbreaking results are expected from all biological disciplines.

SUMMARY POINTS

1. Thelytoky is widespread in solitary and eusocial Hymenoptera and, especially in ants, it has given rise to novel reproductive systems with unprecedented evolutionary consequences, such as the conditional use of sexual and asexual reproduction by queens, as well as male clonality.
2. Thelytoky can have a variety of underlying cytological mechanisms. All well-studied thelytokous eusocial Hymenoptera reproduce via some form of automixis with central fusion, but it is possible that some species employ apomixis. In either case, heterozygosity is initially conserved across the genome but deteriorates over time, especially under automixis with central fusion.
3. In many eusocial Hymenoptera, female development is dependent on heterozygosity at one or more sex-determining loci. This restricts the evolution of thelytoky to mechanisms that preserve heterozygosity, at least at the loci involved in sex determination.
4. Thelytoky in solitary wasps is often induced by endosymbiotic bacteria; however, no such case is currently known to occur in eusocial Hymenoptera. One likely explanation is that in most cases, endosymbiotic bacteria induce thelytoky via gamete duplication, a mechanism that leads to the immediate loss of genome-wide heterozygosity and therefore might be incompatible with sex determination mechanisms in eusocial Hymenoptera. In honey bees, and possibly in other eusocial Hymenoptera, thelytoky instead has a nuclear genetic basis.
5. A number of factors can constrain the evolution of both arrhenotokous and thelytokous parthenogenesis. Because Hymenoptera are ancestrally haplodiploid and arrhenotokous, they must have overcome many of these shared constraints during the early evolution of the clade. This might help explain the apparent ease with which Hymenoptera switch between sexual and asexual reproduction.
6. Thelytoky can have profound ecological consequences. For example, at least in ants, thelytoky seems to be associated with increased invasive potential. Furthermore, thelytokous strains might be prone to act as intraspecific social parasites.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We would like to thank Rockefeller University and the Searle Scholars Program for supporting the research of D.J.C.K., and the Harvard Society of Fellows for supporting the research of C.R. Alex Wild kindly provided the photographs used in **Figure 2**, and Phil Ward generously gave permission to use a modified version of a phylogram, which was first published elsewhere.

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