

## **The need for sperm selection may explain why termite colonies have kings and queens, whereas those of ants, wasps and bees have only queens**

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**Hymenoptera have haploid males which produce sperm by cloning. Sperm selection theory predicts that because termites have diploid males that produce genetically diverse sperm, they may profit from a high sperm surplus (large  $K$ ), whereas Hymenoptera (ants, bees and wasps) should produce few sperm per fertilization (low  $K$ ). Male reproductive “kings”, which continuously provide spermatozoa during the whole life of the queen, allow for a large  $K$ . Available empirical evidence confirms the existence of a large difference in  $K$  between diploid insects, especially Blattodea (Isoptera) ( $K > 1000$ ), and haplo-diploids such as Hymenoptera ( $K < 10$ ). The available data suggests that sperm selection may be an important evolutionary force for species with diploid, but not haploid males.**

All termite species known are social and no termite species has ever been reported to lack a king -male consorts fertilizing the queen(s) continuously during her lifetime [Grasse 1982.]-; whereas no kings exists among social Hymenoptera [Hölldobler & Wilson 1990], be they bees, ants or wasp, despite the occurrence of more than 30000 different social species with widely different reproductive behaviors [Wilson 1971]. Exceptionally among Hymenoptera, ergatoid male ants in the genus *Cardiocondyla*, stay in the colony for long period of time and have life-long spermatogenesis, but they do not qualify as kings as these males foment inbreeding and other winged males leave the nest to engage in nuptial flights with virgin females [Heinze & Hölldobler 1993]. Both groups (Hymenoptera and Isoptera) though, have queens, i.e. fertile females that specialize in the reproductive functions of the colony, with a spermatheca for storing sperm for

variable periods of time. Such a conspicuous difference should have a non trivial explanation. Here I am proposing one.

Traditional informal explanation for this difference by several biologists draws on the different evolutionary history of Blattodea (Isoptera) and Hymenoptera. A special reference is given to the fact that termites derive from roach-like ancestors that lived in the wood and had reduced dispersal, so that males remained associated with females. Recently, based on molecular phylogenetic evidence, termites and cockroaches have been united into a single order Blattodea (Inward et al., 2007). In contrast, the ancestors of eusocial hymenoptera had to look for resources that are scattered in the environment and thus adapted to long periods where males were absent. Although different evolutionary history is certainly important in understanding present adaptations, scenarios of irreversible evolutionary history can normally not be empirically verified, and thus, may not be falsifiable [Jaffe 2007]. We know for example that in innumerable species, males and females exploit different ecological niches where one sex lives in the soil, inside wood, or inside fruits or some kind of organism, and nevertheless the sexes manage to find each other. There is no doubt that evolutionary history explains the differences between both groups of insect that persist despite their myriads of different adaptations. Here I propose an empirically verifiable explanation, without negating the role of other ecological and evolutionary forces, in maintaining “kings” among termite species but not among Hymenoptera species.

Blattodea (Isoptera) are diploid and Hymenoptera are haplo-diploid. Males of the hymenoptera are haploid, whereas those of termites are diploid. Occasionally, some diploid males occur among Hymenoptera species. These males however have a low sperm number and fitness of these males is very low [see Duchateau & Marien 1995 for example]. Thus, we might safely assume that reproductive viable males among Hymenoptera are haploid. As a consequence, all spermatozoa of a hymenopteran male are genetically identical clones, except for the occasional random mutation, whereas spermatozoa of a termite male vary in genetic composition. For example, a diploid male with a genome containing 20 hetero-allelic loci, will produce at least  $2^{20} = 1048576$  genetically different spermatozoa types compared to 1 type for a haploid male.

