Thelytokous parthenogenesis by dealate queens in the myrmicine ant *Monomorium hiten* distributed in Nansei Islands, western Japan, with description of the male

Fuminori Ito[1*], Shogo Makita[1], Hirotaka Nakao[1], Ryota Hosokawa[1], Tomonori Kikuchi[2] and Seiki Yamane[3]

[1]Faculty of Agriculture, Kagawa University, Ikenobe, Miki, Japan
[2]Faculty of Agriculture, Marine Biosystems Research Center, Chiba University, Kamogawa, Japan
[3]Haruyama-cho, Kagoshima, Japan

*Corresponding author: ito.fuminori@kagawa-u.ac.jp

ABSTRACT. Thelytokous reproduction by dealate queens in *Monomorium hiten* Terayama, 1996, collected from four islands of the Nansei Islands (Okinawa Prefecture, Japan) was confirmed in the field and laboratory. Dissection revealed that all dealate queens (N = 38) found in nine field collected colonies were uninseminated. Orphan colonies reared in the laboratory produced alate queens and they laid eggs from which workers or alate queens emerged. Alate queens reared in isolation produced nanitic workers without food supply. Two laboratory colonies produced a total of four males; their morphology is described.

Keywords	Thelytoky, claustral colony foundation, Okinawa, ovary
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INTRODUCTION

Sexual reproduction is widespread in organisms, however, reproduction without males has evolved in several groups independently (Simon et al. 2003). Investigations of the ecological and biological characteristics of such organisms is important for understanding the evolution and maintenance of sex. In ants, knowledge of thelytokous parthenogenesis has accumulated in the past 10 years. So far, 14 species are reported (Rabeling & Kronauer 2013; Masuko 2013, 2014; Lee et al. 2017), of which nine species, asexual populations of *Mycocepurus smithii* (Forel, 1893), the Manazuru population of *Myrmecina nipponica*

Wheeler, 1906, *Monomorium triviale* Wheeler, 1906, *Ooceraea biroi* (Forel,1907), *Platythyrea punctata* (Smith, 1858) in Caribean Islands, *Pristomyrmex punctatus* (Smith,1860), *Strumigenys membranifera* Emery, 1869, *S. hexamera* (Brown, 1958), and *S. rogeri* Emery, 1890, are known as "no male" ants, although some species occasionally produced males but mated individuals are rarely collected (Itow et al.1984; Tsuji & Yamauchi 1995; Rabeling et al. 2009; Ito et al. 2010; Gotoh et al. 2012; Masuko 2013, 2014; Lee et al. 2017). Among the nine species, the queen caste is absent in the Caribbean *P. punctata, P. punctatus* and *O. biroi,* whereas the remaining species have alate or ergatoid queens performing

thelytokous parthenogenesis. To understand the significance of this reproductive system, further knowledge from other ant species is necessary. In this paper, we report on the thelytokous partheno-genesis by dealate queens in *Monomorium hiten* Terayama, 1996, distributed in the Nansei Islands of Japan.

MATERIALS AND METHODS

Ants
Monomorium hiten is known from southern Japan, Taiwan, Vietnam and China (Guénard et al. 2017; antmaps.org 2021), but it remains as a rare species. In the present study, nine colonies were collected: six colonies from Yonagunijima and one colony each from Okinawajima, Ishigakijima, and Iriomotejima (Fig. 1). Nests were found in dead branches or dead acorns fallen on the floor of secondary *Lucidophyllous* forests.

