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Colony composition in the Oriental ectatommine ant, *Gnamptogenys menadensis* in Peninsular Malaysia

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ABSTRACT. Reproductive organization and mode of propagation in social insects can vary under different environmental conditions. To test this hypothesis, colony composition of *Gnamptogenys menadensis* was investigated in 38 colonies from Ulu Gombak, a tropical rain forest zone in Peninsular Malaysia, and compared to earlier data from Karaenta, a tropical monsoon zone in Sulawesi. As in Karaenta, both dealate queens and gamergates (mated egg laying workers) reproduced in Ulu Gombak. Coexistence of dealate queens and gamergates was never observed. The proportion of queen colonies in Ulu Gombak was similar to that in Karaenta, indicating that the occurrence of distinctive dry season in Karaenta does not affect colony reproductive strategies. Based on colony composition and dissection data, the colony life cycle in Ulu Gombak is postulated.

Keywords Arboreal ant, gamergates, reproduction, social structure
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INTRODUCTION

The ectatommine ant genus *Gnamptogenys* contains about 140 species (Bolton 2021), and a variety of reproductive strategies: most species reproduce by alate (AQ) or ergatoid queens (EQ) while other species have gamergates (mated and egg laying workers, G) with alate queens (AQ+G) and/or ergatoid queens (EQ+G) (Pratt 1994; Gobin et al. 1998a; Blatrix & Jaisson 2000; Tanigawa et al. 2002; Ito & Gobin 2008). Therefore, the genus is a good model organism to discuss the diversification of the reproductive strategies in ants.

Colony composition and behavioral characteristics of *G. menadensis* (Mayr, 1887) had been studied in Karaenta, Bantimurung Bulusaraung National Park, South Sulawesi, Indonesia (Gobin et al. 1998ab, 1999, 2001). In Karaenta, most colonies reproduced by gamergates and a few colonies had a dealate queen who monopolized egg laying. In general, alate queens can start new colonies independently (independent colony foundation, ICF) while gamergates show colonial fission, where new colonies are started by groups of workers (Peeters & Ito 2001). The proportion of colonies using ICF and fission can be affected by environmental conditions (Molet et al. 2008;

Cronin et al. 2020). Gobin et al. (1998a) postulated that the abundance of pre-existing cavities available to the Karaenta population as simple nests could favour fission of gamergate colonies in *G. menadensis*. Fission may be selected for under harsh or variable condition (Molet et al. 2008). Actually, in the Australian *Rhytidoponera impressa* group that reproduce by alate queens and/or gamergates, the proportion of queen colonies in northern areas (tropical climate) is larger than that in southern areas (temperate climate) (Molet et al. 2008). Furthermore, in *Myrmecina nipponica* that reproduced by alate queens or ergatoid queens, Cronin et al. (2020) demonstrated that ergatoid queen colonies reproduced by fission is more common in southern-mountain and northern areas than that in southern lowland areas in Japan.

We investigated colony composition of *G. menadensis* in Ulu Gombak, Peninsular Malaysia. According to the Köppen–Geiger climate map, Peninsular Malaysia belongs to tropical rain forest zone where precipitation is more than 2500 mm/year and shows no indication of a distinctive dry season, while Karaenta is in a tropical monsoon zone where the dry season (less than 60 mm/month) is particularly distinct (Fig. 1). The annual rainfall in Karaenta is about 3,500 mm, with a clearly delineated dry season from May to October. In this paper, we test the hypothesis that the proportion of alate queen colonies is higher in the more stable climatic environment of Ulu Gombak than in seasonally variable Karaenta. For this, we report data on colony composition in Ulu Gombak, Peninsular Malaysia, and compare this with earlier data from Karaenta, Sulawesi (Gobin et al. 1998a; Gobin et al. 2001).

