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Oocyte resorption in termite queens: Seasonal dynamics and controlling factors

Tomonari Nozaki [a,b,*,1], Kenji Matsuura [a,2]

[a] Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan
[b] Laboratory of Evolutionary Genomics, National Institute for Basic Biology, Okazaki, Japan

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ABSTRACT

Female insects can resorb their oocytes that could not be oviposited. Oocyte resorption is proposed to be an adaptive mechanism to optimize fitness in hostile environments, recouping resources that might otherwise be lost. Social insects have developed reproductive division of labor, wherein a small number of queens are devoted to egg production. Matured queens are highly specialized in reproduction and are largely dependent on nestmate workers for their nourishment. Therefore, oocyte resorption in the queens should be influenced by social factors such as the amount of available workforce, as well as external and abiotic factors. In this study, we investigated the seasonal dynamics and regulation factors of oocyte resorption in actively reproducing termite queens. We continuously collected the field-nests of the subterranean termite *Reticulitermes speratus* and demonstrated that queens frequently resorbed their oocytes in late summer, even though it is one of the most productive seasons in this species. On the other hand, our laboratory experiment showed that oocyte resorption itself was strongly induced regardless of the season. We also found that the rate of oocyte resorption was influenced by colony size (the number of attending workers). These results suggest that termite queens seasonally resorb their oocytes, yet oocyte resorption itself is regulated by social factors rather than by seasonal factors. Our study provides a unique insight into the regulation of reproduction in social insects.

1. Introduction

The reproductive division of labor is an essential feature in eusocial insects, wherein a small number of reproductive females, "queens", are devoted to egg production. Non-reproductive individuals such as "workers" conduct other tasks including foraging, brood care, and colony maintenance (Wilson, 1971). Queens in mature colonies are completely dependent on nestmate workers for their food, and exhibit behavioral and anatomical specializations for reproduction (Bordereau, 1982; Bordereau and Andersen, 1978; Costa-Leonardo et al., 2013; Nozaki and Matsuura, 2019; Nutting, 1969; Roma et al., 2010; Wheeler, 1996). Under such circumstances, egg production rate of queens can have a significant effect on the colony growth and expansion of their foraging area (Tschinkel, 1988); one may therefore expect that egg production by queens should be dynamically regulated according to the socio-environmental conditions. In fact, ovarian maturation and oogenesis of queens is promoted or inhibited by several social stimuli, such as the amount of workforce for colony maintenance, the number of broods that need care, and the number of queens per colony (Adams and Atkinson, 2008; Brent and Traniello, 2001a, 2001b; Brent et al., 2008; Schrempf et al., 2011; Tschinkel, 1988, 1995; Vargo and Fletcher, 1989).

Oosorption or oocyte resorption is one of the important reproductive strategies of female insects, since they degenerate developing oocytes in response to unfavorable conditions, allowing them to conserve metabolically expensive resources (Bell and Bohm, 1975). To date, a large number of factors that induce oocyte resorption have been identified, including poor nutritional status, environmental changes related to season (e.g., temperature, humidity, and day length), and absence of mates (Barrett et al., 2009; Bell and Bohm, 1975; Kajita and Evans, 2009; Kotaki, 2003; Osawa, 2005). In eusocial Hymenoptera (ants, bees, and wasps), oocyte resorption has been well studied in terms of the suppression of reproduction of workers or subordinate individuals (Bell and Bohm, 1975; Roseler et al., 1980; Van Oystaeyen et al., 2014). For

* Corresponding author at: Center for the Development of New Model Organisms, National Institute for Basic Biology, Okazaki 444-8585, Japan.
E-mail addresses: tomonari64.1.8@gmail.com, nozaki.t@nibb.ac.jp (T. Nozaki).
[1] ORCID: 0000-0003-4358-8118.
[2] ORCID: 0000-0002-9099-6694.

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example, in the buff-tailed bumblebees, *Bombus terrestris*, linear hydrocarbons on the queen's cuticle prevent worker reproduction by both inhibiting the development of ovaries and inducing oocyte resorption (Van Oystaeyen et al., 2014; but also see Amsalem et al., 2015; Padilla et al., 2016). However, there are very few reports on the influence of socio-environmental factors on oocyte resorption of matured and actively reproducing queens.

Termites (Infraorder: Isoptera) have evolved eusociality independent of Hymenoptera despite the outward similarity of their social organization (Thorne, 1997; Thorne and Traniello, 2003). In termites, ovarian development in the queens has been well studied and it has been shown that their egg production is influenced by socio-environmental factors, such as the presence/absence of workers or broods (eggs or larvae), and the number of active queens per colony (Adams and Atkinson, 2008; Brent and Traniello, 2001a; 2001b; 2002;; Brent et al., 2008; Shellman-Reeve, 1990). On the other hand, documentation on oocyte resorption has been far less, although Watson (1972), exceptionally, observed oocyte resorption for the first time in the queens of a harvester termite, *Hodotermes mossambicus* in laboratory-initiated colonies. Investigation of oocyte resorption in field-collected queens can provide further information to better understand the controlling factors and the functional significance of oocyte resorption in termites.