Thelytokous reproduction by queens
One of the nine colonies (FI15-98) collected in Yonagunijima in November of 2015 was reared in a styrene container (10.0 x 6.5 x 2.8 cm) with a bottom layered of plaster of Paris for keeping the humidity high. A slide glass was put over a depression (ca 1.5 cm x 3 cm with ca 5 mm depth) excavated in plaster floor, which served as a nest chamber. The ants were kept in the laboratory at 20~28℃. A few mealworm fragments and diluted honey were given as prey twice or thrice per week. After three months' culture, in February of 2016, all queens, a few hundred workers and the brood of FI15-98 were transferred to a new nest, leaving ca 500 workers and several immatures in the original nest. Both the queenright and orphan (Orphan 1) colonies were kept under the same conditions. This procedure was repeated along the increase of the original colony, producing further three orphan colonies (Orphan-2, 3, and 4). After separation, Orphan-1 produced new
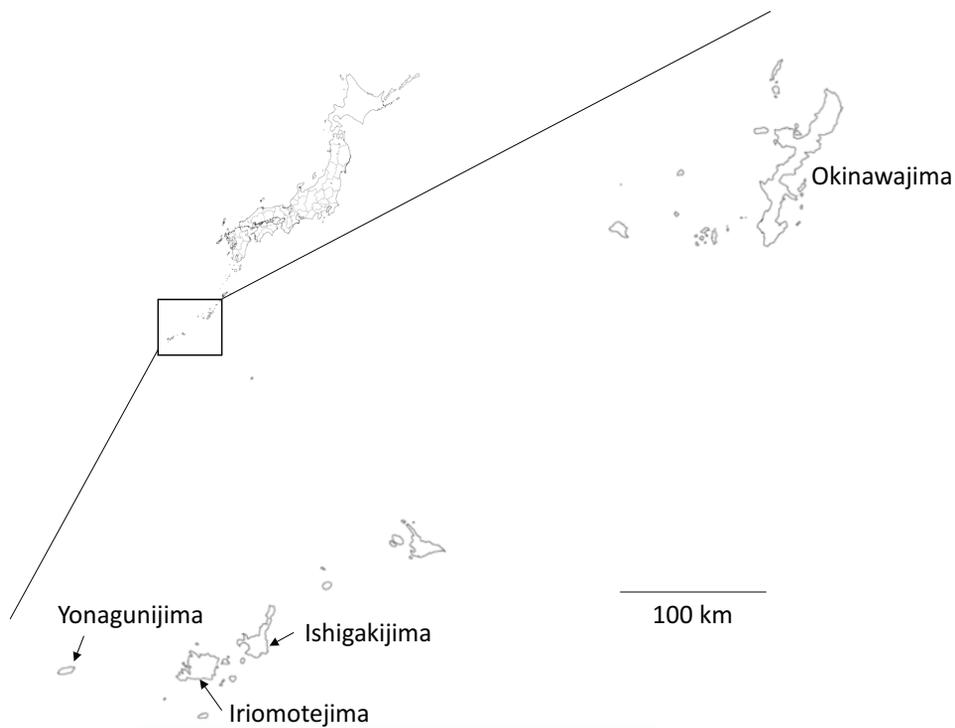
Fig.1. Collecting sites of *Monomorium hiten* in Japan.

queens that laid several eggs from which workers emerged. This colony was then split into two colonies in September of 2016: one had new dealate queens (Orphan-1-1) while the other had no queens (Orphan-1-2), and both were reared in the laboratory until February of 2017.

All field-collected queens were dissected under a binocular microscope to check for their reproductive condition immediately after collection in the field or after the end of laboratory culture. After all queens of colonies FI18-190 and SM18-1 were dissected, the remaining workers and brood were reared in the same manner as for colony FI15-98.

Colony foundation
Queens of some polygynous ant species can't disperse and found new colonies independently, even if they have wings (e.g. *Linepithema humil* (Mayr, 1868), Passera 1994). To confirm the ability of independent colony foundation by queens,

eight alate queens produced in Orphan-1-2 were kept individually in a small glass tube (φ5 mm x 46 mm) plugged by a moist cotton ball. The tubes with queens were kept in an incubator (24 degree 12L 12D). Prey was not given. The number of immatures produced by each queen was recorded every two or three days. The head width of workers (N = 32) in a mature colony and these (N = 11) in founding colonies established in the laboratory was measured under a binocular microscope.

Reproduction by workers
To confirm the reproductive ability of workers, 20 workers of FI15-98 were carefully dissected to check for the absence or presence of ovarioles. In addition, 50 workers and 20 larvae were isolated from this colony, and kept as described above. The occurrence of eggs was checked a few times a week for two months.

Fig. 2. A male, queens and workers of *Monomorium hiten*.

Table 1. Colony composition of *Monomorium hiten* collected in the Nansei Islands, Japan. The colonies are listed in order of colony size from smallest to largest.

