

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

S. Aron, I. Timmermans and M. Pearcy

*Biol. Lett.* published online 9 February 2011  
doi: 10.1098/rsbl.2010.1189

---

### Supplementary data

["Data Supplement"](#)

<http://rsbl.royalsocietypublishing.org/content/suppl/2011/02/04/rsbl.2010.1189.DC1.html>

### References

[This article cites 11 articles, 6 of which can be accessed free](#)

<http://rsbl.royalsocietypublishing.org/content/early/2011/02/04/rsbl.2010.1189.full.html#ref-list-1>

### P<P

Published online 9 February 2011 in advance of the print journal.

### Subject collections

Articles on similar topics can be found in the following collections

[behaviour](#) (1798 articles)

[evolution](#) (2421 articles)

### Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

---

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include the digital object identifier (DOIs) and date of initial publication.

---

To subscribe to *Biol. Lett.* go to: <http://rsbl.royalsocietypublishing.org/subscriptions>

---

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

S. Aron\*, I. Timmermans and M. Pearcy

*Evolutionary Biology and Ecology, CP 160/12, Université Libre de Bruxelles, Brussels, Belgium*

\*Author for correspondence ([saron@ulb.ac.be](mailto:saron@ulb.ac.be)).

**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 polyethism, improved resistance to diseases and/or reduced within-colony conflicts [9].

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2010.1189> or via <http://rsbl.royalsocietypublishing.org>.

In Hymenoptera, males develop from haploid eggs by arrhenotokous 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 arrhenotokous 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 (42.82° N, 2.99° 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 (*Ccur11*, *Ccur46*, *Ccur58* and *Ccur63b*) [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<sup>-1</sup> 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-350HD 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 (arrhenotokous, thelytokous or fertilized) and maternity (queen or worker) of the eggs laid were very low (electronic supplementary material). The probability of considering a queen-laid egg as arising from thelytokous parthenogenesis, while it was in fact fertilized, ranged from 1.93 × 10<sup>-10</sup> to 0.0039 across colonies. The probability of considering a queen-laid egg as haploid while it was diploid homozygous ranged from 1.21 × 10<sup>-11</sup> to 0.0002 across colonies; the probability of regarding an egg as haploid whereas it was produced by thelytokous parthenogenesis and homozygous at all four loci ranged from

0.0011 to 0.06 across colonies. Finally, all eggs sampled harboured maternal alleles at all loci, indicating that worker reproduction is absent or, at least, not significant in queenright colonies. The presence of only two haploid or eight diploid eggs laid by workers in our sample would have been detected with a probability higher than 0.96.

### 3. RESULTS

Colonies contained  $983.4 \pm 111.3$  workers (mean  $\pm$  s.e.; range: 272–2090); no brood was found at the time of collection. Over the 27 colonies sampled, 16 (59.2%) held diploid eggs produced by thelytokous parthenogenesis, 23 (85.2%) haploid eggs produced by arrhenotokous parthenogenesis and four (14.8%) exclusively diploid fertilized eggs. There was significant variation in the proportion of asexually produced and sexually produced eggs laid by queens over time ( $\chi^2$ -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 ( $\chi^2$ -test for homogeneity 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  $8$ , respectively, 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 parthenogenesis. Thus, while queens initially laid an equal proportion of fertilized and thelytokous eggs (figure 1c), workers selectively rear new reproductive queens from parthenogenetic eggs ( $\chi^2$ -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 sexuals. 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.

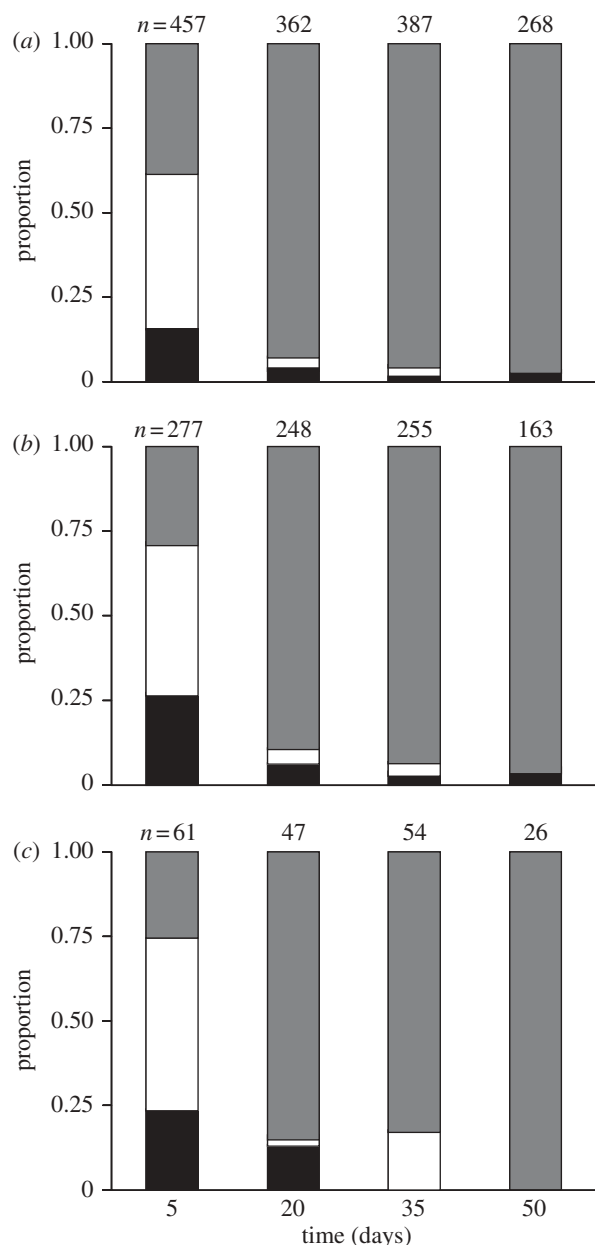


Figure 1. Proportions of fertilized (grey bars), arrhenotokous (white bars) and thelytokous (black bars) eggs laid by queens of *C. cursor* 5, 20, 35 and 50 days after they resumed egg laying in early spring. (a) All colonies ( $n = 27$ ); (b) colonies where queens produced thelytokous eggs ( $n = 16$ ); (c) colonies having reared adult sexual females ( $n = 3$ ). Total number of eggs typed is indicated above the bars.

