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Thelytokous parthenogenesis in dealate queens of the ponerine ant *Anochetus shohki*

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ABSTRACT. Two orphan colonies of *Anochetus shohki* were collected from Yonaguni Island, Okinawa, Japan. Alate queens emerged from one of the orphan colonies, which had cocoons at the time of field-sampling. These queens exhibited an enlarged pronotum, which is a trait typical of non-claustral foundresses. They shed their wings in a few days, and then started laying eggs 24 days later. These eggs eventually developed into workers. After some of these workers eclosed, dissections revealed that queens possessed well-developed ovaries with six ovarioles (3 + 3) and workers lacked ovaries. Spermathecae of these queens were empty, and no males emerged during the study period. These findings indicate that uninseminated dealate queens in *A. shohki* can produce workers *via* thelytokous parthenogenesis, at least in orphan colonies. This represents the first report of thelytoky not only in *Anochetus* but also in the Ponerini tribe, and it is the second such report in the Ponerinae subfamily.

Keywords thelytoky, alate queen, Ponerinae, ovary
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INTRODUCTION

Hymenoptera exhibit a haplodiploid genetic system, where females and males arise from fertilized diploid and unfertilized haploid eggs, respectively. Ants, with the exception of some socially parasitic species, are eusocial, and their sexual reproduction are monopolized by one or a few mated queens in their colonies. However, there are 19 ant species known to exhibit thelytokous parthenogenesis, in which an unmated female produces females (Grasso et al., 2000; Masuko, 2013, 2014; Rabeling & Kronauer, 2013; Lee et al., 2018; Idogawa et al., 2021; Ito et al., 2021; Wang et al., 2023). Seventeen of these thelytokous ant species belong to Myrmicinae and Formicinae, one belongs to Dorylinae, and the other belongs to Ponerinae. Determining the number of thelytokous species and the significance of thelytoky for their life cycle and evolution presents a great challenge for myrmecologists (Rabeling & Kronauer, 2013).

The genus *Anochetus*, which belongs to the ponerine ant subfamily, comprises 115 species and is distributed worldwide, particularly in tropical and subtropical regions (Fisher, 2010; Bolton, 2022). *Anochetus* ants, along with *Odontomachus* (Ponerinae), *Myrmoteras* (Formicinae), and some of the tribe Attini (Myrmicinae), are known as trap-

jaw ants (Larabee & Suarez, 2014). Their colony size is usually less than 100 individuals (Brown, W. L., 1978). To my knowledge, 25 out of 115 *Anochetus* species are known to possess an alate/dealate queen that independently founds a new colony (Brown, W. L., 1978; Villet et al., 1991; Ito & Ohkawara, 1994; Torres et al., 2000; Gobin et al., 2006; Kugler & Ionescu, 2007; Fisher & Smith, 2008; González-Campero & Elizalde, 2008; Tinaut et al., 2011; Shattuck & Slipinska, 2012; Satria et al., 2017; Leong et al., 2018; Chen et al., 2019), whereas nine species are known to possess an ergatoid (wingless) queen that performs dependent colony foundation (Brown, W. L., 1978; Lattke, 1986; Villet et al., 1991; Torres et al., 2000; Gobin et al., 2006; Fisher & Smith, 2008; Satria et al., 2017), and both alate and ergatoid queens have been reported in three other species (Brown, W. L., 1978; Tinaut et al., 2011). Further studies are required to understand how the reproductive strategies of these species have evolved divergently.

Anochetus shohki is the only *Anochetus* species distributed in Japan and is limited to Ishigaki, Iriomote, Miyako, and Yonaguni Islands, which are located on the southwestern edge of Japan (Terayama et al., 2014; Hisasue et al., 2019). Although alate queens and workers have been found in this species, there is no further information on their reproductive biology. In this study, I report thelytokous parthenogenesis by dealate queens in an orphaned colony of *A. shohki* collected from Yonaguni Island.