Colony code	Locality	Date collected	No. queens (mated)	No. workers
FI20-62	Yonagunijima	2020.03	1 (0)	13
FI18-190	Iriomotejima	2018.11.	3 (0)	22
HN17-1	Okinawajima	2017.04	2 (0)	ca 40
TK-1	Yonagunijima	2005.01	10 (0)	57
FI20-61	Yonagunijima	2020.03	5 (0)	66
SM18-1	Ishigakijima	2018.09.	4 (0)	ca 100
FI20-35	Yonagunijima	2020.03	4 (0)	ca 400
FI15-98	Yonagunijima	2015.11	5 (0)	ca 500
FI20-36	Yonagunijima	2020.03	4 (0)	530

Description of the male

During laboratory culture, two colonies produced a few males. Male morphology is for the first time described for *M. hiten* (see Appendix).

RESULTS

Thelytokous reproduction by queens

All but one colony had multiple dealate queens (Table 1). Colony size varied from 13 to 530 workers. All dealate queens (N = 38) from nine colonies were uninseminated. A spermatheca was found in all dissected queens; however, none of these contained sperm. The total number of ovarioles ranged from 16 to 26. All queens had developed oocytes and no remarkable difference was found in the ovarian development of coexisting queens of the same colony.

The colony Orphan-1 from FI15-98 produced several workers and four alate queens that emerged in March 2016. Males did not emerge in this orphan colony. These four alate queens subsequently shed their wings inside the nest chamber, and started to lay eggs which developed into workers. Orphan-1 was split into two colonies, of which one had four dealate queens with several workers and brood (Orphan-1-1) and the other contained only workers and brood (Orpahn-1-2). Orphan 1-1 continued to produce workers and alate queens, and in February 2017, it contained 99 dealate queens and ca 1000 workers. On the other hand, Orphan-1-2 produced 17 alate queens

and several workers, and these queens shed their wings and laid eggs that developed into workers.

Each of the other three orphan colonies (Orphan-2, 3, and 4) produced five to 20 alate queens, and these new queens laid eggs after dealation, and all these colonies produced several alate queens and numerous workers during three years. One male emerged in Orphan-2 (Fig. 2).

Production of workers from eggs laid by unmated queens that emerged in the laboratory was also confirmed for the other colonies collected in Iriomotejima (FI18-90) and Ishigakijima (SM18-1). Colony FI18-190 produced three males over six months.