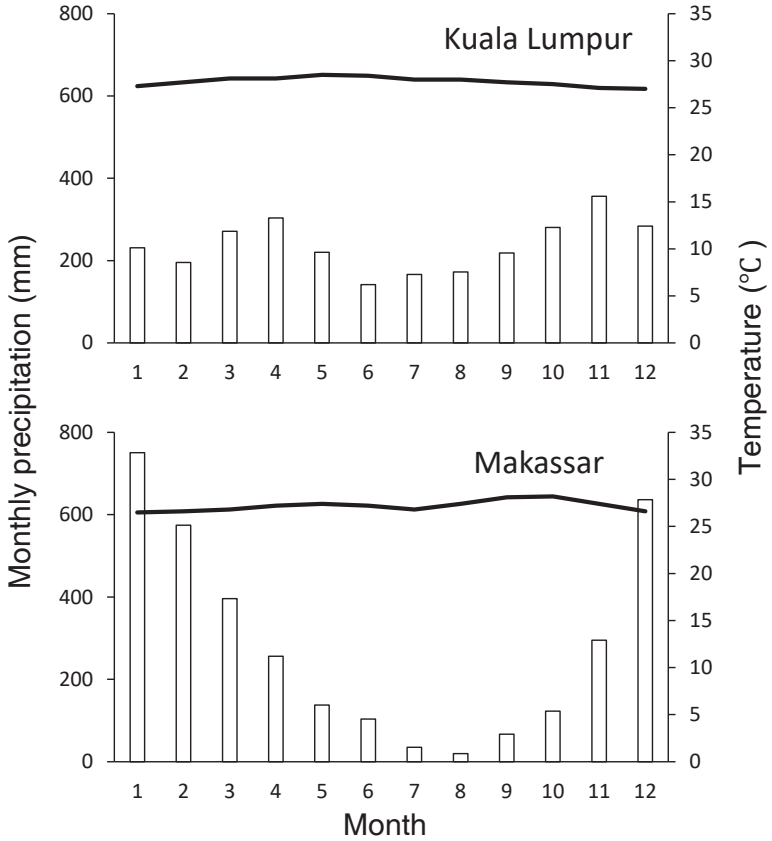

Fig 1. Monthly change of average precipitation (white bars) and temperature (solid line: calculated from 30 years data from 1991 to 2020) in Kuala Lumpur (near Ulu Gombak) and Makassar (near Karaenta). Data were obtained from homepage of Japan Meteorological Agency.

MATERIALS and METHODS

Colonies of *G. menadensis* were collected around Ulu Gombak field research center, Peninsular Malaysia on five separate periods: July and November in 1998, and January, March, and May in 1999. In each sampling period, we collected 7 to 11 colonies of *G. menadensis*. Nests of *G. menadensis* are mainly found in and around small humid valleys in Ulu Gombak. In the field, small pieces of cookies were used as baits to track foraging workers to their nests. Colonies were found in dead branches attached to living trees, in decayed branches that had fallen to the ground, and also between the base of the leaves and stems of rattan plants. The nests were carefully removed from the locations, placed into plastic bags and brought back to the field center to be sorted. All individuals were collected by breaking open the nests. All adult females were dissected to determine their reproductive condition (insemination, ovarian development, yellow bodies) using a binocular microscope immediately after collection. Brood was enumerated at each developmental stage separately. In six colonies, the number of eggs was not individually counted but was recorded qualitatively. Cocoons of 10 colonies were dissected to verify the sex and castes of pupae. The ovariole number of dissected females was counted for 49 mated workers from 17 colonies and 136 virgin workers in two colonies. The head width of females in 13 colonies with diverse colony sizes was measured with a micrometer, or a digital camera (SHIMADZU moticam 2000) attached to a binocular microscope. For colonies with less than 50 workers, all individuals were measured, while for colonies with more than 50 individuals, all gamergates as well as randomly selected 40 to 80 virgin workers were measured. In this paper, we used the term "gamergates" for mated workers with developing oocytes and/or the presence of yellow bodies.

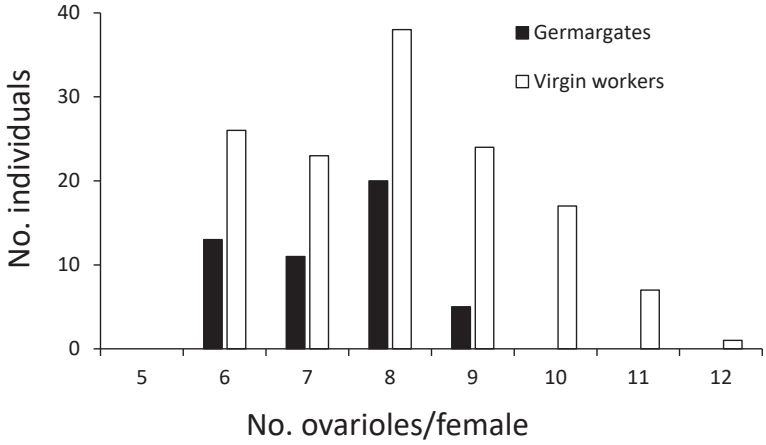

Fig. 2. Frequency distribution of ovariole numbers of gamergates and virgin workers. Ovariole numbers were counted for 49 gamergates from 17 colonies and 136 virgin workers from two colonies (FI99-2, BG99-21).