Reticulitermes is one of the best studied termite genera in terms of reproductive biology (reviewed in Lainé and Wright, 2003; Matsuura, 2017; Thorne et al., 1999; Vargo and Husseneder, 2009). The processes of oogenesis and ovarian development have been described in detail (Grandi and Chicca, 1999; Ishitani and Maekawa, 2010; Su et al., 2014; 2015), and hormonal regulation has also been examined (Elliott and Stay, 2007; Ishitani and Maekawa, 2010; Maekawa et al., 2010). In *R. speratus*, mature field colonies contain more than 100,000 foraging workers, tens to hundreds of queens, and one king (Fig. 1A, Matsuura et al., 2009, 2018; Tsunoda et al., 1999; Yashiro and Matsuura, 2014). Queens continuously produce eggs over several years, and the body and ovary size of queens varies significantly seasonally, especially between summer and winter, reflecting the reproductive ability at the time (Fig. 1B). Seasonal patterns of colony-level egg production in Japan have been reported in detail by Matsuura et al. (2007); queens begin to produce eggs in May, just after the swarming season (Takematsu, 1999), and the rate of egg production was estimated to reach its maximum in the summer season (June and July), when the activity in the colony was at its peak in this species (Fuchikawa et al., 2012). By October, almost all eggs were hatched and no eggs were observed inside their nests during winter (November-April, Matsuura et al., 2007, Fig. 1C).

In this study, we investigated the seasonal dynamics and regulation factors of oocyte resorption in *R. speratus* queens. We first established oocyte categories and described the cytological features of oocyte