Independent colony foundation by queens

Of eight alate queens kept individually in small glass tubes, six queens laid eggs and produced workers (Fig. 3). The duration of each developmental stage, calculated from the appearance of the first individual of each developmental stage, is shown in Table 2. In total, it took 48 days to complete development from egg stage to emergence of adult workers at 24°C. When the first worker emerged, the number of remaining pupae was 0 to three (average 1.67 ± 1.21SD, n = 6), thus queens could produce on average 2.7 workers during claustral colony foundation (Table 3). Worker body size produced by claustral queens was significantly smaller than that in mature colonies (Fig. 4, Welch two-sample t-test, t = 8.69, df = 20.26, p < 0.0001).
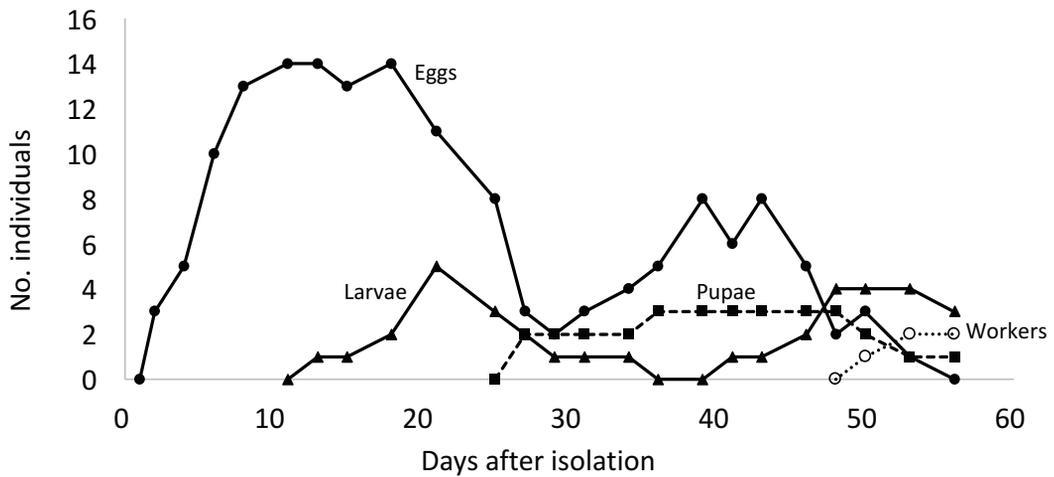
Fig. 3. Development of an incipient colony founded by a single queen of *Monomorium hiten* produced in Orphan 1- 2 of FI15-98. A representative example is shown.
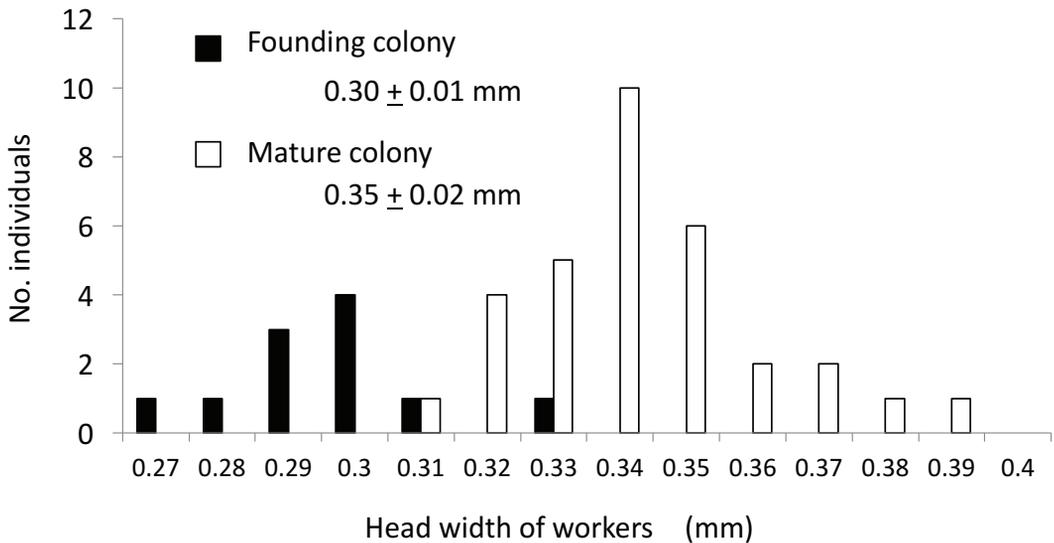
Fig. 4. Head width distribution of workers in founding and mature colonies.

Table 2. Days for development of each developmental stage under 24 degree, based on the data from six founding colonies. Sample size of all developmental stage was six.

	Average days for development
Eggs	12.1 ± 2.2
Larvae	16.7 ± 3.5
Pupae	19.8 ± 1.3
Total	48.3 ± 3.6

Worker sterility

We did not find ovaries in dissected workers (N = 20). Also, when 50 workers were kept with 20 larvae, they never laid eggs. These results indicate the complete sterility of *M. hiten* workers as in other *Monomorium* species.

Table 3. Number of brood produced by queens during claustral colony foundation. Alate queens emerged in Orphan 1- 2 of FI15-98 were isolated.

	No. individuals when the first worker emerged			
Queen code	Eggs	Larvae	Pupae	Workers
Cl 3	1	5	3	1
Cl 4	3	4	2	1
Cl 5	0	1	1	1
Cl 6	3	3	3	1
Cl 7	0	5	1	1
Cl 8	0	0	0	1

DISCUSSION

Our results demonstrate thelytokous parthenogenesis by dealate queens occurs in *Monomorium hiten,* at least for the population distributed in the four islands of the Nansei Islands. Four males emerged in the laboratory. Such occasional male production has been known in the thelytokous species *Pristomyrmex punctutus* (Itow et al. 1984; but mated individuals have never been collected). Further study of *M. hiten* is necessary to understand why males are produced in this species. In the genus *Monomorium*, *M. triviale* is also known as a thelytokous species in which virgin ergatoid queens reproduce (Gotoh et al. 2012). Queen morphology differs between these species, but their nest site and habitat preferences seems to be similar: both are found in dead plant remains present on relatively sunny forest floors.