MATERIALS AND METHODS

Ants

Two orphan colonies of *A. shohki* were found nesting in the soil under porous stones on June 2, 2022 on the forest floor along the Tabaru River on Yonaguni Island, Okinawa, Japan. The first colony (colony A) contained only six workers, whereas the second colony (colony B) contained 17 workers and five cocoons. These colonies were transported to the laboratory at Tamagawa University, where they were reared in plastic containers (7.7 × 10.8 × 3.2 cm) filled with moistened plaster, maintained at 25°C, and fed springtails (Collembola) three times a week.

Examination of reproductive females

After the emergence of five alate queens, newly produced eggs were found (see **Results**). On day 111, when some of the broods pupated, all surviving adults in colony B (three dealate queens and eight workers; Table 1) were painted to distinguish them from newly emerged adults. Twenty-four days later (day 135), all painted adults and three newly eclosed workers were dissected to examine their reproductive status, including the number of ovarioles and mature oocytes and the presence of yellow bodies. One dealate queen and three workers died within this 24-day period, so they were not included in the examination.

Table 1. Eclosion of alate queens and their production of offspring in colony B of *Anochetus shohki*. All dates were in 2022.

Date	Workers	Queens	Eggs	Larvae	Pupae
22 June (day 1)	17	0	0	0	5
13 July (day 22)	NA	1	0	0	0
25 July (day 34)	NA	4	0	0	0
29 July (day 38)	NA	5*	0	0	0
6 August (day 46)	NA	4	1	0	0
1 September (day 72)	NA	4	>15	3	0
10 October (day 111)	8	3	NA	NA	NA
6 November (day 138)	10†	0	3	9	16

* One alate queen was fixed (Fig. 1A). † All of these workers were parthenogenetically produced by an uninseminated dealate queen (or queens).

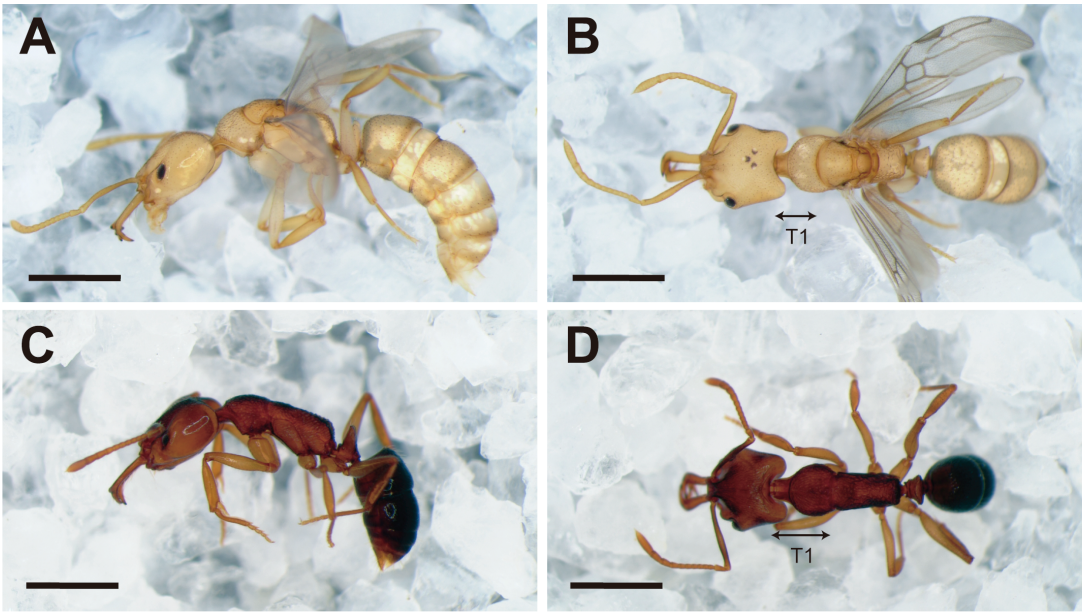

Fig. 1. Alate queen and worker of *A. shohki*. Lateral (A) and dorsal (B) views of an alate queen just after eclosion. Lateral (C) and dorsal (D) views of a worker produced by an unmated queen. Although the body color of newly eclosed queens was pale, it became darker, similar to that of workers, within a few weeks. T1 indicates the pronotum. Scale bars: 1 mm.