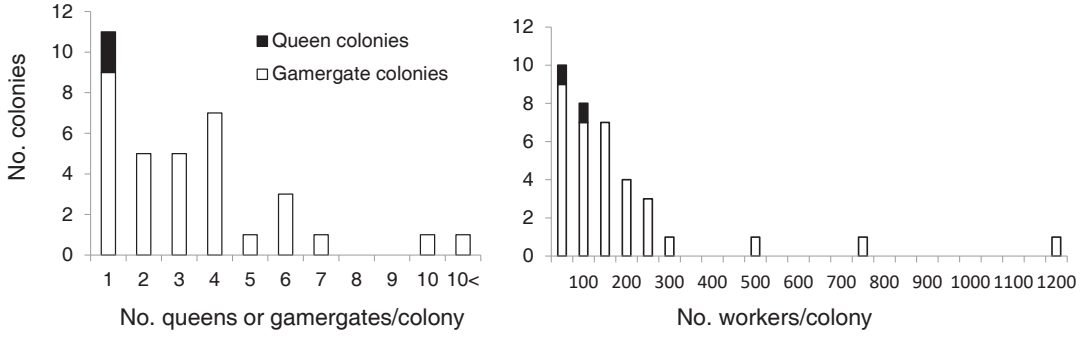

Fig. 3. Number of workers, gamergates, and queens in colonies of *G. menadensis* in Ulu Gombak

