

Sexual Cooperation: Mating Increases Longevity in Ant Queens

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Summary

Divergent reproductive interests of males and females often cause sexual conflict [1, 2]. Males of many species manipulate females by transferring seminal fluids that boost female short-term fecundity while decreasing their life expectancy and future reproductivity [3, 4]. The life history of ants, however, is expected to reduce sexual conflict; whereas most insect females show repeated phases of mating and reproduction, ant queens mate only during a short period early in life and undergo a lifelong commitment to their mates by storing sperm [5]. Furthermore, sexual offspring can only be reared after a sterile worker force has been built up [5]. Therefore, the males should also profit from a long female lifespan. In the ant *Cardiocondyla obscurior*, mating indeed has a positive effect on the lifetime reproductive success of queens. Queens that mated to either one fertile or one sterilized male lived considerably longer and started laying eggs earlier than virgin queens. Only queens that received viable sperm from fertile males showed increased fecundity. The lack of a trade-off between fecundity and longevity is unexpected, given evolutionary theories of aging [6]. Our data instead reveal the existence of sexual cooperation in ants.

Results and Discussion

Sexual conflict arises because the reproductive interests of males and females in many species differ greatly; females benefit most from a high lifetime reproductive output, whereas males attempt to gain maximum fitness from each mating event. During copulation, males can enhance their own short-term benefits by transferring substances that increase female egg-laying rates [7, 8] but have a negative effect on their re-mating probability, lifespan, and lifetime reproductive output [3, 9–14]. Conflict between the sexes is high in promiscuous mating systems [15–17] and decreases when, as in monogamous animals, the same partners are likely to re-mate

later in life and thus have converging interests. Experimentally enforced monogamy in the normally promiscuous fruit flies, in fact, led to the evolution of less-harmful males [18].

Lifelong commitment between two partners is rare in nature [19] but is typical for social insects. In termites, the queen and the king live together for their entire lifespan, inside a well-protected “royal cell” in the heart of the nest [20]. In ants, males and virgin queens mate during a short period early in life, after which the males die. The queens store the sperm of their mates in a spermatheca and use it to fertilize eggs throughout their entire lives, which in some species can last decades [5]. During the first months or even years after mating, the sperm is exclusively used to produce large numbers of workers, which form the “soma” of the colony. Like somatic cells of a multicellular organism, workers are typically sterile and do not directly contribute to future generations but, instead, rear the queen’s sexual offspring—the equivalent of the germ line in multicellular organisms. Sexuials are produced only after this first period of colony growth, and the sexual output of mature colonies depends on the size of the worker force that has been built up [21]. Both ant queens and males therefore benefit from mating only after the time lag of a long ergonomic phase; males would not profit from increasing the short-term fecundity of the queens at the cost of their longevity. Hence, the two specific life-history parameters of ants—a lifetime pair bond and late reproduction—are expected to reduce sexual conflict and, in its place, to promote cooperation between males and queens [20, 21]. In the stable associations of social insects, both sexes should benefit from a long female lifespan, which allows a lengthy period of sexual reproduction once the colony has reached sexual maturity.

We tested the effect of mating on the lifespan and reproductive success of queens of the ant *Cardiocondyla obscurior*. We compared three types of queens: virgin queens (VQ, $n = 34$); queens that mated with a single fertile male (of the wingless male morph [5]) (MQ, $n = 47$); and queens that mated with a single male (of the wingless male morph [5]) that was previously sterilized by X-ray irradiation (minimum 60 Gy; $2.95 \pm$ standard deviation, 0.12 Gy/min) (SQ, $n = 18$). These latter queens had experienced copulation and stored sperm in their spermathecae. This sperm, however, was not viable (i.e., it was motionless and in clumps, as revealed by dissections of the spermathecae of five queens) and did not fertilize eggs. Eggs laid by such queens, and also by virgin queens, were therefore expected to develop into males as a result of haplodiploid sex determination. Each queen was housed in an individual nest box with 20 workers, which corresponds to the average natural colony size. The number of workers was kept constant over the course of the experiment, during which colonies were kept on a high-nutrition diet (i.e., fed three times a week ad libitum with honey and pieces of dead cockroaches). The number of eggs and the survival of the queen were checked twice per week. At

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Figure 1. Fecundity of *Cardiocondyla* Queens

The egg-laying rate of queens receiving viable sperm during copulation was higher than that of virgin queens and queens that mated with a previously sterilized male transferring non-viable sperm (ANOVA on ln transformed number of eggs laid per week: $F = 112.1$, $p < 0.001$; Post-Hoc Bonferroni t-Test all pairwise: VQ-SQ: $p = n.s.$, VQ-MQ and SQ-MQ: $p < 0.001$; mean eggs/week and sample size: VQ: 6.4 [$n = 13$], SQ: 6.1 [$n = 18$], MQ: 20.6 [$n = 21$]). Graphical presentation of the back transformed means \pm standard deviations.

the same time, short observation scans were performed, revealing that all three groups of queens stayed permanently in the central nest area containing the brood. Furthermore, no other behavioral differences between queens (e.g., in general activity or brood care) or between workers in the handling of these queens, could be detected in the different colonies.