Rabeling and Kronauer (2013) discussed that six of eleven thelytokous ants (including species with males) are "invasive species" and thelytoky is adaptive for such "invasive species" because these ants may suffer inbreeding depression and the inability to find males. The term "invasive ants" is usually used for species which have a negative impact on native biota (Holway et al. 2002). However, Rabeling and Kronauer (2013) use "invasive" for species that often invade via human commerce and can establish in new places, namely, so-called "exotic species". They considered *M. triviale* as an "invasive" species. But whether *M. triviale* and *M. hiten* are exotic species or not remains uncertain, because their relatively limited distribution seems different from typical exotic species, which have a

widespread distribution. The habitat preference of both species also differs from typical exotic ants. In our experience, the colonies of *M. triviale* and *M. hiten* have only been found inside or at the edge of forests, whereas thelytokous exotic species such as *O. biroi, S. membranifera,* and *S. rogeri* are common in urban parks (Ito et al. 2010; Kitahiro et al. 2014; Lin pers. comm.).

Besides the benefits of thelytoky for exotic ants pointed out by Rabeling and Kronauer (2013), Ito *et al.* (2010) discussed that thelytokous reproduction without males seems to be adaptive for living in open, disturbed habitat, where exotic species found. This is because the high intrinsic rate of natural increase through the reduction of male production is favored in such disturbed habitat (MacArthur & Wilson1967; Tsuji & Tsuji 1986). However, habitat preference of the two thelytokous *Monomorium* species indicates that this case may not fit the argument made by Ito *et al.* (2010).

In the present study, *M. hiten* colonies easily produced many alate queens and workers in the laboratory. Alate queens shed wings in the nest chambers, after which they established polygynous colonies. If this happens in the field, the colony density could be locally very high, because of subsequent colony fission. However, so far, we collected only nine colonies, despite a lot of effort to discover *M. hiten* in the Nansei Islands. These facts indicate that some ecological factors may inhibit the increase of colony size and/or colony fission in the field. This situation is quite different from the case of *M. triviale* because this species is locally very abundant where it was found (Ito, unpublished). One of the remarkable differences

between the two species is in their queen morphology: alate queens of *M. hiten* may be able to fly and start a new colony independently as shown in the present study, whereas *M. triviale* queens are wingless, which makes colony fission the obligate way for colony foundation. This difference of queen morphology may be related to the difference in local abundance between these two thelytokous species.

Monomorium is one of the largest genera, containing 296 valid species (Bolton 2021), some of which are very common species. Some species are well known as tramp species (Passera 1994). However, biological knowledge is still insufficient except for the notorious pest species, *M. pharaonis* (Linnaeus, 1758) (e.g., Børgesen 2000; Wetterer 2010a; Boonen & Billen 2016) and the tramp species, *M. floricora* (Jerdon, 1851) (e.g., Way & Bolton 1997; Ozaki et al. 2000; Eow et al. 2004; Wetterer 2010b). Knowledge of reproductive structure and ecology in several species of *Monomorium* will help develop our understanding of reproduction without males in ants.

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APPENDIX

Description of male of *Monomorium hiten* (Fig. 5)

