Naturwissenschaften (2010) 97:725–728
DOI 10.1007/s00114-010-0688-5

ORIGINAL PAPER

Thelytokous parthenogenesis by queens in the dacetine ant *Pyramica membranifera* (Hymenoptera: Formicidae)

Fuminori Ito · Yoshifumi Touyama · Ayako Gotoh · Shungo Kitahiro · Johan Billen

Received: 12 January 2010 / Revised: 4 March 2010 / Accepted: 26 May 2010 / Published online: 16 June 2010

Abstract Thelytokous parthenogenesis in which diploid females are produced from unfertilized eggs, was recently reported for some ant species. Here, we document thelytokous reproduction by queens in the polygynous species *Pyramica membranifera*. Queens that emerged in the laboratory were kept with or without workers under laboratory conditions. Independent colony founding was successful for a few queens if prey was provided. All artificial colonies, which started with a newly emerged queen and workers produced new workers and some of the colonies also produced female sexuals. Some of the female sexuals shed their wings in the laboratory and started formation of new polygynous colonies. Workers had no ovaries and thus, were obligatorily sterile.

Keywords Thelytoky · Parthenogenesis · Polygyny · Ants

Introduction

In Hymenoptera, the majority of species show a haplodiploid sex-determining system. Females originate from fertilized eggs and are diploid while males develop from unfertilized eggs through arrhenotokous parthenogenesis (Cook and Crozier 1995). In a few species, however, unmated females may produce diploid daughters from unfertilized eggs through thelytokous parthenogenesis. In ants, recent studies using genetic analysis have resulted in rapid progress of the knowledge of thelytokous parthenogenesis and three distinct reproductive strategies of asexuality occur (Himler et al. 2009; Rabeling et al. 2009): (1) facultatively thelytokous parthenogenesis, in which males contribute to produce workers, but daughter queens are clonally produced by thelytoky; (2) worker reproduction of females with a trend towards queen loss; (3) strict queen thelytoky with sterile workers. So far, (1) is known from *Cataglyphis cursor, Wasmania auropunctata*, and *Vollenhovia emeryi* (Pearcy et al. 2004; Fournier et al. 2005; Kobayashi et al. 2008) and (2) has been shown in *Cerapachys biroi*, *Messor capitatus*, *Platythyrea punctata*, and *Pristomyrmex punctatus* (Itow et al. 1984; Tsuji 1988; Tsuji and Yamauchi 1995; Heinze and Hölldobler 1995; Schilder et al. 1999; Grasso et al. 2000; Hartmann et al. 2005). The third type, (3), is known only in *Mycocepurus smithii* (Himler et al. 2009; Rabeling et al. 2009) and possibly in *Monomorium triviale* (Sasaki and Tsuji, pers. comm.). Although the number of ant species showing thelytokous parthenogenesis has almost doubled during the last 10 years, the phenomenon is still not well documented and understood. Therefore, more research of other species is needed to better understand the significance of this peculiar reproductive system. Here, we report on the occurrence of thelytokous parthenogenesis by queens in *Pyramica membranifera* in western Japan. Furthermore, we briefly review the habitat preference of thelytokous parthenogenesis species found in Japan and discuss its significance.

Communicated by Jürgen Heinze

F. Ito (✉) · A. Gotoh · S. Kitahiro
Faculty of Agriculture, Kagawa University,
Ikenobe, Miki,
Kagawa 761-0795, Japan
e-mail: ito@ag.kagawa-u.ac.jp

Y. Touyama
Shichifuku Co., Ltd.,
Hiroshima 732-0001, Japan

J. Billen
Zoological Institute, Katholieke Universiteit Leuven,
Naamsestraat 59, box 2466, 3000 Leuven, Belgium

Materials and methods

Ants

Pyramica membranifera, is known as a tramp species, which has been spread through human trade through the warmer regions of the world (Brown and Wilson 1959). The species is well adapted to open areas and often inhabits cultured areas. Investigation of ant fauna in several sites in western Japan indicated that they are never found inside forests and exclusively collected in urban parks in residential areas (Kitahiro et al., unpublished). They nest in the soil and are polyphagous arthropod predators (Wilson 1953). Queens have been collected and described (Wheeler 1933), however, males have never been described. Although they are not a common species in Japan, they are found from the western part of Honshu to Kyushu. So far only their predatory behavior and their prey items have been reported (Wilson 1953).