As predicted, colonies of queens that had mated with a fertile male produced both female and male offspring, whereas both virgin queens and queens that had mated with a sterilized male produced exclusively male offspring. Egg-laying rates were three times higher in the queens mated with fertile males compared to both groups of male-producing queens (Figure 1). Independent of the large differences in egg-laying rates, queens that had mated with either a fertile or a sterilized male lived approximately 50% longer than virgin queens (26 versus 18 weeks, Figure 2). In addition, they started

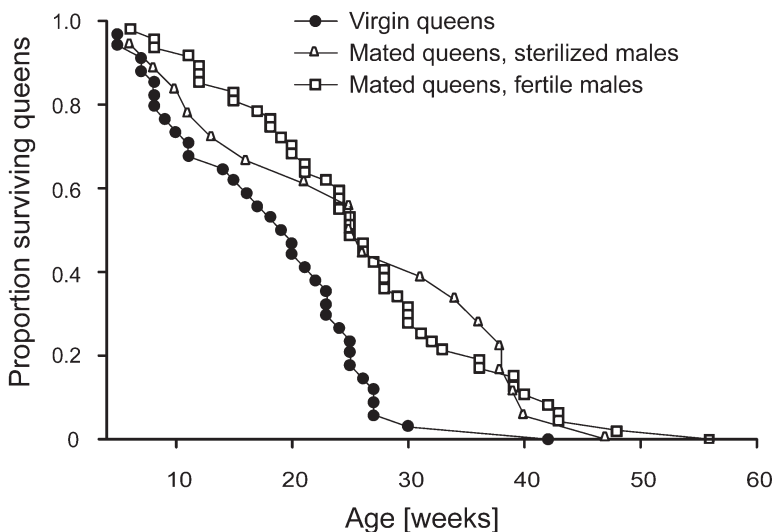


Figure 2. Lifespan of *Cardiocondyla* Queens
Mated queens lived longer than virgin queens, irrespective of whether their mate transferred viable or non-viable sperm (survival analysis for multiple samples: $\chi^2 = 11.0997$, $p < 0.004$; Cox's F-Test: VQ-SQ: $p = 0.013$, VQ-MQ: $p < 0.001$, SQ-MQ: $p = n.s.$; mean queen life span in weeks and sample size: VQ: 18.2 [$n = 34$], SQ: 25.8 [$n = 18$], MQ: 26.0 [$n = 47$]). The graph shows the proportion of surviving queens (Kaplan-Meier).

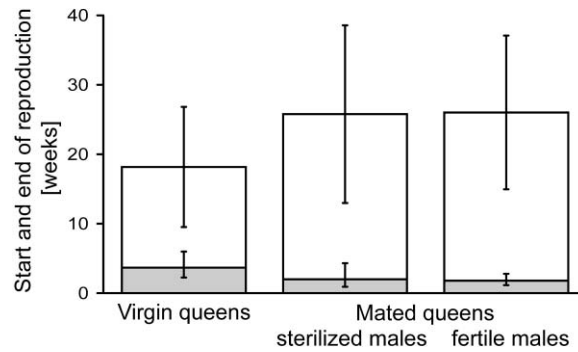


Figure 3. Duration of the Reproductive Phase in *Cardiocondyla* Queens

The onset of reproduction was more delayed in virgin queens compared to queens that mated with either one fertile or one sterilized male (ANOVA on ln transformed queen age at first egg-laying: $F = 6.2$, $p < 0.01$; Post-Hoc Bonferroni t-Test all pairwise: VQ-SQ: $p = 0.01$, VQ-MQ: $p < 0.001$, SQ-MQ: $p = n.s.$; mean day of first egg-laying and sample size: VQ: 25.7 [$n = 15$], SQ: 13.9 [$n = 18$], MQ: 12.5 [$n = 14$]). Because egg-laying continued until queen death, queen lifespan is equivalent to the end of the reproductive phase (for statistics, mean values, and sample size, see Figure 2). The graph shows the means \pm standard deviations of the start (back transformed data) and the end of egg-laying in the queens' lifespan (given in weeks). Gray: nonreproductive phase. White: reproductive phase.

laying eggs earlier than virgin queens (Figure 3). Therefore, mated queens experienced a 2-fold increase of their lifetime reproductive period: first, through an earlier onset of reproduction (by about 2 weeks); and second, through a longer lifespan (by approximately 8 weeks, Figure 3). Even though fecundity was equally low in virgins and queens that had mated with a sterilized male, the longer egg-laying period of the latter resulted in a 1.5-fold higher reproductive output (estimated total egg number laid during the lifetime: VQ = 93; SQ = 146). Because the queens that mated with fertile males also showed increased fecundity, their lifetime reproductive success (estimated total egg number = 499) was much

higher than that of the two groups of male-producing queens.