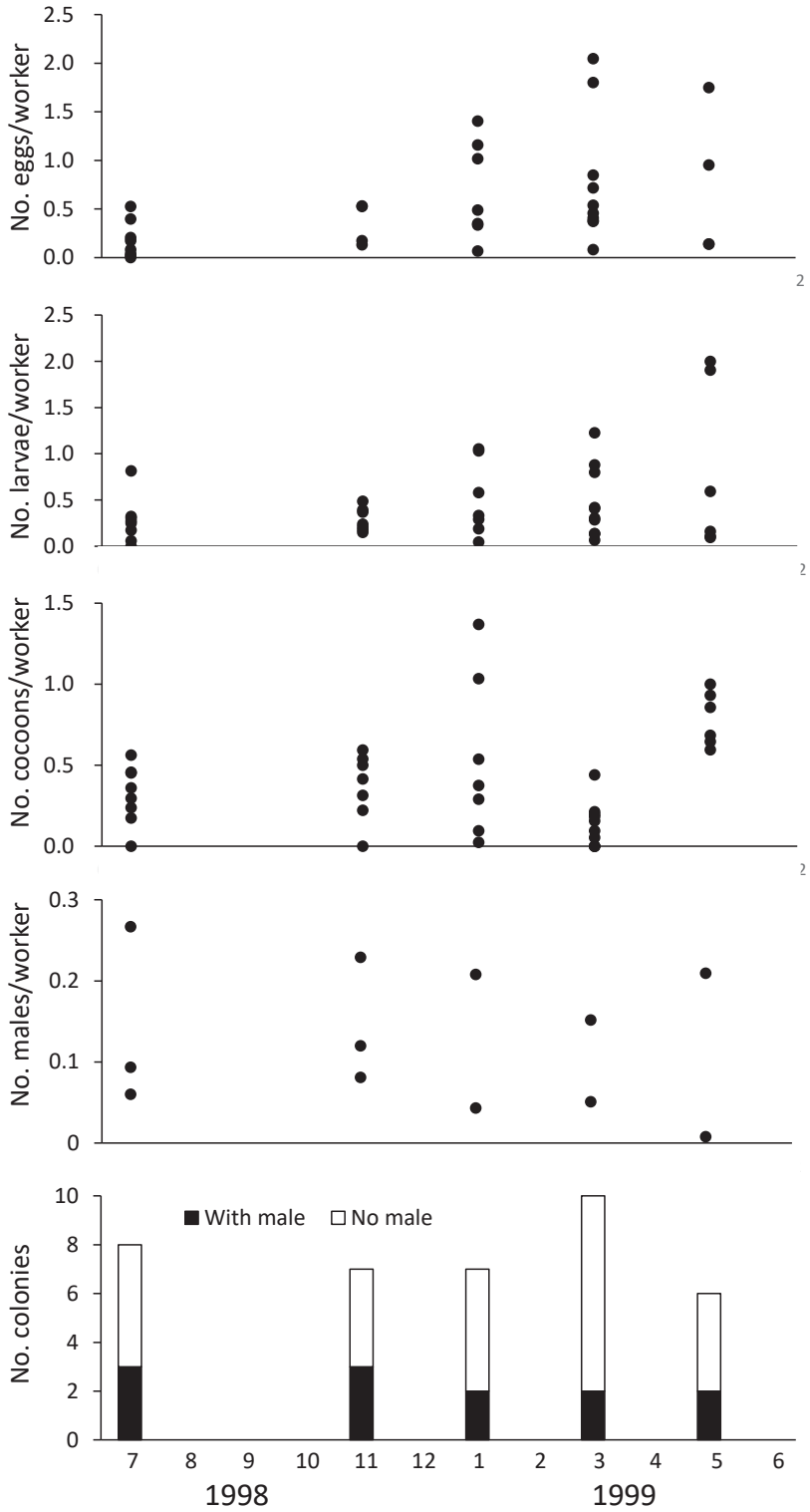

Fig. 4. Brood and male production. Number of colonies producing males is shown at the bottom. Number of eggs, larvae, cocoons and males per worker for each colony were plotted. For males, only data of male producing colonies are shown.