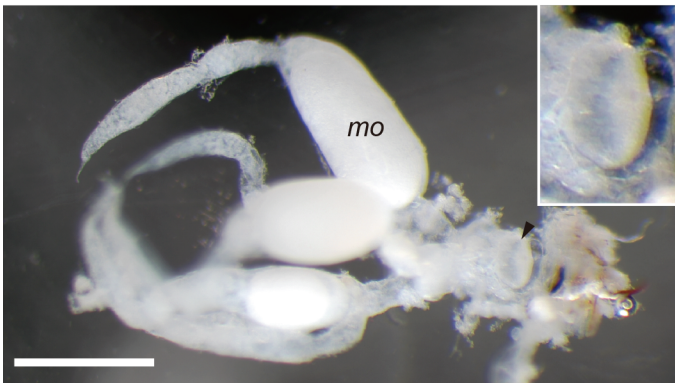

Fig. 2. Ovary of an unmated queen in *A. shohki*. This ovary was composed of six ovarioles with one mature oocyte (*mo*). A spermatheca is indicated by an arrowhead and enlarged in the inset image. Scale bar: 500 µm.

RESULTS

All five pupae originally found in colony B eclosed into alate queens between day 22 and 38 (Table 1). Their thoraces exhibited typical characteristics of non-claustral foundresses (Keller et al., 2014), with an enlarged pronotum (T1) resembling that of workers (Fig. 1). One of the queens was fixed in 70% ethanol (Figs. 1A, B), whereas the others were kept in their colony, where they shed their wings within a few days. The first egg was found on day 46, and more than 15 eggs were found on day 72. Given that males were not present during this period, the queens remained unmated.

To determine the egg layers, two dealate queens, five field-collected workers, and three newly eclosed workers were dissected and examined their reproductive status. All eight workers lacked ovaries, whereas both queens had six ovarioles (3 + 3) with one or two mature oocytes but no apparent yellow bodies (Fig. 2). The spermathecae of the queens were empty (Fig. 2). Ad-

ditionally, three live workers in colony A lacked ovaries. These results indicate that the egg layers were dealate queens. Then, all broods produced from day 46 onward in colony B developed into workers (Figs. 1C, D).

DISCUSSION

The present study indicates that unmated dealate queens of *A. shohki* can parthenogenetically produce workers, at least, in an orphan colony. Failure to observe the yellow bodies could be attributed to the relatively brief duration (approximately three months) during which the queens were laying eggs. This study represents the first report of thelytokous parthenogenesis not only in *Anochetus* but also in Ponerini, the major tribe that includes >95% of Ponerinae species (taxonomy based on the AntCat 2023: https://antcat.org/catalog/430052), and the second report in Ponerinae after Heinze & Hölldobler (1995) described thelytoky in *Platythyrea punctata*, which belongs to the tribe Platythyreini. However, because no queen-right colonies were found in the field, it remains unknown whether the reproduction of alate queens is via obligatory thelytoky or is mainly sexual but with facultative thelytoky.

In contrast to previous reports on alate queens, no males were found in any habitat of *A. shohki* (Terayama, 1996; Hisasue et al., 2019), providing circumstantial evidence that dealate queens in this species may reproduce thelytokously. Males have been reported in 26 *Anochetus* species (Brown, W. L., 1978; Torres et al., 2000; Kugler & Ionescu, 2007; Fisher & Smith, 2008; Satria et al., 2017; Chen et al., 2019). Although occasional male production has been observed in two other thelytokous ants, *Ooceraea biroi* (Kronauer et al., 2012) and *Pristomyrmex punctatus* (Itow et al., 1984; Yamada & Eguchi, 2016), many reports on *Anochetus* males suggest that sexual reproduction is more common than thelytokous reproduction in this genus (Torres et al., 2000).

