Ant queens adjust egg fertilization to benefit from both sexual and asexual reproduction

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An enduring problem in evolutionary biology is the near ubiquity of sexual reproduction despite the inherent cost of transmitting only half the parent’s genes to progeny. Queens of some ant species circumvent this cost by using selectively both sexual reproduction and parthenogenesis: workers arise from fertilized eggs, while new queens are produced by parthenogenesis. We show that queens of the ant Cataglyphis cursor maximize the transmission rate of their genes by regulating the proportion of fertilized and parthenogenetic eggs laid over time. Parthenogenetic offspring are produced in early spring, when workers raise the brood into sexuals. After the mating period, queens lay mostly fertilized eggs that will be reared as the non-reproductive caste.

Keywords: ant; parthenogenesis; sex; Cataglyphis

1. INTRODUCTION
The near-ubiquity of sexual reproduction in multicellular organisms has long puzzled evolutionary biologists, because sexuality is associated with strong fitness costs [1,2]. Most notably, sexually reproducing individuals incur a ‘twofold’ genetic cost because they transmit only half of their genes to the next generation, while asexually reproducing individuals pass all their genes to each offspring. Asexual reproduction, therefore, results in a twofold increase of per capita reproductive output compared with sexual reproduction.

Recent findings have shown that queens of social insects can circumvent the twofold cost of sex by taking advantage of the social caste system in order to benefit from the advantages of both sexual and asexual reproduction [3–5]. In some species, queens use alternative modes of reproduction for the production of the reproductive (queen) and non-reproductive (worker) female castes: new queens are produced asexually by thelytokous parthenogenesis, while workers are produced by normal sexual reproduction [5–8]. By selectively using asexual and sexual reproduction, mothers increase the transmission rate of their genes to their reproductive daughters, while maintaining genetic diversity in the worker force. Genetic diversity enhances colony performance by genetic polymethism, improved resistance to diseases and/or reduced within-colony conflicts [9].

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In Hymenoptera, males develop from haploid eggs by arthrotokous parthenogenesis and females from diploid eggs. In the ant Cataglyphis cursor, 96.4 per cent of daughter queens arise from thelytokous parthenogenetic eggs, while 97.5 per cent of daughter workers arise from fertilized eggs [6]. A distinctive feature of the species is that there is no overwintering brood. Sexuals develop from the eggs laid during the first days after queens resume egg laying in early spring. All the eggs laid later in the season are reared into the worker caste [10].

We hypothesize that such a schedule in caste production exerts strong selection on queens to regulate the proportion of fertilized and parthenogenetic eggs laid over time. Production of thelytokous eggs should be maximal in early spring, when the workers raise the brood into sexuals. Apart from this period, queens should lay exclusively fertilized eggs, which will be raised into a genetically diverse worker force. Here, we tested this hypothesis by monitoring the proportion of parthenogenetic and fertilized eggs among diploid (female) eggs laid by queens at the beginning of the season. We also examined possible variations in the proportion of haploid (male) eggs laid as a function of the reproductive cycle. Finally, we analysed the proportion of new virgin queens reared from parthenogenetically—and sexually produced eggs.

2. MATERIAL AND METHODS
Colonies of the ant C. cursor are headed by a single queen mated with up to eight males (effective mating frequency, 3.79) [11]. Workers have retained ovaries and can produce males by arthrotokous parthenogenesis and females by thelytokous parthenogenesis [9]. However, behavioural observations and detailed genetic analyses have shown that workers never reproduce in the presence of a queen [10,11].

Our study population was located at St-Hyppolite, France (42° 52' N, 2° 29' E). Twenty-seven colonies of C. cursor were excavated in early March, before queens resumed egg laying. They were maintained under laboratory conditions (26 ± 2°C, 12 L:12 D), and were fed on cockroaches and sugar water. About half the eggs laid by the queens from each colony were collected at four periods: 5, 20, 35 and 50 days after queens started laying. These periods cover the timing of sexual and worker production under laboratory conditions [10]. The length of the egg stage is 13 days in the laboratory; thus, an interval of 15 days between two successive collections prevented the sampling of eggs from the same period twice. The eggs were preserved at −80°C for subsequent genetic analyses.

A total of 1474 eggs (mean ± s.e. = 54.6 ± 3.15; n = 27 colonies) and the mother queens from the 27 colonies were genotyped at four microsatellite loci (Cat21, Cat46, C4666 and Cat466b) [6]. These markers exhibited 20, 15, 15 and 11 alleles, respectively, in the study population. Individual egg DNA was extracted by homogenization in a digestive solution (200 µg ml⁻¹ proteinase K, Biogene) and incubated for 12 h at 55°C. Genomic DNA was precipitated with ethanol following standard protocols. Adult female DNA was purified by phenol/chloroform. Amplifications by PCR were carried out following the fluorescent analysis protocols described in Pearcy et al. [6]. The amplified products were separated on an ABI Prism 3730 automated sequencer and sized against Rox-500HD standard (Applied Biosystems, USA). To determine the proportion of virgin queens reared from parthenogenetically or sexually produced eggs, 29 new queens produced under laboratory conditions (n = 3 colonies) were genotyped.

