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Short Communication

Successful reproduction of unmated *Tropilaelaps mercedesae* and its implication on mite population growth in *Apis mellifera* colonies

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ABSTRACT

Successful reproduction by unmated *Tropilaelaps mercedesae* is reported here for the first time. Of the eight mature daughters that did not have male mates within their natal cells, four produced both mature sons and daughters, and four produced mature daughters only. Overall, 78% of the new daughters that had no egg-laying experience, and 84% of the foundresses that had or had not laid previously reproduced. Both inoculum daughter and foundress mites were collected from tan-bodied pupae and inoculated immediately. Therefore, our results suggest that phoresy is not required for reproduction in tropilaelaps mites. The ability of virgin females to lay both males and females (deuterotoky), and to reproduce without spending a phoretic period on adult bees may play major roles in tropilaelaps mites' competitive advantage over varroa mites in *Apis mellifera* colonies.

1. Introduction

Tropilaelaps mercedesae is a serious parasite of *Apis mellifera* L. colonies in mainland Asia. All aspects of tropilaelaps mites' biology and ecology are unknown; available information about them is summarized in de Guzman et al. (2017). The fecundity of tropilaelaps mites ranged from 0.79 to 2.1 progeny in brood cells of *A. mellifera* (Buawangpong et al., 2015; Khongphinitbunjong et al., 2013; Woyke, 1987a). However, high proportions (up to 64%) of foundress tropilaelaps mites produced only one progeny while only about 1/3 of females produced at least two (Kavinseksan, 2003; Ritter and Schneider-Ritter, 1988), indications that female offspring being laid more frequently. Increased production of mature daughters only by females has been reported (Khongphinitbunjong et al., 2013). In spite of these low fecundity and high production of females without male males, tropilaelaps mites are more abundant than *Varroa destructor* in Asia (Burgett et al., 1983).

Survival of tropilaelaps mites on adult bees is about two days, but longer on pupae (> five days) (Koeniger and Muzaffar, 1988; Rinderer et al., 1994; Woyke, 1987b) and four-day old larvae (~4 weeks) (Woyke, 1994). Although considered to be a main driver to their population increase, the short survival of tropilaelaps mites on adult bees is believed to limit their distribution. Here, we compared the fecundity of foundress and daughter tropilaelaps mites, and the necessity of phoretic period for their successful reproduction.

2. Materials and methods

Inoculum mites were collected from three *A. mellifera* colonies highly infested with *T. mercedesae* in Chiang Mai, Thailand. Tan-bodied (TB) or older pupae were examined since the first daughter and son are expected to have already emerged at this time. For each infested cell, all mites were counted, and sex and stage differentiated to determine the presence or absence of a foundress, a mature adult daughter and a mature adult son. Daughters were differentiated from foundress mites based on pigmentation; foundress mites are darker in color than daughters. The number of exuviae reaffirmed the number of mature daughters or sons. We collected 100 female mites (70 foundresses and 30 daughters). Each female was eventually categorized as one of the following: (1) foundress with no male progeny within the brood cell, (2) foundress with a male progeny within the brood cell, (3) young daughter with no male sibling within the brood cell, and (4) young daughter with male sibling within the brood cell. Each female was then inoculated immediately (one person collected mites while another inoculated mites) into a frame of brood containing 4th instar larvae (L4)

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Table 1
Comparative fecundity and numbers of different stages of daughter and foundress tropilaelaps mites (mean ± standard error) that have been removed from tan-bodied (TB) or older pupae and immediately inoculated into 4th instar larvae (L4). For each parameter, no differences were detected ($P > 0.05$).

Mite status	Total survived	Progeny					Total Progeny	Ratio (M:F)
		Larva	Protonymph	Deutonymph	Male	Daughter		
Daughter, no male	14	0	0.21 ± 0.15	0.21 ± 0.11	0.50 ± 0.20	1.00 ± 0.30	1.93 ± 0.32	1:2
Daughter, male	8	0	0.13 ± 0.13	0.63 ± 0.32	0.25 ± 0.16	1.00 ± 0.38	2.00 ± 0.50	1:4
Foundress, no male	34	0.03 ± 0.03	0.29 ± 0.12	0.38 ± 0.10	0.56 ± 0.12	0.62 ± 0.14	1.88 ± 0.23	1:0.93
Foundress, male	12	0	0.08 ± 0.08	0.17 ± 0.11	0.50 ± 0.15	1.08 ± 0.29	1.83 ± 0.39	1:2.16

and capped with gelatin caps as described by de Guzman et al. (2013). We used this technique to ensure that each brood cell was not naturally infested with tropilaelaps mites. Thereafter, the test brood frames were placed in an incubator at 34.5 °C and 70% relative humidity. Mite reproduction was assessed after 10 days or when pupae were tan-bodied. The progeny was counted, and the stages and sex differentiated. The number of exuviae was also noted.