Investigation of colony composition

In Takamatsu, Ozu (Shikoku Island), Hatsukaichi, Hiroshima (Honshu Island), and Kagoshima (Kyushu Island), *P. membranifera* colonies were collected in urban parks and on the campuses of Kagawa University and Kagoshima University between July and October from 2001 to 2006 (Table 1). Colony composition was assessed just after sampling for all but one colony (FI01-260). Just after sampling, 15 queens contained in these colonies were dissected to check their reproductive status. Queens were dissected under a dissecting microscope and ovary development (number of developing and mature oocytes) and the absence or presence of yellow bodies was recorded. Then, ovaries with spermathecae were removed by forceps. The reproductive organs were put into a drop of water on a slide glass and were covered with a cover slip. The absence or presence of sperm in the spermathecae was confirmed under a light microscope. After the experiments mentioned below, all other wingless queens in the field-collected colonies were also dissected.

Colonies were kept in artificial nests (10.0×6.2×2.7 cm) and were placed in an incubator (25°C, 14L10D) for the following experiments. The bottom of each nest box was covered with plaster and brood chambers (ca. 3.0×3.0×0.3 cm) were excavated in the plaster floor. The chambers were covered with glass plates. For each experiment, we used plaster nests with nest size of 8.5×5.5×1.8 cm or 7.2×5.0×1.2 cm with brood chambers ca. 2.0×2.0×0.3 cm in size.

Experiment 1: independent colony foundation

To examine whether queens can independently start a colony, 12 alate (winged) queens that emerged in colony FI04-01 during laboratory culture were kept individually in artificial nests. Five of the 12 queens were kept without prey to check for the possibility of claustral colony foundation. The remaining seven queens were kept under a semi-claustral condition and were offered a few collembola two or three times per week. Colony development was checked three times per week. A solitary dealated (wingless) queen collected in Kagoshima was also kept with a continuous prey supply.

Experiment 2: queen thelytoky

To check for thelytokous production of workers and queens by virgin queens, a dealate queen that emerged in the laboratory and has shed its wings in the nest chamber and 15 workers were kept in an artificial nest, and the colony development was monitored for 1 year. Collembola were provided as prey two or three times per week. The colony development was checked three times per week. Queens that emerged from the colonies in the laboratory were used for this experiment, ten queens from colony FI04-01, five from FI01-260, and two from FI04-11. Furthermore, in colony FI06-30, two queens collected in the field were kept separately with several workers, and brood production was observed for 1 year.

Table 1 Colony composition of *P. membranifera*

Colony code	Locality	Latitude and longitude	No. of individuals		
			Dealate queens	Workers	Alate queens
FI01-260	Takamatsu	34.20.34 N, 134.02.15 E	5 (2)	ca. 50	0
FI04-1	Hiroshima	34.20.26 N, 132.27.46 E	14 (8)	333	92
FI04-4	Hiroshima	34.20.26 N, 132.27.46 E	2	5	1
FI04-10	Ozu	33.30.09 N, 132.33.00 E	3 (3)	92	0
FI04-11	Ozu	33.30.09 N, 132.33.00 E	6 (2)	241	0
FI06-30	Hatsukaichi	34.18.45 N, 132.18.06 E	2	320	2
–	Kagoshima	31.34.17 N, 130.32.42 E	1	–	–

Number of queens dissected just after sampling is shown in parenthesis. Boxes without entries have no code for this solitary queen

Experiment 3: worker reproduction

To investigate worker egg-laying activity, 10 to 20 workers with some larvae from a colony were isolated and kept in an artificial nest for 2 or 3 months with collembola supplied as prey. Egg-laying activity was monitored two or three times per week. In total, 100 workers from three colonies were used for this experiment. After the experiment, workers were dissected under a dissecting microscope to check for the presence or absence of ovaries.

Results

Colony composition

Only six queenright colonies were collected (Table 1). All colonies had multiple queens (average, 5.3±SD 4.5; range, 2–14, N=6), and an average of 196 workers (198±SD 129; range 5–333, N=5). In Kagoshima, a solitary dealate queen was collected from the soil. One colony produced numerous pupae and adults of alate queens. No males were found. All queens collected in the field had empty spermatheca and 2+2 ovarioles with some developing oocytes and accumulation of yellow body (just after sampling N=15, after laboratory culture N=18).

Experiment 1: independent colony foundation

Isolated alate queens shed their wings from within 1 to 39 days after isolation (mean, 11.3±SD 10.6 days, N=12). All five queens kept without prey never laid eggs and died 35 to 100 days after isolation, while three of the seven queens that were kept with a continuous supply of prey laid eggs after 38 to 71 days. Two of these three queens produced seven workers each until the queens' deaths (after 152 days and 203 days, respectively). The remaining queen produced 26 workers and two alate queens over 1 year. These two alate queens died outside the nest chamber without dealation. The remaining four queens with prey supply died without egg laying between 30 and 76 days after isolation. The solitary queen collected in Kagoshima, which was confirmed to be a virgin by dissection after the experiment, also produced three workers.