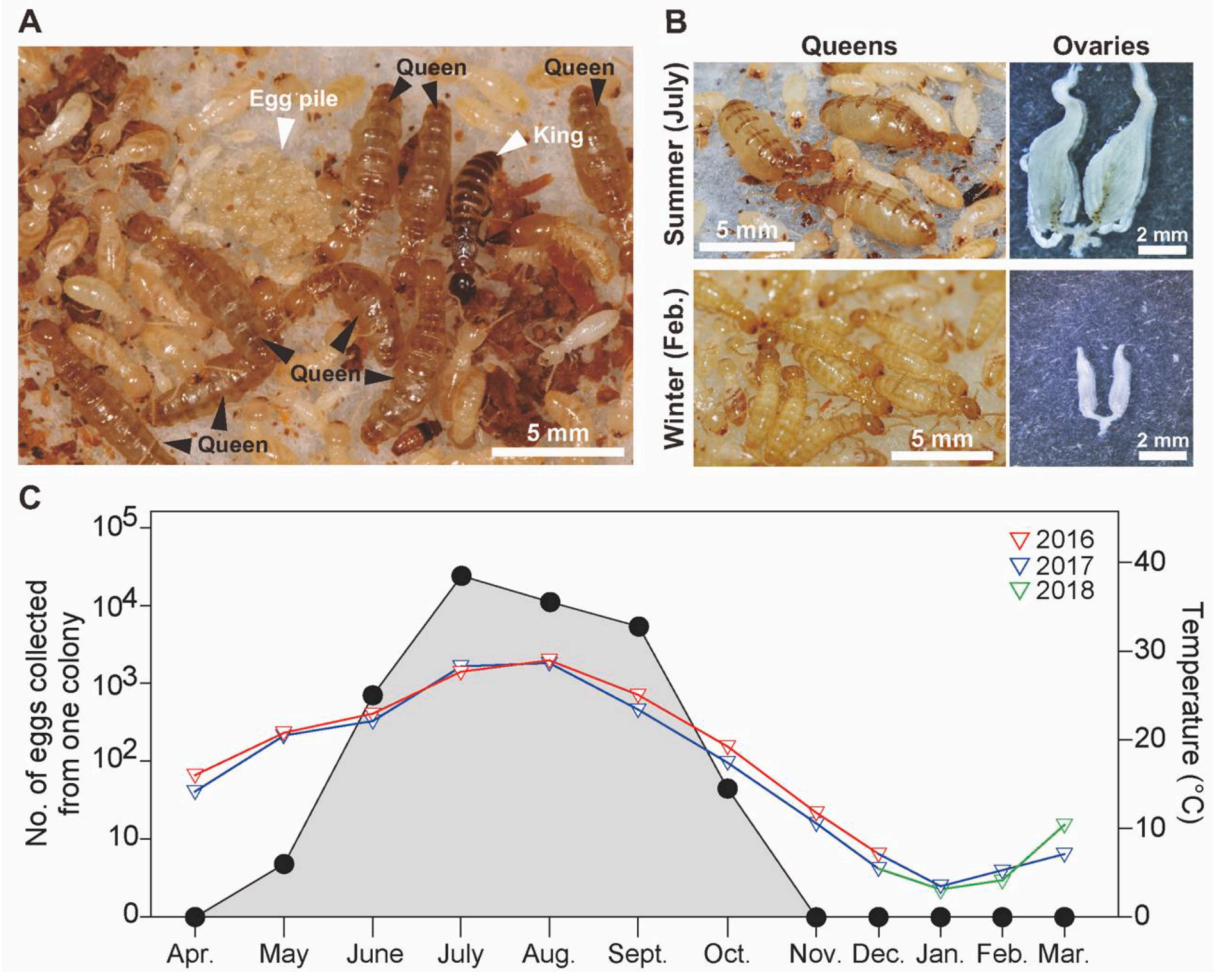

Fig. 1. Queens of *Reticulitermes speratus* and their seasonality in egg production. **(A)** Mature field colonies comprise a number of neotenic queens, which continuously produce eggs over several years. **(B)** The size of queens and their ovaries vary drastically across these seasons. **(C)** The colonies of *R. speratus* show a distinct annual pattern of egg production. The number of eggs per colony was adapted and modified from Matsuura et al. 2007. The colored lines indicate the average temperature in Kyoto city, where most of the termite colonies in this study were collected. The red, blue, and green color correspond to data of 2016, 2017 and 2018, respectively. Meteorological data were obtained from the Japan Meteorological Agency (http://www.jma.go.jp/jma/indexe.html). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

resorption, based on the results of the apoptosis assay and confocal microscopic observation of follicle cells. We sampled field colonies throughout the season and detected seasonality in the oocyte resorption in the queens. Finally, we conducted a laboratory experiment to analyze the effect of a socio-environmental factor, the number of attending workers, on oocyte resorption in the queens' ovaries.

2. Materials and methods

2.1. Collection of termite queens

From 2016 to 2019, colonies of *R. speratus* were collected from pine or cedar forests of central and western Japan. Rotten woods containing their colonies were transported to the laboratory and carefully dissected to extract termites. Each colony contained multiple queens, which were nymph-derived neotenics, as previously reported (Matsuura et al., 2009; 2018;; Yashiro and Matsuura, 2014). In total, 27 colonies were collected and 271 neotenic queens were used in this study (details in Table S1).

2.2. Anatomical structure of ovaries and oocyte categories in termite queens

To elucidate the anatomical structure of the ovaries and establish oocyte categories, 47 queens from five colonies (IW160519, MO160519, NA160530, WA160618, and OO160505, Table S1) were dissected in phosphate-buffered saline (PBS: 33 mM KH_2PO_4, 33 mM Na_2HPO_4, pH 6.8) under a stereomicroscope (Olympus SZX7, Olympus). Generally, termite ovaries consist of a large number of panoistic ovarioles, which have no specialized nurse cells (Fig. 2, Chapman et al., 2013; Weesner, 1969); therefore, we carefully loosened and counted ovarioles using fine forceps. The head width of queens was also measured as a proxy of body size. The queens were kept in a plastic container filled with moist mixed sawdust placed in the dark at 25 °C with their nestmates until use (which did not exceed one week). We classified basal oocytes into four types—undeveloped oocytes, pre-vitellogenic oocytes, vitellogenic oocytes, and irregularly shaped oocytes—based on morphological differences observed during the dissection of the ovarioles (see Results section). We measured the size of these oocytes, using six other queens from three colonies (KU170802, TA160806, and NI160823, Table S1). These queens were dissected on the day of sample collection and included in the assessment of seasonality. Oocytes were randomly chosen and the length (a) and diameter (b) of each oocyte were measured under a stereomicroscope with a digital imaging system (FLVFS-LS; Flovel). The volumes of oocytes (V) were estimated using the formula $V = 4\pi ab^2/3$ (Matsuura and Kobayashi, 2007). We also recorded the size of "eggs in the oviduct". The size of them corresponds to that of the mature vitellogenic oocytes, which were ready for ovulation. To determine whether irregularly shaped oocytes were degenerated, we also conducted an apoptosis assay. Ovarioles containing irregularly shaped oocytes were stained with the Vybrant™ Apoptosis Assay Kit no. 4 (Invitrogen). The kit contains the dyes YO-PRO1, which can enter apoptotic but not healthy cells, giving them a green fluorescence (Attisano et al., 2013). Thus, the healthy cells remain unstained, while apoptotic oocytes show green fluorescence. All procedures were performed according to the manufacturer's protocols. Briefly, the dissected ovarioles were stained with YO-PRO1 (1 mM/mL) in PBS for 30 min and observed using a DM IL LED inverted microscope (Leica Microsystems). In this assay, we used three queens from the colonies OO160505 and MO160519 (Table S1). The queens were kept in a plastic container filled with moist mixed sawdust placed in the dark at 25 °C with their nestmates until use (which did not exceed one month).