Structure. Head in full-face view as long as broad (excluding eyes), round, with weakly convex posterior margin and round posterolatera corner; clypeus slightly broader than long, demarcated from frons by distinct sulcus, with straight anterior margin, in profile rather strongly and roundly convex; eyes large, strongly bulging, in full-face view with anteriorly convergent inner margins; lateral ocelli located close to posterior margin of head, with distance between lateral ocelli twice as long as their diameter; Mandible narrow, with parallel basal and outer (ventral) margins and three teeth on masticatory margin, basal margin weakly serrate; antenna 13-merous, with scape slightly shorter than pedicel and first flagellar segment combined, and all flagellomeres slightly longer than broad. Mesosoma, in dorsal view, elongate, ca. 1.75 times as long as broad, broadest in front of wing bases; mesoscutum occupying almost half of mesosomal dorsum; pronotum almost hidden beneath mesoscutum; scutoscutellar sulcus posteriorly strongly concave medially; metanotum short, distinctly demarcated anteriorly and posteriorly with narrow but deep sulci. Mesosoma, in profile, 1.7 times as long as high; pronotum with steep anterior slope and large lateral face; mesoscutum with weakly convex dorsal outline and steep anterior slope; mesopleuron large with oblique mesopleural sulcus separating dorsal area from larger ventral area; metapleuron separated from mesopleuron by broad sulcus, but vaguely delimited from propodeum; separation of upper metapleuron from lower metapleuron distinct; dorsal outline of propodeum gently sloping posteriad, rounding into steeper posterior slope. Petiole with short pedicel, in profile longer than high; its node distinctly tapering apically with rather sharp apex, with gently sloping anterior slope and steeper and shorter posterior slope; venter of petiolar sternite weakly convex; in frontal view node widened toward apex, with straight dorsal (apical) margin and round dorsolateral corner; postpetiole in dorsal view as broad as and much shorter than petiole, in profile as high as petiole, with anterior and posterior margins rather parallel; its node in dorsal view broader than long; subpostpetiolar process low, with sharply defined anterior and posterior extremities. Gaster elongate, broadest at first tergite, then gradually tapering posteriad; with gaster in profile anterior face of first tergite low, detached from posterior margin of postpetiole.

Sculpture. Body extensively smooth and shiny; eye encircled with narrow belt of dense short carinae; area between mandibular base and anterior margin of eye coarsely sculptured; antenna except for scape and pedicel entirely densely microsculptured and mat; lateral and posterior face of postpetiole sculptured and mat.
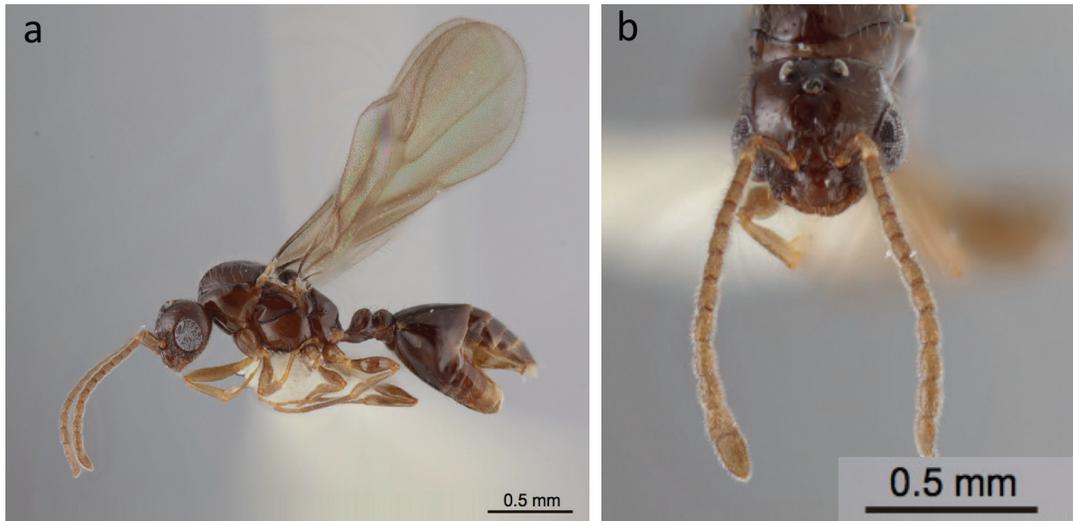
Fig. 5. Habitus (a) and head (b) of a male.

Pilosity and color. Dorsum of body with relatively sparse standing hairs; scape with suberect hairs (distinctly shorter than diameter of scape) on its anteroventral margin, those on dorsal margin much shorter; petiole ventrally without standing hairs but postpetiole ventrally with a few standing hairs; flagellomeres with dense suberect pubenscence; dense appressed short hairs present on outer faces of tibiae of all legs. Body brown to dark brown, gaster darker than head and mesosoma; legs brown to light brown, with tibiae slightly yellowish.

Wing venation. Forewing: pterostigma distinct; costal and subcostal veins close to each other; median, marginal and first submarginal cells closed; second submarginal cell incompletely closed; first discoidal cell absent; submedian and second discoidal cell not separated. Hind wing: venation much reduced, without distinctly closed cell.