Experiment 2: queen thelytoky

All 17 queens that emerged in the laboratory produced 7 to 39 workers, and ten of the 17 queens produced a few alate queens during 1 year. In three colonies, newly emerged queens shed their wings in their natal nests and started to lay eggs. For comparison with the case of independent colony foundation, we used the data of queens from the same colony (FI04-1, N=10). The ratio of successful colony foundation was significantly higher in dependent foundation (all ten colonies) than independent foundation with prey (three of the seven colonies, Fisher's exact probability test, P=0.015). The pre-oviposition period in the dependent colony foundation was significantly shorter (3.6 days ± 1.0 SD, N=10) compared to the independent foundation (56±13, N=3, Mann–Whitney U test, P< 0.001). Further comparison of demographic characteristics could not be carried out because too few independently founding colonies survived long enough to allow comparison. Both queens in FI06-30 produced several workers, and one of the queens also produced an alate queen. We, thus, confirmed thelytokous parthenogenesis by virgin queens from five different cities, Takamatsu, Ozu, Hiroshima, Hatsukaichi, and Kagoshima.

Experiment 3: worker reproduction

Isolated workers never laid eggs during 2 months. Dissection of workers revealed that the workers had no ovaries (N=80), indicating that they were obligatorily sterile.

Discussion

Our investigations on *P. membranifera* in the field and in the laboratory have revealed a novel life history for this ant species. Queens show thelytokous reproduction with production of both workers and alate queens that can start new colonies independently in a non-claustral way, although the efficiency of independent colony foundation is remarkably low. Our data on colony composition and queen reproductive condition in the field is still limited because collection of field colonies is quite difficult. Furthermore, genetic analysis has not been carried out. However, we confirmed the production of alate queens and/or workers by virgin queens from five isolated localities in western Japan. Thus, thelytokous reproduction by queens with sterile workers seems to be a common reproductive system of *P. membranifera* in Japan. All colonies collected in the field have multiple queens, and some of the newly emerged queens were adopted into their natal colony in the laboratory. In the field, colonies were often distributed very close to each other (personal observation). These results indicate that this species is a secondarily polygynous ant and its colonies may proliferate by colonial fission as many other polygynous ants. Thus, *P. membranifera* shows a new case of thelytoky, which represents a new combination of life history traits: independent colony foundation by alate queens and colony fission. The present results, together with recent reports of the unusual reproductive systems of ants (reviewed by Keller 2007 and Heinze 2008), show that

life history and reproductive structure in ants can be unexpectedly varied among species.

In Japan, there are five thelytokous ant species, *V. emeryi*, *P. punctatus*, *P. membranifera, C. biroi*, and *M. triviale* (Kobayashi et al. 2008; Itow et al. 1984; Tsuji 1988; Tsuji and Yamauchi 1995; Sasaki and Tsuji, pers. comm). Males are either very rare or have not been found to date in all species except *V. emeryi* where workers are produced by sexual reproduction. In the other four species, mated individuals have not been collected. No thelytokous species has been reported in their congeners of these four genera. As in *P. membranifera*, *Pristomyrmex punctatus*, *M. triviale* and *C. biroi* are also found in open, disturbed areas (Tsuji and Yamauchi 1995; Tsuji 1988). Except for the case of *Monomorium,* where many species of the genus prefer to nest in such habitat, the habitat preference of the three other species is exceptional when compared to the other congeneric species. *Pristomyrmex yaeyamaensis* distributed in Nansei isl. and four *Pristomyrmex* species in the Oriental tropics are found inside forests (Yamane and Terayama 1999; Tsuji et al. 1997). *Cerapachys* species are abundant in forests, but are rare in artificial open sites while *C. biroi* is very common in such habitat (Ito et al. 2001 and unpublished). Among the 18 Japanese species of *Pyramica*, *P. membranifera* is the only species that is exclusively collected in urban parks in residential quarters (Japanese ant database group 2003; Kitahiro et al. unpublished). Theoretically, the high intrinsic rate of natural increase is favored in such open habitat which experiences frequent disturbance (MacArthur and Wilson 1967). In ants, secondarily polygynous ant species, which have a high intrinsic rate of natural increase because of short pre-reproductive period, are common in open habitat (Tsuji and Tsuji 1996). The reduction or complete abandonment of male production apparently contributes to the high intrinsic rate of natural increase, therefore, such thelytoky may be favorable in an open habitat.