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 determining 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 relatedness 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 queens were among the most populous of our sampling. Furthermore, the number of sexuals produced is positively associated with colony size (Spearman rank correlation:  $r_s = 0.55$ ,  $n = 27$ ,  $p < 0.007$ ).

Interestingly, our data also strongly suggest that workers of *C. cursor* exert some proximate control over the parthenogenetic origin of reproductive females. Multiple mating by queens of *C. cursor* aligns the reproductive optima of queens and workers, because workers are on average more closely related to their parthenogenetic sister queens ( $r = 0.50$ ), than they would be to sexually produced sister queens ( $r = 0.42$ ) [6,11]. Therefore, workers can enhance their inclusive fitness by preferentially rearing the clones of their mother as new reproductive queens, rather than their half-sisters. Consistent with this prediction, all sexual females produced in our experiments developed from thelytokous parthenogenetic eggs, while about half (53%) of the diploid eggs laid in early spring were fertilized (figure 1c). Despite the small sample size ( $n = 29$  new queens from  $n = 3$  colonies), this result lends credence to the hypothesis that workers favour the rearing of their parthenogenetic sisters into the reproductive caste. However, we cannot rule out the hypothesis of a genetic effect on caste determination, favouring development of thelytokous eggs into queens.

In conclusion, this study shows that queens of the ant *C. cursor* optimize the probability that parthenogenetic eggs will develop into reproductive daughters and maximize the transmission of their genes to the

next generation by regulating the proportion of parthenogenetic and fertilized eggs laid over time.

We thank C. Lenoën for her help, and the FRIA and the Belgian FRS-FNRS for their financial support.

- 1 Maynard Smith, J. 1978 *The evolution of sex*. Cambridge, UK: Cambridge University Press.
- 2 Bell, G. 1982 *The masterpiece of nature: the evolution and genetics of sexuality*. Berkeley, CA: University of California Press.
- 3 Fournier, D. & Aron, S. 2009 No-male's land for an Amazonian ant. *Curr. Biol.* **19**, R738–R740. (doi:10.1016/j.cub.2009.7.021)
- 4 Himler, A. G., Caldera, E. J., Baer, B. C., Fernandez-Marin, H. & Mueller, U. G. 2009 No sex in fungus-farming ants or their crops. *Proc. R. Soc. B* **276**, 2611–2616. (doi:10.1098/rspb.2009.0313)
- 5 Matsuura, K., Vargo, E. L., Kawatsu, K., Labadie, P. E., Nakano, N., Yashiro, T. & Tsuji, K. 2009 Queen succession through asexual reproduction in termites. *Science* **323**, 1687. (doi:10.1126/science.1169702)
- 6 Percy, M., Aron, S., Doums, C. & Keller, L. 2004 Conditional use of sex and parthenogenesis for worker and queen production in ants. *Science* **306**, 1780–1783. (doi:10.1126/science.1105453)
- 7 Fournier, D., Estoup, A., Orivel, J., Foucaud, J., Jourdan, H., Le Breton, J. & Keller, L. 2005 Clonal reproduction by males and females in the little fire ant. *Nature* **435**, 1230–1234. (doi:10.1038/nature03705)
- 8 Ohkawara, K., Nakayama, M., Satoh, A., Trindl, A. & Heinze, J. 2006 Clonal reproduction and genetic caste differences in a queen-polymorphic ant, *Völlenhovia emeryi*. *Biol. Lett.* **2**, 359–363. (doi:10.1098/rsbl.2006.0491)
- 9 Boomsma, J. J., Kronauer, D. J. C. & Pedersen, J. S. 2009 The evolution of social insect mating systems. In *Organization of insect societies* (eds J. Gadau, J. Fewell & E. O. Wilson), pp. 1–25. Cambridge, MA: Harvard University Press.
- 10 Cagniant, H. 1979 La parthénogenèse thélytoque et arrhénotoque chez la fourmi *Cataglyphis cursor* Fonscolombe. Cycle biologique en élevage des colonies avec reine et des colonies sans reine. *Insectes Soc.* **26**, 51–60. (doi:10.1007/BF02283912)
- 11 Percy, M. & Aron, S. 2006 Local resource competition and sex-ratio in the ant *Cataglyphis cursor*. *Behav. Ecol.* **17**, 569–574. (doi:10.1093/beheco/arj067)
- 12 de Menten, L., Fournier, D., Brent, C., Passera, L., Vargo, E. L. & Aron, S. 2005 Dual mechanism of queen influence over sex ratio in the ant *Pheidole pallidula*. *Behav. Ecol. Sociobiol.* **58**, 527–533. (doi:10.1007/s00265-005-0964-0)
- 13 Okamoto, M. & Ohkawara, K. 2010 Egg production and caste allocation in the clonally reproductive ant *Völlenhovia emeryi*. *Behav. Ecol.* **21**, 1005–1010. (doi:10.1093/beheco/arq093)
- 14 Pamilo, P. 1991 Evolution of colony characteristics in social insects. 1. Sex allocation. *Am. Nat.* **137**, 83–107. (doi:10.1086/285147)