Thelytokous parthenogenesis in ants is classified into three types: (i) unmated workers producing workers by thelytoky, (ii) mated queens producing workers sexually but new queens by thelytoky, and (iii) queens producing sterile workers and new queens by thelytoky (Himler et al., 2009). The results of the present study support the third type of thelytoky in *A. shohki*. Thelytoky in *P. punctata*, where workers produce workers parthenogenetically (Heinze & Hölldobler, 1995), corresponds to type (i). Therefore, *A. shohki* is suspected to be the first case of type (iii) thelytoky in the subfamily Ponerinae. However, whether thelytoky occurs in field-collected colonies and/or is limited to the Yonaguni population remain unknown. Further studies investigating the reproductive strategy of *A. shohki* using queen-right colonies collected from Yonaguni Island as well as other islands would help fill these knowledge gaps and contribute to our understanding of reproduction in *Anochetus* species.

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REFERENCES

Bolton B, 2022. An online catalog of the ants of the world. Available from http://antcat.org. (accessed on 12 Nov 2022)..

Brown, W. L. J, 1978. Contributions toward a reclassification of the Formicidae. Part VI. Ponerinae, tribe Ponerini, subtribe Odontomachiti. Section B. Genus Anochetus and bibliography. *Studia Entomologica* 20: 549–638.

Chen Z, Yang Z, Zhou S, 2019. Review of the ant genus *Anochetus* Mayr, 1861 (Hymenoptera, Formicidae) from China, with revival of the valid status of *Anochetus gracilis*. *Journal of Hymenoptera Research* 68: 49–74.

Fisher BL, 2010. Biogeography. In: Lach, L., Parr, C.L., Abbott, K.L. (Eds.), Ant Ecology. Oxford University Press, Nex York, pp. 18–37.

Fisher BL, Smith MA, 2008. A revision of Malagasy species of *Anochetus* Mayr and *Odontomachus* Latreille (Hymenoptera: Formicidae). *PLoS ONE* 3: 1–23.

Gobin B, Ito F, Peeters C, Billen J, 2006. Queen-worker differences in spermatheca reservoir of phylogenetically basal ants. *Cell and Tissue Research* 326: 169–178.

González-Campero MC, Elizalde L, 2008. A new species of *Anochetus* (Hymenoptera: Formicidae: Ponerini) from Argentina and Paraguay, associated with a leaf cutter ants. *Entomotropica* 23: 97–102.

Grasso DA, Wenseleers T, Mori A, Le Moli F, Billen J, 2000. Thelytokous worker reproduction and lack of *Wolbachia* infection in the harvesting ant *Messor capitatus*. *Ethology Ecology & Evolution* 12: 309–314.

Heinze J, Hölldobler B, 1995. Thelytokous parthenogenesis and dominance hierarchies in the ponerine ant,*Platythyrea punctata*. *Naturwissenschaften* 82: 40–41.

Himler AG, Caldera EJ, Baer BC, Fernández-Marín H, Mueller UG, 2009. No sex in fungus-farming ants or their crops. *Proceedings of the Royal Society B: Biological Sciences* 276: 2611–2616.

Hisasue Y, Tsuji N, Nishiya K, 2019. Records of *Anochetus shohki* Terayama, 1996 from Iriomote Island and Yonaguni Island, Ryukyus, Japan (Hymenoptera: Formicidae). *Pulex* 98: 788–789.

Idogawa N, Sasaki T, Tsuji K, Dobata S, 2021. Comprehensive analysis of male-free reproduction in Monomorium triviale (Formicidae: Myrmicinae). *PLoS ONE* 16: e0246710.

Ito F, Ohkawara K, 1994. Spermatheca size differentiation between queens and workers in primitive ants - Relationship with reproductive structure of colonies. *Naturwissenschaften* 81: 138–140.

Ito F, Makita S, Nakao H, Hosokawa R, Kikuchi T, Yamane S, 2021. Thelytokous parthenogenesis by dealate queens in the myrmicine ant *Monomorium hiten* distributed in Nansei Islands, western Japan, with description of the male. *Asian Myrmecology* 14: 1–9.