Because the four microsatellite loci were highly polymorphic, potential sources of error on the genetic origin (arthrotokous, thelytokous or fertilized) and maternity (queen or worker) of the eggs might arise from parthenogenetic and females by thelytokous parthenogenesis [9]. However, behavioural observations and detailed genetic analyses have shown that workers never reproduce in the presence of a queen [10,11].

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When workers raise the diploid brood into female sex-
proportion of fertilized eggs and thelytokous eggs laid
over time. Thelytokous eggs are laid in early spring,
proportion of fertilized and thelytokous eggs
regulate the
exclusively diploid fertilized eggs. There was signifi-
variation in the proportion of asexually produced and sexually produced eggs laid by queens
over time (χ^2-test for homogeneity between 5 and
20 days = 254.4, p < 0.0001; figure 1a). Whereas the
proportion of diploid eggs produced by thelytokous
parthenogenesis was close to 0.16 when queens
resumed egg laying, it rapidly decreased to 0.03–
0.04 after three weeks and reached 0.0 after 50 days.
A similar trend was observed for the production of
haploid eggs. Their proportion attained 0.45 in early
spring and fell to 0.03 on day 20. Conversely, the
proportion of diploid fertilized eggs laid reached 0.39 at
the onset of the reproductive period, and after three
weeks all eggs laid were fertilized.

The same pattern was observed when considering
the 16 colonies where queens produced thelytokous
eggs (figure 1b). Most of the parthenogenetic eggs
(haploids and diploids) were produced at the onset of
the laying period. Their proportion dramatically
decreased within a few weeks, while an increasing
proportion of fertilized eggs were laid (χ^2-test for hom-
ogeneity between 5 and 20 days = 194.3, p < 0.0001).

From the 16 colonies where queens had produced
eggs by thelytokous parthenogenesis, three (18%) raised new virgin queens (n = 11, 10 and 8, respec-
tively, which is small but typical for the species). Genetic analyses showed that all 29 new queens
lacked paternal alleles (inferred from mother–offspring genotypic comparisons) at the four loci,
indicating that they had been produced by partheno-
genesis. Thus, while queens initially laid an equal
proportion of diploid and thelytokous eggs (figure 1c),
workers selectively rear new reproductive
queens from parthenogenetic eggs (χ^2-test = 18.6,
p < 0.0001).

4. DISCUSSION
Our results show that queens of C. cursor regulate the
proportion of fertilized eggs and thelytokous eggs laid
over time. Thelytokous eggs are laid in early spring, when workers raise the diploid brood into female sex-
uals. The proportion of parthenogenetic eggs laid
decreases rapidly, and after three weeks all the eggs
arise from sexual reproduction and give rise to workers.
Thus, by laying parthenogenetic eggs at the onset of
the reproductive period, queens maximize the rate of
transmission of their genes to the next generation.

Previous studies have shown that ant queens can
exert substantial proximate control over caste ratio at
the egg stage. Queens of Pheidole pallidula use two
complementary mechanisms in this respect: by deter-
ing the proportion of female eggs laid and by hormonally biasing the development of female eggs
into either a worker or reproductive form [12]. More
recently, queens of Vollenhovia emeryi were shown to
invest more resources in producing queen-destined
eggs compared with other castes (i.e. male or
worker), in accordance with asymmetries of related-
ness with the progeny [13]. In this species, both
sexes are produced clonally and the queen’s relatedness
to her female and male sexual offspring is 1 and 0,
respectively. To our knowledge, the present study on *C. cursor* provides the first evidence that queens of social insects can control fertilization ratio at the egg stage *over time*, to benefit from the advantages of both sexual and asexual reproduction.

Queens produced eggs by thelytokous parthenogenesis in 16 experimental colonies, but these eggs were reared into new adult queens in three colonies only. The fate of the thelytokous eggs in the 13 remaining colonies is unclear; they may be eliminated or, more likely, they developed into workers. Previous studies indeed showed that workers may also arise from queen parthenogenesis in *C. cursor* [6]. Moreover, field collections show that few colonies produce female sexuals and, in this situation, their number is very limited (median, 5; range, 1–17) [11]. Intrinsic factors such as colony size may be prime factors in accounting for differences in the production of sexuals among colonies [14]. Consistent with this, field observations revealed a strong association between colony size and sexual production in *C. cursor*, with larger colonies showing higher investment in reproduction [11]. The present study is in agreement with this result: the three colonies rearing new reproductive clones of their mother as new reproductive queens, respectively. To our knowledge, the present study on *C. cursor* provides the first evidence that queens of social insects can control fertilization ratio at the egg stage *over time*, to benefit from the advantages of both sexual and asexual reproduction.

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