

Research article

How to fool a virgin: Artificial dealation triggers oviposition in virgin *Lasius niger* queens

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Abstract. Once inseminated, ant queens rapidly shed their wings and start to lay eggs. Here we test whether there is a causal link between dealation and oviposition in the ant *Lasius niger*. We show that artificially dealated virgin queens start to lay eggs shortly after wing removal, whereas winged virgin queens hardly ever lay eggs. Dealate virgins do, however, produce fewer eggs than mated queens. These findings indicate that dealation does induce egg-laying, and that other factors, such as mating and/or insemination, further stimulate oviposition under natural conditions.

Keywords: Oviposition, dealation, *Lasius niger*, egg-laying, virgin queens.

Introduction

For ant queens mating marks the beginning of a new life involving complex behavioral and physiological changes. Once mated, ant queens rapidly shed their four wings by tearing them off with their hind legs (Forel, 1922; Hölldobler and Wilson, 1990). Over the following weeks, wing muscles are broken down and metabolized, freeing energy that the queens use to produce eggs and to provide food for the developing brood. While it has long been known that wing muscle histolysis and oviposition are induced upon insemination (Jones et al., 1978; Davis et al., 1989), it is unknown whether the shedding of wings, which normally follows insemination, also contributes to this phenomenon.

Circumstantial evidence that dealation itself might play a role in initiating oviposition comes from the fire ant *Solenopsis invicta*. In this species virgin queens are prevented from laying eggs by a pheromone that the functional queen releases. But when not in contact with this pheromone for several days, most virgin queens shed their wings and start to lay eggs (e.g. Fletcher and Blum, 1981; Vargo and Laurel, 1994;

Vargo, 1997). Whether there is a causal link between dealation and the production of eggs is, however, not established.

In this study we test whether artificial dealation of virgin queens can trigger oviposition in the ant *Lasius niger*. This experiment was performed because we needed a large number of male, i.e., unfertilized eggs for another study. Since preliminary tests showed that winged virgin *L. niger* queens in isolation did not dealate and start to lay male eggs on their own, we tried artificial dealation.

Material and methods

121 virgin *L. niger* queens were collected from 12 field nests on 15 July 2005 (four to 16 per nest). Approximately half of the queens of each nest had their wings removed with soft forceps (66 in total), whereas the other half was left untouched (55 in total). We then transferred queens in pairs to glass vials with a moist cotton ball inside, having ensured that pairs consisted of nest-mates subjected to the same treatment. In both treatment groups eggs were counted and removed every two to three days for the first two weeks. A final egg-number assessment took place another two weeks later. To verify that the eggs laid were not exclusively trophic eggs (i.e., eggs that serve as food and cannot develop), we checked for the presence of embryos in a subset of 20 to 30 eggs per nest (Leica S6D microscope, 64 x magnification). We also tested with a chromosome squash method (van Dijken, 1991; Aron et al., 1994), whether metaphasic chromosomes were present in a total of 35 eggs from seven nests. The presence of metaphasic chromosomes indicates that cells are dividing and hence that eggs are viable.

In order to compare the egg-laying rate of virgin queens to that of mated queens, we also collected 40 mated queens after a local mating flight on 15 July 2005. Mated queens were transferred to glass vials as described above and their eggs were counted and removed two and four days after the transfer.

Results and discussion

Within two days of the treatment, the two groups of virgin queens differed substantially in terms of their reproductive

status. While none of the vials with winged virgins contained eggs, eggs were already present in 39% of the vials with dealate virgins. After two weeks, dealate virgins had produced on average 7.9 ± 6.6 eggs, which was significantly more than the 0.1 ± 0.4 eggs laid by winged virgins (Fig. 1, Wilcoxon signed rank test, $n = 12$, $p < 0.01$). In fact, only one winged virgin pair had produced eggs during these two weeks. In the third and fourth week following dealation, the difference in egg production between the two groups of virgin queens was even more pronounced. Dealate virgins laid on average 18.2 ± 11.8 eggs, which was significantly more than the 0.9 ± 2.2 eggs laid by winged virgins (Fig. 1, Wilcoxon signed-rank test, $n = 12$, $p < 0.01$). These findings demonstrate that dealation is able to induce egg-laying behavior independently of mating and insemination. Dealation possibly acts as a mechanical stimulus that initiates or accelerates flight-muscle degeneration, which, in turn, provides the resources necessary for laying eggs. It is thus possible that rapid wing-shedding after mating is an adaptive behavior that allows ant queens to boost their egg-laying rate during the first few days or weeks of the nest-founding stage.

Three lines of evidence suggest that a considerable number of eggs laid by dealate virgin queens were viable. First, we observed no trophic eggs, which, in *L. niger* appear to be round and whitish (Baroni Urbani, 1991). All eggs observed using a microscope had the shape of an ellipse and were embryonate. Second, chromosome squashes revealed active cell division in at least 29% of the eggs tested. Finally, after the end of the experiment some eggs laid by virgin queens developed into larvae.