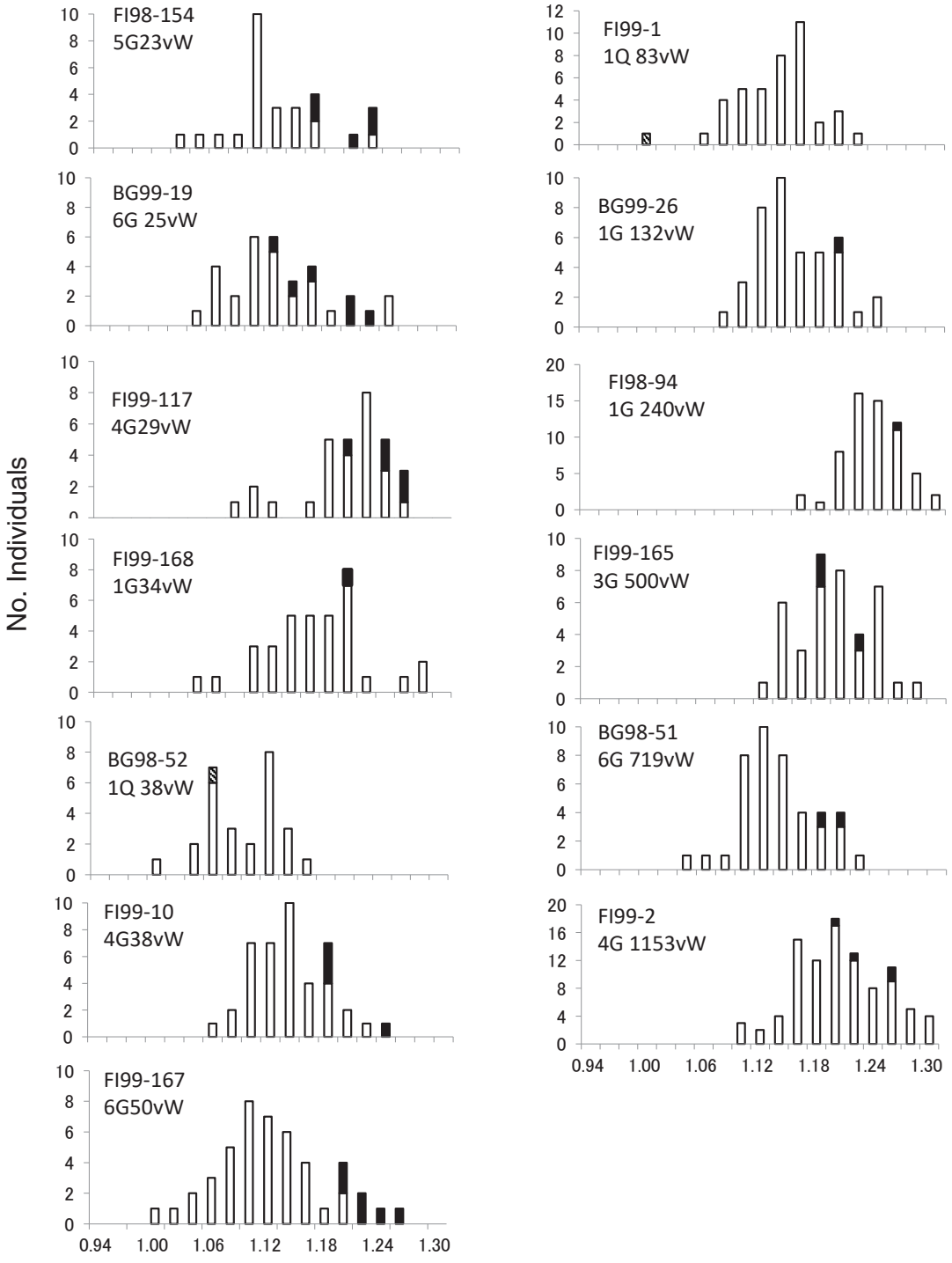

Fig. 5. Frequency distribution of head width of queens, gamergates and workers in 13 colonies of *G.* menadensis in Ulu Gombak. Q: dealate queen (oblique line), G:gamergate (black), vW:virgin worker (white). Number of gamergates and virgin workers in each colony is also shown under colony codes.

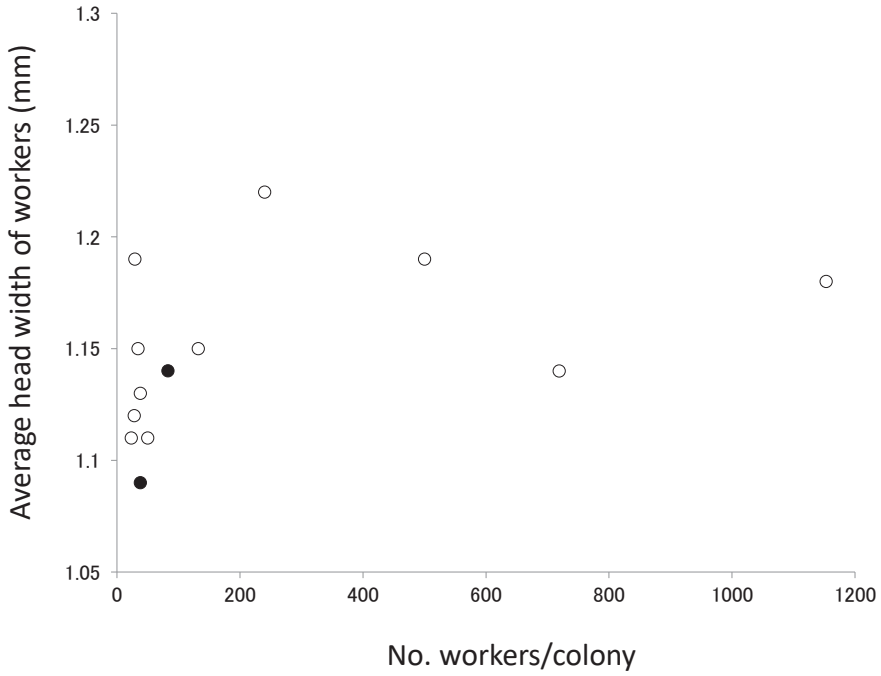

Fig 6. Relationship between colony size and average head width of workers. Black: queen colonies, White: gamergate colonies.