3. Results and discussion

Of the 100 females introduced, 21 foundresses and seven daughters died probably due to either old age for the foundress or death of their bee hosts. Both foundress and daughter mites produced about two progeny ($\chi^2 = 0.03$, $P = 0.998$) (Table 1). Of the 28 surviving foundresses, which were known to have reproduced at least one progeny before inoculation, 20 (71%) reproduced again and 8 (29%) did not reproduce after inoculation. Of the 21 foundresses that did not have progeny before mite inoculation, 16 (76%) reproduced and 5 (24%) remained non-reproductive. While 25–50% of the foundress mites produced both mature sons and daughters, about 20–25% did not reproduce. Six foundresses produced two males each (four of which also had a mature daughter each and two produced mature sons only). Overall, the sex ratio of progeny favored females over males (M:F = 1:2 for daughter mites and 1:1.5 for foundresses).

With the eight introduced daughters presumed to be mated because of the presence of adult male siblings in their natal cells, two produced both males and females, and three produced mature daughter and nymphs (Fig. 1). For the 14 presumably unmated daughters (no mature male mates present within their natal cells) that we inoculated into brood cells, four (29%) were able to produce both mature female and male offspring (a characteristic of a deuterotokous type of reproduction), four produced mature daughters only (29%) (a characteristic of a theletokous type of reproduction) and only one daughter (7%) produced a mature male (a characteristic of arrhenotokous type of reproduction).

Karyotype or sex determination has not been studied in the genus *Tropilaelaps* (under the family Laelapidae) although haplodiploidy is thought to be involved (Dong et al., 2017). However, the laelapid mites, *Hypoaspis aculeifer* and *Cosmolaelaps miles*, are reported arrhenotokous

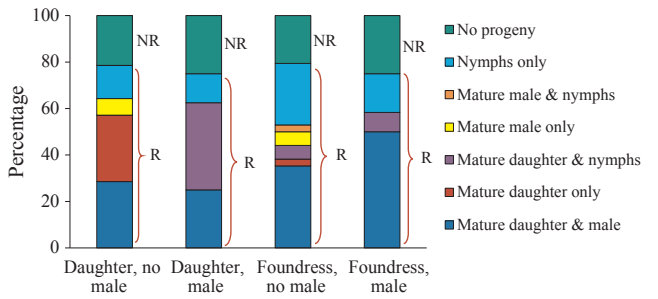

Fig. 1. Proportion of reproductive (R) and non-reproductive (NR) daughter or foundress tropilaelaps mites that have been removed from tan-bodied (TB) or older pupae and immediately inoculated into 4th instar larvae (L4).

(de Jong et al., 1981), and *Cosmolaelaps jaboticabalensis* reproduces by thelytoky (Moreira et al., 2015). Like theletoky, deuterotoky allows new females to start infestations without prior mating. High proportions of tropilaelaps females that produced mature daughters only (thus, no male mates) had been observed (Khongphinitbunjong et al., 2013). In this study, 29% of the unmated daughters produced mature daughters only. If unmated tropilaelaps mites are indeed capable of deuterotoky, then their population will continue to grow as long as suitable brood is available. Whether this ability of producing both males and females by unmated tropilaelaps females is a type of reproduction mode for these mites, however, is unclear. Given that some of the foundress and daughters (13 of the 14 surviving daughters) were collected from recapped cells, daughters may have been mated first before the males left the cells. However, the number of exuviae (molt) matched the number of new adults present within the brood cells. It is also possible that free-roaming males were able to invade opened infested brood cells, quickly mated with the new females and left before the cells were recapped by bees. However, of the 1335 adults that we collected free-roaming on the combs and some from capped brood, 94% were females. Also, about 70% of the foundress that did not lay sons to re-mate with them produced at least one progeny when re-inoculated immediately into L4. This ability of female to store sperm reduces the necessity for males or sons re-mating with mothers to replenish stored sperm. Nevertheless, whether or not the progeny produced by unmated mature daughters are reproductively functional needs further study.

It is also possible that endosymbionts are responsible for the successful reproduction of unmated tropilaelaps females. The genome of *T. mercedesae* collected from China has recently been completed. Accordingly, *T. mercedesae* is associated with a symbiotic bacterium, *Rickettsiella grylli*-like, and a part of the Wolbachia gene was also detected (Dong et al., 2017). The authors speculated that the *R. grylli*-like bacterium may have replaced Wolbachia in the past. Wolbachia is a well-known master manipulator of mite reproduction (Breeuwer and Jacobs, 1996). Whether or not *R. grylli*-like symbiont is a reproductive parasite of tropilaelaps mites has yet to be established.

Further, we inoculated both foundress and daughter mites immediately after collecting them from TB pupae. Hence, our study suggests that phoresy on adult bees is not necessary for successful reproduction in tropilaelaps mites. While varroa mites may not lay the first time they enter a cell, about 77% (17 of 22) of the new daughters (without prior egg-laying experience) laid about two progeny per female. Successful removal of tropilaelaps-infested brood has been observed in Thai *A. mellifera* (Khongphinitbunjong et al., 2013). However, the contribution of hygienic behavior on mite growth may be brief or insignificant because of the lack of phoretic period and that mating is not always required. It is likely that foundress or daughter mites exposed by hygienic behavior can re-invade new hosts and resume reproduction immediately. The results of this study may help explain the competitive advantage of tropilaelaps mites and why they are more successful parasites of *A. mellifera* than varroa mites in Asia. As far as dispersal is concerned, our observations suggest that even unmated tropilaelaps females can start a new population.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jip.2018.02.010.

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