Many organisms produce large number of sperm, much more than that required to fertilize the female's eggs [Sivinski 1980]. Many mechanisms can account for a large number of sperm. One explanation claims that sperm surplus accelerates adaptation as several advantageous combinations of genes can be selected by focusing on the sperm, before producing offspring, thanks to intra- and inter-ejaculate sperm selection. As expressed by Sivinski [1980], "The best sperm to survive in a female, penetrate a particular ovum, and complement that egg's genotype, may be a statistical rarity... In a sense, an ejaculate would serve as a lek from which the egg would choose its mate". Clearly, not all sperm reach the spermatheca and that arriving there may be ejected from the spermatheca [Baer 2005]. Sperm plasma, vaginal fluids, and morphological or biochemical features in the female's reproductive tract may kill many spermatozoa, and spermatozoa-spermatozoa competition may further reduce the number of sperm reaching the ovum. Sperm selection has been proposed as a likely reason accounting for the large sperm surplus observed in many species [Cohen 1999, Ball & Parker 2003, Jaffe 2004]. Sperm selection, as an evolutionary mechanism, is highly efficient and evolutionary stable. Computer simulations showed that sperm selection is an evolutionary stable strategy and that adaptation to high sperm numbers is achieved in digital diploid male but not in haploids. This result is explained by the fact that sperm selection favors the most fit allele combinations, only if genetic variance among spermatozoa exists [Jaffe 2004]. Empirical evidence suggests that sperm selection, rather than sperm competition, explain some features of human sperm ejaculates [Jaffe et al. 2006]. Indirect evidence for sperm selection has been published also for the dung beetle [Simmons & Kotiaho 2007] where sexy-sperm is favored by morphological characteristics of the female reproductive duct.

If we accept that sperm selection is at work in biological evolution, the difference between haploid and diploid males is highly relevant. A large number of sperm confers advantages to diploid organisms because it allows, among other benefits, selection of spermatozoa expressing the fittest combination of alleles responsible for basic metabolic functions, producing fitter offspring. Sperm of haploid males are clones and therefore offer no advantage for sperm selection. Haplodiploids though have the advantage that beneficial genes are more likely to be spread more rapidly than in diploids [Brueckner 1978], if no sperm selection mechanism is at work. Computer simulations show that this reasoning is sound [Jaffe 2004], suggesting that evolution

should favor the production of large number of spermatozoa by diploid, but not by haploid males. This kind of selection happens in addition to selection acting on males, as males with deleterious mutations will not reproduce. Thus, individual selection and sexual selection acting on males is always important as the occurrence of combats between males of social insects, especially among hymenoptera, shows [Lee & Starr 2007]. Few reports of these combats exist partly because behavior of males in mating swarms is practically unknown for most species of Hymenoptera and Isoptera and many other types of male-male competition may occur.

It is often believed that spermatozoa express the genotype of the diploid parent so that all spermatozoa from a single individual are phenotypically identical. This assumes that the haploid genotype lies dormant and is not expressed. This mechanism has been proposed as a way to prevent selfish alleles competing within the ejaculate (See Hurst & Peck 1996 for a revision). Although the working of such a mechanism can not be excluded in all cases, it clearly does not apply in many cases. Spermiogenesis allows the expression of the haploid genome before chromatin condensation and the formation of the final spermatozoon (Hecht 1995). Other direct or indirect evidence for unique spermatozoa phenotypes has been found (Skalhegg et al 2002, Ren et al 2001, Evans et al 2003). For example, among sperm of diploid cockroaches, large phenotypic intra- and inter-ejaculate variance exist (Harris et al 2007) and the sperm phenotypes of this cockroach have a strong genetic basis (Moore et al 2004). Thus, even if only a few genes are expressed phenotypically, and these genes are central to basic protein networks (Jeong et al 2001), spermatozoa selection would be an important driving force of evolution in sexual diploid organisms (Jaffe 2004). Other physiological and developmental mechanisms, such as germline competition (Hastings 1989) would also achieve a similar result.

Let us define  $K$  as the average number of sperm used per fertilization of one successful ovum, which also expresses the sperm-use efficiency or the amount of sperm surplus. The little empirical data available that allows calculating  $K$  is summarized in Table 1. The value of  $K$  for all the species of Hymenoptera studied so far is very low, whereas  $K$  for diploid insects is large.  $K$  is very large in the few species of Blattodea (roaches and termites) for which it is known. Unfortunately very little data is available for termites. The high values of  $K$  for termites ( $>1000$ ) is due to the fact that males

inseminate females frequently [Raina, et al 2003]; whereas for ants and bees the available data indicates a  $K < 10$ , even in species where queens are polygamous [Baer & Boomsma 2004].

All colonies of social insects have queens which produce a large number of workers (females in the case of ants, males and females in the termite). Hymenoptera and termite differ in that among hymenoptera, queens receive one to several male ejaculates once in their life, during the nuptial flight, Sperm are stored for later use during their whole life in the queen's spermatheca. Termite queens, that also have a functional spermatheca, are inseminated continuously during their life, often more than once a day [Raina et al 2003], receiving up to thousands of male ejaculates over their lifetime.