RESULTS

Colony composition: Reproduction in colonies of *G. menadensis* in Ulu Gombak was either by a dealate queen (N = 2 colonies) or gamergates (N = 33 colonies) (Table 1, Appendix 1). In three colonies, we did not detect mated individuals nor virgin workers with active ovaries, even when brood including eggs was abundant, suggesting that part of the colony was missed during collection. Coexistence of mated queens and gamergates was never observed. Both of the two queen colonies were monogynous, having a single mated egg laying dealate queen per colony, and all workers in those two colonies were confirmed to be virgin. The queen in FI 99-168 (personal colony code) had seven ovarioles (4-3), though unfortunately the data for the queen in the second colony were lost.

The number of ovarioles in virgin workers was slightly higher than that in gamergates (Fig. 2, Welch two sample test, t = -3.739, df = 127.44, p = 0.00028). This was probably because a large proportion of dissected virgin workers belonged to the largest colony (colony code FI99-2), which contained many large sized workers (see below). Among gamergate colonies, nine colonies were monogynous with a single gamergate, while the remaining 24 colonies had several gamergates, with a maximum of 14 in one colony (average 3.52 ± 2.8 SD, Fig. 3). The number of virgin workers per colony varied greatly, ranging from 23 to 1153 (161 ± 225 SD, Fig.3). The colony size of the two queen colonies was relatively small, with 38 (personal colony code, BG98-52) and 83 (FI99-1) workers respectively (Fig. 3). The colony size was not related to the number of gamergates (Spearman's rank correlation, p = 0.66).

Some of the virgin workers had a few developing oocytes, however, chorionated reproductive eggs (as defined by Gobin et al. 1998b) or yellow bodies were absent in nearly all virgin workers, confirming that these individuals do not lay reproductively-destined eggs, as reported earlier in *G. menadensis* from the Karaenta population (Gobin et al. 1998a) and observed in the laboratory (Gobin et al. 1998b). Only reproductive eggs can develop into male or female offspring, whereas *G. menadensis* workers can also lay trophic eggs that cannot develop further (Gobin et al. 1998b).

Table 1. The number of colonies in which queens or gamergates reproduced in *Gnamptogenys menadensis* in two sites. [1]Gobin et al. (1998).

	Queens	Gamergates	Orphan	Total
Ulu Gombak, Peninsular Malaysia	2	33	3	38
Karaenta, Sulawesi[1]	2	34	0	36

Table 2. Reproductive condition of gamergates in three colonies where ovary development varied among gamergates. Characterization of yellow bodies: - no yellow bodies, I: few pale yellow-bodies, II: moderate amount of yellow bodies, III: dense yellow bodies.

Collection month	Colony code	Individual code	Yellow bodies	No. developing oocytes
July	FI98-156	G1	II	0
	(No. workers = 60)	G2	-	2
	FI98-154	G1	I	1
	(No. workers = 23)	G2	II	0
		G3	II	0
		G4	II	1
		G5	I	2
March	FI99-117	G1	II	6
	(No. workers = 29)	G2	II	0
		G3	II	0
		G4	II	0

In three of 24 colonies with multiple gamergates, there were remarkable differences in ovarian development among gamergates (Table 2). In FI99-117, which contained four gamergates and 25 virgin workers, all four gamergates had dense yellow bodies, however, developing oocytes were found in only one of these. In FI 98-156, which contained two gamergates and 58 virgins, one gamergate had no developing oocytes but had yellow bodies, while the other had two developing oocytes without yellow bodies. Similarly, in FI98-154, which contained five gamergates and 18 virgin workers, two gamergates had yellow bodies but no developing oocytes while the other three had one or two developing oocytes. These colonies may indicate the occurrence of recent gamergate replacement, or suggest reproductive skew among gamergates.

In the remaining 21 colonies with multiple gamergates, the number of developing oocytes and quantity of yellow bodies were almost similar among gamergates. In two colonies (FI99-167, which contained six gamergates and 32 virgin workers, and BG99-19, which contained six gamergates and 25 virgin workers), the yellow bodies of all gamergates were tiny and pale yellow. Probably these two colonies were recently founded via colonial fission; though another possibility is that worker mating occurred after the death of reproductive females.

All brood stages were found throughout the year (Fig. 4). Alate males were present in 12 of 38 colonies. Male production occurred throughout the year, as in every sampling period, at least some of colonies included males. The proportion of male producing colonies was not statistically different among the five sampling periods (Fisher's exact test, $p = 0.89$). Male numbers varied from one to 225 males. The smaller colonies rarely produced males. No alate queens were collected and the production of alate queen pupae was observed only in a single colony (FI99-175)

collected in May of 1999. In this colony, we did not find any mated individuals, though 165 virgin workers, one male, 84 cocoons and several larvae and eggs included. Of the 84 cocoons, 14 were pupae of alate queens.