Although artificial dealation induced egg-laying, dealate virgins laid considerably fewer eggs than mated queens. In the first four days after mating, mated queens laid on average 9.8 ± 1.7 eggs. This was significantly more than the average 2.6 ± 2.2 eggs produced by dealate virgins in a comparable time span shortly after dealation (calculated as four-day average over the first two weeks of the experiment) (Fig. 2, t-test, $df = 30$, $p < 0.001$). Even during the third and fourth week of the experiment, when dealate virgins had increased their egg production, they still laid significantly fewer eggs (four-day average over the third and fourth week after dealation = 6.1 ± 3.9 eggs) than mated queens (Fig. 2, t-test, $df = 30$, $p < 0.001$). These results suggest that in mated queens factors other than dealation also enhance the rate of egg-laying.

In addition to clarifying the role of dealation in egg-laying, our findings are of practical interest. In ants it is often impossible to discriminate male eggs from female eggs based on morphological criteria. Thus, artificial dealation is an easy method for obtaining male (haploid) eggs in great numbers. This can be useful for studies in which the brood sex ratio needs to be manipulated or for various types of developmental studies.

Another method for obtaining male eggs has been recently described by Mir et al. (2003) for the ant *Solenopsis invicta*. In the monogynous (single-queen) form of this species virgin queens spontaneously dealate and start to lay eggs upon separation from the reproductive queen (Fletcher and

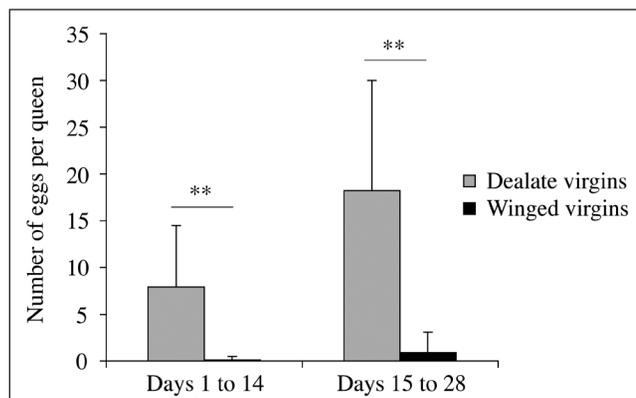


Figure 1. Number of eggs laid by artificially dealated and winged virgin *L. niger* queens over a two-week period. Depicted are mean and standard deviation calculated on the means per nest ($n = 12$). ** $p < 0.01$.

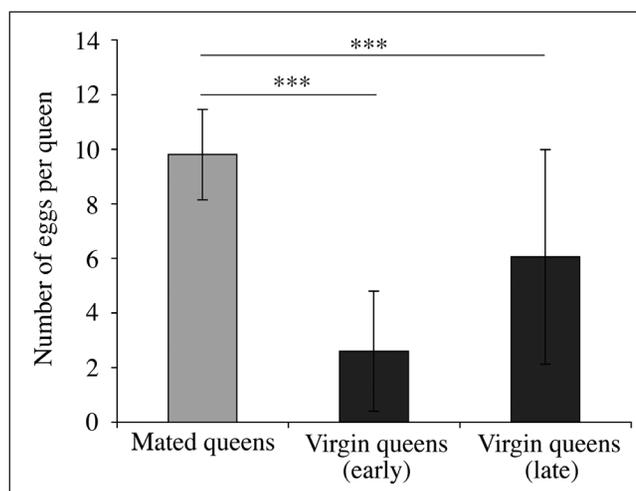


Figure 2. Number of eggs laid by mated queens versus dealate virgin queens over a four-day period. Depicted are mean and standard deviation. Sample sizes were $n = 20$ for the mated queens (means from 20 queen pairs) and $n = 12$ for the virgin queens (means from 12 nests). *** $p < 0.001$

Blum, 1981; Keller and Ross, 1999). Thus, large numbers of male eggs can be obtained by setting up experimental colonies with virgin queens and workers (Mir et al., 2003). However, the ability of virgin queens to dealate and quickly initiate reproduction in the absence of a reproductive queen is not a common feature of all ant species. In *L. niger* none of the winged virgin queens shed her wings spontaneously throughout the experiment; and even in *Solenopsis geminata*, a species closely related to *S. invicta*, virgin queens did not dealate when separated from the reproductive queen for two weeks (Vargo and Porter, 1993). In addition, *L. niger* and *S. geminata* virgins do not initiate egg-laying as quickly as *S. invicta* queens. Vargo and Porter (1993) suggest that in *S. invicta* rapid dealation and egg-laying by virgin queens may have evolved because of frequent queen loss due to periodic flooding of the nest sites. Our finding that winged *L. niger* virgins

do not readily dealate and lay eggs when isolated is consistent with this hypothesis, since this species does not live in a frequently disturbed environment.

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