Itow T, Kobayashi K, Kubota M, Ogata K, Imai HT, Crozier RH, 1984. The reproductive cycle of the queenless ant *Pristomyrmex pungens*. *Insectes Sociaux* 31: 87–102.

Keller RA, Peeters C, Beldade P, 2014. Evolution of thorax architecture in ant castes highlights trade-off between flight and ground behaviors. *eLife* 3: e01539.

Kronauer DJC, Pierce NE, Keller L, 2012. Asexual reproduction in introduced and native populations of the ant *Cerapachys biroi*. *Molecular Ecology* 21: 5221–5235.

Kugler J, Ionescu O, 2007. *Anochetus bytinskii*, a new ant species from Israel (Hymenoptera: Formicidae). *Israel Journal of Entomology* 37: 287–298.

Larabee FJ, Suarez A V, 2014. The evolution and functional morphology of trap-jaw ants (Hymenoptera: Formicidae). *Myrmecological News* 20: 25–36.

Lattke JE, 1986. Two new species of neotropical *Anochetus* Mayr (Hymenoptera: Formicidae). *Insectes Sociaux* 33: 352–358.

Lee C-C, Hsu S-F, Yang C-C (Scotty), Lin C-C, 2018. Thelytokous parthenogenesis in the exotic dacetine ant *Strumigenys rogeri* (Hymenoptera: Formicidae) in Taiwan. *Entomological Science* 21: 28–33.

Leong CM, Tsai WH, Terayama M, Shiao SF, Lin CC, 2018. Description of a new species of the genus *Anochetus* Mayr (Hymenoptera: Formicidae) from Orchid Island, Taiwan. *Journal of Asia-Pacific Entomology* 21: 124–129.

Masuko K, 2013. Thelytokous parthenogenesis in the ant *Strumigenys hexamera* (Hymenoptera: Formicidae). *Annals of the Entomological Society of America* 106: 479–484.

Masuko K, 2014. Thelytokous parthenogenesis in the ant *Myrmecina nipponica* (Hymenoptera: Formicidae). *Zoological Science* 31: 582–586.

Rabeling C, Kronauer DJC, 2013. Thelytokous parthenogenesis in eusocial Hymenoptera. *Annual Review of Entomology* 58: 273–292.

Satria R, Viet BT, Eguchi K, 2017. New synonymy and redescription of *Anochetus mixtus* Radchenko, 1993, and distinction from the other members of the *Anochetus rugosus* group (Hymenoptera: Formicidae: Ponerinae). *Asian Myrmecology* 9: e009006.

Shattuck SO, Slipinska E, 2012. Revision of the Australian species of the ant genus *Anochetus* (Hymenoptera: Formicidae). *Zootaxa* 28: 1–28.

Terayama M, Kubota S, Eguchi K, 2014. Encyclopedia of Japanese ants (In Japanese). Asakura Shoten, Tokyo.

Terayama M, 1996. Taxonomic studies on the Japanese Formicidae, part 2. Seven genera of Ponerinae, Cerapachyinae and Myrmicinae. *Nature and Human Activities* 1: 32.

Tinaut A, Bensusan K, Guillem R, 2011. Redescription of the queen of *Anochetus ghilianii* (Spinola, 1851) (Hymenoptera, Formicidae). *Boletín de la Asociación Española de Entomologia* 35: 157–167.

Torres JA, Snelling RR, Jones TH, 2000. Distribution, ecology and behavior of *Anochetus kempfi* (Hymenoptera: Formicidae) and description of the sexual forms. *Sociobiology* 36: 505–516.

Villet MH, Crewe RM, Duncan FD, 1991. Evolutionary trends in the reproductive biology of ponerine ants (Hymenoptera: Formicidae). *Journal of Natural History* 25: 1603–1610.

Wang C, Sung P-J, Lin C-C, Ito F, Billen J, 2023. Parthenogenetic reproduction in *Strumigenys* ants: an update. *Insects*.

Yamada A, Eguchi K, 2016. Description of the male genitalia of *Pristomyrmex punctatus* (Smith, 1860) (Hymenoptera, Formicidae, Myrmicinae). *Asian Myrmecology* 8: 87–94.