Body size: Body size of workers varied from 1.00 to 1.30 mm in head width. Figure 5 shows the frequency distribution of worker and queen head width in 13 colonies. Queen heads were smaller than most of workers, particularly the head width of the queen in FI99-1, which was the smallest among all females. The queen in BG98-52 also had a smaller head width than the majority of workers. Overall, head width of virgin workers varied among smaller colonies, while larger colonies had larger workers (Fig. 6). The average body size of workers in a small, single queen colony (BG98-52, 38 workers) was the smallest, indicating that this colony might have been started via independent foundation by a dealate queen. Mated workers were usually among the larger workers in a colony (Fig. 5), though this trend was mostly apparent in smaller colonies with less than 100 workers. In two colonies that contained gamergates with pale yellow bodies as mentioned above (FI99-167 & BG99-19), the larger workers were again the ones that were mated. This was particularly obvious in FI 99-167, where the largest six workers were mated. In BG99-19, three mated workers were bigger individuals whereas the remaining three were medium sized workers.

DISCUSSION

Comparison with Karaenta, Sulawesi: As in Karaenta, Sulawesi, *G. menadensis* reproduction in colonies in Ulu Gombak, Peninsular Malaysia, can occur via either dealated queens or gamergates. Contrary to our expectations, the observed proportion of queen colonies in the population of Ulu Gombak was similar to that in Karaenta, as studied by Gobin et al. (1998a). In both populations, there is only a single reproductive female in the queen colonies, while polygyny was common in gamergate colonies. The number of gamergates per colony in Ulu Gombak (3.52 ± 2.79) was not different to that in Karaenta (5.14 ± 3.99) ($t = 1.549$, $df = 34.697$, $p = 0.131$). While the colony size (the number of workers) tended to be larger in Ulu Gombak (average 165 ± SD 225) than in Karaenta (96 ± 63), this difference was not significant ($t = -1.898$, $df = 30.3$, $p = 0.067$). Environmental differences (i.e. the presence or absence of a distinctive dry season) between the two sites does not apparently affect the social organization of *G. menadensis*. In other words, environmental differences between the two sites may be insufficient to give rise to divergent social organization in this species.

Reproductive structure: The reproductive structure of colonies in AQ + G ant species varies from species to species. For example, many dealate queens and gamergates are able to co-exist in a single colony in *Pseudoneoponera tridentata* (Smith, 1858) (Sommer et al. 1994), while dealate queens and gamergates never coexist and both AQ colonies and G colonies are polygynous in *Gnamptogenys striatula* Mayr, 1884 (Blatrix & Jaisson 2000). *Harpegnathos saltator* Jerdon, 1851 seems to be similar to *G. menadensis*: queen colonies are always monogynous and gamergate colonies are polygynous (Peeters et al. 2000). This means that alate queens function as random dispersers, although workers mate only when they inherit the colonies. Similar patterns are known in *Platythyrea* sp. and *Ectomomyrmex leeuwenhoeki sumatrensis* (Forel, 1901) (Ito 2016; Ito et al. 2007). In these cases, the function of mated workers is similar to the adopted queens in secondarily polygynous species without gamergates. Compared to other AQ+G species, a remarkable characteristic that is obvious in *G. menadensis* is that the queen body size is smaller than most workers (as measured by head width). Such small sized queens are unusual in ants. Other known examples are ergatoid queens of *Mystrium oberthueri* Forel, 1897 and dealate queens of *Brachyponera luteipes* (Mayr, 1862) (Molet et al. 2007; Kikuchi et al. 2008). Furthermore, the ovariole number of queens in *G. menadensis* was smaller than that of workers. This may also be related to the small body size of queen: a positive relationship between body size and ovariole number has been reported in some ant species, e.g. *Myopopone castanea* (Smith, 1860) (Ito 2010) and *Myrmecina froggatti* Forel, 1910 (Ito et al. 1994).