Acknowledgement We thank K. Yamamoto, H. Mizoguti, and T. Kameyama for their help in field research, Sk. Yamane for his hospitality and accommodation help in Kagoshima, K. Tsuji for stimulating discussion and providing unpublished information on *M. triviale*, and L. Keller, J. Heinze, P. Loutes, and several anonymous referees for useful comments on the manuscript. This research was supported by grants in aid for scientific research from JSPS [C, No. 14540579 (principal investigator, F. Ito); A, No. 17207003 (principal investigator, S. Higashi); B, No. 18370012 (principal investigator, K. Tsuji), for young scientists (A. Gotoh)] and by research grant OT 01/24 from the Katholieke Universiteit Leuven Research Fund.

References

Brown WL, Wilson EO (1959) The evolution of the dacetine ants. Q Rev Biol 34:278–294

Cook JM, Crozier RH (1995) Sex determination and population biology in the hymenoptera. Trend Ecol Evol 10:281–286

Grasso D, Wenseleers T, Mori A, Le Moli F, Billen J (2000) Thelytokous worker reproduction and lack of *Wolbachia* infection in the harvesting ant *Messor capitatus*. Ethol Ecol Evol 12:309–314

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

Hartmann A, Wantia J, Heinze J (2005) Facultative sexual reproduction in the parthenogenetic ant *Platythyrea punctata*. Insect Soc 52:155–162

Heinze J (2008) The demise of the standard ant. Myrmecol News 11:9–20

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, Ferandez-Marin H, Mueller UG (2009) No sex in fungus-farming ants or their crops. Proc R Soc B 276:2611–2616

Ito F, Yamane Sk, Eguchi K, Noerdjito WA, Kahono S, Tsuji K, Ohkawara K, Yamauchi K, Nishida T, Nakamura K (2001) Ant species diversity in the Bogor botanic garden, West Java, Indonesia, with descriptions of two new species of the genus *Leptanilla* (Hymenoptera, Formicidae). Tropics 10:379–404

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

Japanese ant database group (2003) Ants of Japan. Gakken, Tokyo, Japan

Keller L (2007) Uncovering the biodiversity of genetic and reproductive systems. Time for a more open approach. American society of naturalists E.O. Wilson award winner address. Am Nat 169:1–9

Kobayashi K, Hasegawa E, Ohkawara K (2008) Clonal reproduction by males of the ant *Vollenhovia emeryi* (Wheeler). Entomol Sci 11:167–172

MacArthur RH, Wilson EO (1967) The theory of island biogeography. Princeton Univ. Press, Princeton, NJ

Pearcy M, Aron S, Doums C, Keller L (2004) Conditional use of sex and parthenogenesis for workers and queen production in ants. Sci 306:1780–1783

Rabeling C, Lino-Neto J, Cappellari SC, Dos-Santos IA, Mueller UG, Bacci M Jr (2009) Thelytokous parthenogenesis in the fungus-gardening ant *Mycocepurus smithii* (Hymenoptera: Formicidae). Plos One 4:e6781

Schilder K, Heinze J, Hölldobler B (1999) Colony structure and reproduction in the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera, Formicidae). Insect Soc 46:150–158

Tsuji K (1988) Obligate parthenogenesis and reproductive division of labor in the Japanese queenless ant *Pristomyrmex pungens*. Behav Ecol Sociobiol 13:247–255

Tsuji K, Tsuji N (1996) Evolution of life history strategies in ants: variation in queen number and mode of colony founding. Oikos 76:83–92

Tsuji K, Yamauchi K (1995) Production of females by parthenogenesis in the ant, *Cerapachys biroi*. Insect Soc 42:333–336

Tsuji K, Ito F, Ohkawara K (1997) A preliminary report on colony composition and foraging behavior in three species of genus *Pristomyrmex* ants in Indonesia and Malaysia. Ari 21:5–7 (in Japanese with English abstract)

Wheeler WM (1933) An ant new to the fauna of Hawaiian Islands. Proc Hawai Entomol Soc 8:275–278

Wilson EO (1953) The ecology of some north American dacetine ants. Ann Entomol Soc Am 46:479–495

Yamane Sk, Terayama M (1999) A new species of the genus *Pristomyrmex* Mayr from Japan, and a proposal of a new synonym of species in the genus *Camponotus* Mayr (Hymenoptera; Formicidae). Mem Myrmecol Soc Jpn 328(1):17–24