The low egg-laying rate of virgin queens matches previous observations in other ants [22]. Similarly, virgin females of many nonsocial insects are characterized by a lower fecundity and delayed oviposition compared to mated females [23]. Virgin females of solitary insects, as well as self-fertilizing nematodes, typically live longer than mated individuals [14, 24–28]. In contrast to this widespread negative effect of mating on longevity, virgin *Cardiocondyla* queens lived shorter than mated queens, even if males were infertile as a result of irradiation. The egg-laying rate of these latter queens was as low as that of virgins, which suggests that fertilization of eggs by viable sperm is necessary for a *Cardiocondyla* queen to reach maximum fecundity. The long lifespan of mated queens cannot result from potential preferential care of workers directed toward highly fecund queens (laying fertilized eggs) because queens mated with sterilized males lived as long as the queens mated with fertile males but produced the same low number of (unfertilized) eggs as the short-lived virgins.

The difference between the egg-laying rates of both types of mated queens of *C. obscurior*, and the similarity of their lifespan, further indicates that no trade-off exists between investment in reproduction and longevity. *Cardiocondyla* queens pay no apparent cost of high fecundity in terms of longevity—an observation not only indicated by comparisons between groups of queens but also further supported by the absence of a significant correlation between individual egg-laying rates and lifespan within any of the groups (Spearman Rank correlation: VQ, $r_s = 0.44$, $p > 0.12$; SQ, $r_s = -0.19$, $p > 0.45$; MQ, $r_s = -0.11$, $p > 0.78$). Moreover, both groups of mated queens started laying eggs earlier than virgin queens and also lived considerably longer.

Our data on longevity and reproduction in *C. obscurior* conflict with predictions from evolutionary theories of aging [6, 29, 30] and, more generally, of life-history evolution [31, 32] given that a trade-off between these two life-history parameters is consistently found in many organisms [24, 33, 34]. For example, fruit flies selected for a long lifespan showed reduced early fecundity [35], whereas flies with experimentally lowered egg-laying rates lived longer than control females [36]. It has previously been shown that queens of social insects typically live much longer than sterile workers [5, 37]. However, this alone does not allow us to conclude that reproduction causes increased longevity because reproducing individuals differ from non-reproductives in numerous life-history aspects (e.g., morphology, physiology, and behavior), including their sheltered way of life in the center of the nest [38, 39]. A low extrinsic mortality rate generally promotes internal longevity [31] in queens [39] and even in non-reproducing indoor workers [40]. Previous research on aging in social insects did not distinguish between the effects of reproduction itself and those of the low extrinsic mortality rate on the longevity of reproducing individuals. In contrast, our study compares the lifespan of groups of reproducing individuals that differ in fecundity but otherwise share the same life history (e.g., their reproductive and risk-avoidance behavior). The study therefore gives the first

convincing evidence that the longer lifespan of reproducing individuals among social insects is independent of the reproductive investment, but can instead be associated with the mating act or substances received through mating itself.

Whereas our study describes the positive effect of single mating versus nonmating on both female lifespan and lifetime fecundity, previous work has shown that, compared to female crickets mating with unattractive males, females mating with preferred males had either increased reproductive success or increased longevity (while the onset of oviposition was delayed) [41]. In crickets and several other species, a protein-rich spermatophore is transferred as a nuptial gift to the females during copulation. Multiple mating (as opposed to single mating) either increased or did not affect female longevity in such species [19, 42]. These results even held true when spermatophores were experimentally removed [41, 43]. In species without nuptial feeding, in contrast, lifespan generally decreases with increased mating rate [13, 19]. Ant males do not transfer nuptial gifts to the females, and multiple mating is rare [5]. Nevertheless, we predict a similar positive effect of multiple mating on lifespan, as observed in our study with single mating, because both the lifetime-pair bond (now with several partners) and the late onset of reproduction after colony maturation remain important unchanged life-history parameters promoting sexual cooperation. We further suggest that sexual cooperation might not be restricted to social insects but could be generally found throughout the animal kingdom wherever partners show a lifetime commitment to each other.

At present, it is unknown whether *Cardiocondyla* males transmit substances that lengthen female lifespan, directly or indirectly, for example by activating the production of antioxidants in the female body. Recent work on honeybees revealed higher expression levels of such compounds in the environment of the spermatheca in mated than in nonmated females [44]. These compounds are assumed to ensure the survival of the stored sperm throughout the long reproductive phase of the queens, and it remains to be tested whether they might similarly be involved in prolonging the life of the queens.

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