The body size of alate queens of *G. menadensis* suggests that this kind of reproductive is well specialized for random long-distance dispersal, and may have a relatively shorter life span than large sized queens of other ant species. Low frequency and small colony size of queen colonies seems to be consistent with this argument. Besides, the low frequency of queen-producing colonies (only a single colony observed in this study) also supports the rare occurrence of colonies with queens. In the case of *Harpegnathous saltator*, where queen body size is larger than that of workers, more than half of all colonies contain reproductive queens, and colony size of queen colonies is not relatively small (Peeters et al. 2000).

Colony life cycle: Based on our results of colony composition and reproductive condition of mated individuals, colony life cycle of *G. menadensis* in Ulu Gombak is postulated as follows. Alate queens start new colonies independently and raise workers. When the queen dies, workers become able to mate and as such ensure the continuation of the colony. In such colonies, as shown by Gobin et al. (2001), dominance behavior frequently occurs, and some top-ranking workers exhibits sexual calling behavior and mate outside the nest. Hierarchy determination seems to be related to body size, as shown in small colonies with gamergates (e.g. FI98-154, BG99-19, FI99-117, FI99-10, & FI99-167): larger workers may become dominant, and mate with males. Colonies that have lost their reproductive female(s), i.e. after queen death or fission of a group of virgin workers, some workers may mate with males, as likely happened recently in two colonies (FI99-167, BG99-19) that contained gamergates with pale yellow bodies. Interactions among gamergates are not known, but in some cases, gamergate number seems to decline, as some sterile gamergates with many yellow bodies were found. Gobin et al. (1999) showed experimentally that virgin workers will immobilize reproducing workers with low fertility in the presence of fertile reproductives. Dense distribution of nests in some areas suggests that colonial fission may occur in gamergate colonies such as described by Gobin et al. (1998a), though we were not able to verify this in the present study.

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Appendix 1. Colony composition of *Gnamptogenys menadensis* collected in Ulu Gombak. DQ: dealated queens, G:gamergates, VW: virgin workers, M:males, C: cocoons, L: larvae, E: eggs, +:present but not counted.

	Number of individuals						
Colony Code	**DQ**	**G**	**VW**	**M**	**C**	**L**	**E**
Collected in July 1998							
FI98-154	0	5	23	0	4	7	4
BG-UG98-18	0	2	50	0	18	3	4
BG-UG98-12	0	1	59	0	14	19	31
FI98-156	0	2	60	0	+	+	+
BG-UG98-19	0	2	197	0	89	54	78
FI98-94	0	1	240	64	135	196	49
FI98-155	0	0	322	30	147	56	5
FI98-100	0	1	351	21	104	88	14
Collected in Nov. 1998							
BG-UG98-52	1	0	38	0	19	15	20
BG-UG98-53	0	3	46	0	0	7	8
BG-UG98-49	0	4	105	0	33	39	+
BG-UG98-50	0	4	113	0	67	55	60
BG-UG98-45	0	2	167	20	37	35	+
BG-UG98-44	0	4	262	60	141	63	34
BG-UG98-51	0	6	719	58	298	130	+

Collected in Jan. 1999							
FI99-10	0	4	38	0	26	20	22
FI99-9	0	2	43	0	1	2	15
FI99-6	0	0	59	0	60	17	59
FI99-1	1	0	62	0	18	64	87
FI99-8	0	1	106	0	9	18	46
FI99-7	0	1	111	4	50	54	31
FI99-2	0	4	1153	225	406	362	73
Collected in March 1999							
BG99-22	0	4	21	0	0	6	43
BG99-19	0	6	28	0	11	22	45
FI99-117	0	4	29	0	0	4	11
BG99-20	0	1	37	0	0	5	3
BG99-23	0	7	46	0	9	14	39
BG99-27	0	14	74	0	7	31	53
BG99-26	0	1	132	20	28	54	49
BG99-21	0	3	186	0	29	149	85
BG99-25	0	3	188	8	29	194	85
BG99-18	0	10	209	0	11	14	85
Collected in May 1999							
FI99-168	0	1	34	0	18	40	20
FI99-167	0	6	50	0	32	64	56
FI99-176	0	1	80	0	68	8	10
FI99-247	0	3	109	0	50	7	10
FI99-175	0	0	165	1	84	21	+
FI99-165	0	3	500	88	250	250	+