It is surprising how little data exist on number of sperm produced by males. Data for termites is especially scarce. More data is available for hymenoptera showing that haplo-diploid species have much lower  $K$  than diploid insects. Other species of termites might have a lower  $K$  than the Formosa termite here reported, but it seems reasonable to assume that because sperm selection requires a much larger sperm surplus in Blattodea (Isoptera) but not Hymenoptera, termites profit from a large sperm supply. As the cost for storing very large number of sperm in a spermatheca may be high [Tschinkel 1987, Baer, Armitage, Boomsma 2006], termites achieve large amounts of sperm by maintaining males copulating frequently with the queens. On the other hand, the relatively low sperm requirements for Hymenoptera can be handled in such a way that a few ejaculates can be stored for later use, so that hymenoptera colonies can dispense with males after copula.

It is not likely that the results showing a low  $K$  are due to some other idiosyncrasy of the hymenoptera, as taxonomically unrelated species such as rotifers, which have haploid males, show similar low values of  $K$ . Rotifer males store sperm in a bag that they transfer to females during mating. Fertilization takes place inside the females. Data for other animals and plants seem to follow a similar trend. The case of aphids and mites is interesting. Estimates of  $K$  for aphids give very low values and reproduction in some aphids is sperm limited (Dagg 2002). Yet aphids, although similar in many aspects to haplo-diploid insects, differ in their genetic reproductive system and merit a separate treatment. In general, species with males producing low number of sperms

makes them likely to get into situations where sexual reproduction is sperm limited, so that their females are likely to evolve the potential for asexual reproduction.

More empirical data is needed to test this hypothesis. Even if the hypothesis is eventually falsified, stimulating research in these aspects of the reproductive biology of social insects and of other diploid and haplo-diploid organisms should improve our understanding of evolution. Certainly, biological evolution when looked at from the perspective of gametes [Poore & Fagerström 2001], appears quite different than when its analysis is centered on the organism or the gene.

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**Table 1: Empirical data for sperm surplus (*K*) in Insecta and Rotifera.**

<b>Taxon</b>	<b><i>K</i></b>	<b>Referente</b>
<b>HAPLO-DIPLOID INSECTS</b>		
<i>Various Hymenoptera</i>	<10	Page 1986, Boomsma et al 2005
<b>Hymenoptera: Formicidae</b>		
<i>Eciton bucheli</i> , <i>Dorylus molestus</i>	<5	Kronauer & Boomsma 2007
<i>Iridomyrmex humilis</i>	<10	Séller & Passera 1992
<i>Cardiocondyla obscurior</i>	<10	Heinze personal communication
<i>Solenopsis invicta</i>	<5	Tschinkel & Porter 1988
25 species of ants	<5	Tschinkel 1987
<b>Hymenoptera: Apidae</b>		
<i>Apis spp</i>	<10	Kraus et al 2004, Königler et al 1989, 1994 2000,
<i>Bombus spp</i>	<10	Tasei et al 1998, Röseler 1973
<b>Hymenoptera: Chalcidoidae</b>		
<i>Eupelmus orientalis</i> , <i>Dinarmus basalis</i>	<5	Damiens et al 2002
<b>HAPLO-DIPLOIDS ROTIFERA</b>		
<i>Brachionus plicatilis</i>	<5	Ruttner-Kolisko 1983, Snell & Hoff 1987, Snell & Childress 1987
<i>Epiphanes senta</i>	<10	Schröder 2003
<b>DIPLOID INSECTS:</b>		
Various diploid insects	>10	Sivinski 1979
<i>Drosophila melanogaster</i>	>50	Pitnick, Miller, Reagan, Holland 2001
<i>Aedes aegypti</i>	>20	Sivinski 1980, Jones 1968
<i>Papilio xuthus</i>	>1000	Watanabe & Hachisuka 2005
<i>Grylodes sigillatus</i> , <i>Gryllus veletis</i> , <i>Gryllus texensis</i> ,	>500	Schaus & Sakaluk 2001
<b>Blattodea: Blaberidae</b>		
<i>Nauphoeta cinerea</i>	>300	Moore et al 2004
<b>Blattodea: Blattellidae</b>		
<i>Blattella germanica</i>	>100	Cochran 1979
<b>Blattodea: Termitidae</b>		
<i>Coptotermes formosanus</i>	>1000	Raina et al 2007
<i>Nasutitermes corniger</i>	>>100	Issa and Perdomo personal communication

<sup>1</sup> Inferred from the fact that sexual reproduction is often sperm limited