In order to determine the detailed cytological features of each category of oocytes, we observed the oocytes using a confocal laser-scanning microscope (CLSM; FV1000, Olympus). For this, we used two queens from the colony TY190622 (Table S1). The queens were kept in a plastic container filled with moist mixed sawdust placed in the dark at 25 °C with their nestmates until use (which did not exceed one week). Dissected and disentangled ovarioles containing each type of oocyte were fixed with 4% paraformaldehyde in PBS for 30 min. Fixed ovarioles were washed thrice in 0.3% Triton X-100 in PBS (PBS-T) for 15 min. The ovariole cells were then stained with 4,6-diamidino-2-phenylindole (DAPI) (1 μg/mL; Dojindo) for the nuclei and Alexa Fluor™ 488 phalloidin (66 nM/mL; Thermo Fisher Scientific) for the cytoskeleton (F-actin). After 1 h at room temperature, the cells were washed thrice with

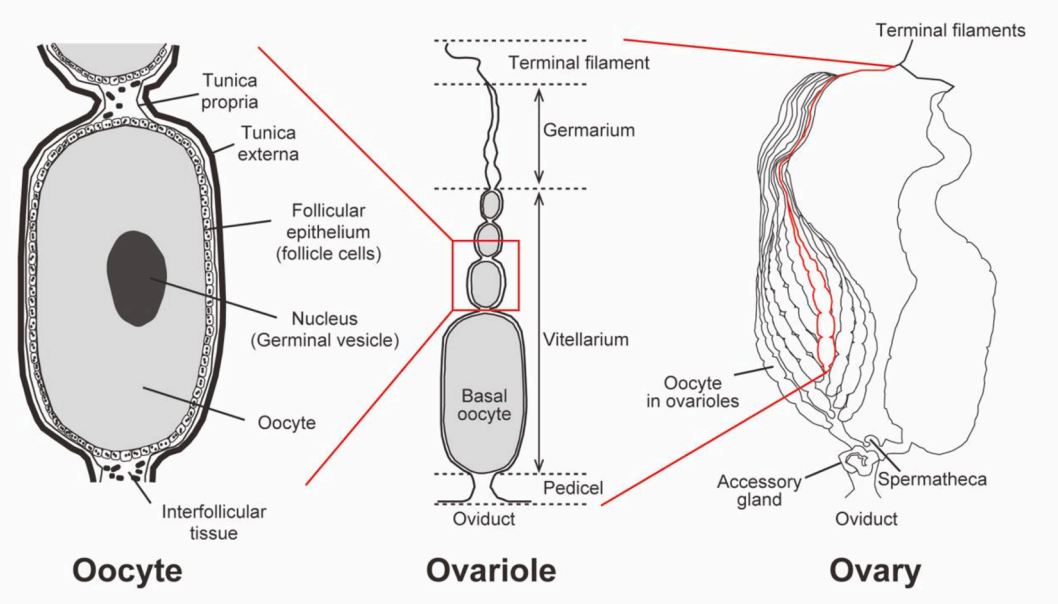

Fig. 2. Diagrams of ovary, ovariole, and oocytes in termites. These illustrations were drawn according to Weesner (1969), Grandi and Chicca (1999), and Chapman et al. (2013). Termite ovaries consist of large number of panoistic ovarioles, which have no specialized nurse cells in the germarium. Each ovariole contains a series of oocytes and the most advanced oocytes are located in the closest position to the lateral oviduct (basal oocytes). Each oocyte has a single large nucleus (germinal vesicle) and is surrounded by the follicular epithelium, which is a layer of binucleated follicle cells. Follicles (oocytes and follicle cells) are enclosed by noncellular layers, the tunica propria and externa. After choriogenesis, mature oocytes are ovulated through the oviduct.

PBS-T for 15 min. Sample oocytes were observed under a CLSM (FV1000, Olympus) and the morphology of follicle cells was visualized by DAPI/phalloidin staining and differential interference contrast (DIC) microscopy. During the observations using the CLSM, we focused on the follicle cells (Fig. 2), since the morphology of the cells is a key characteristic not only for defining the oocyte stage in termites (Grandi and Chicca, 1999; Oguchi et al., 2016; Su et al., 2014), but also for oocyte resorption in insects in general (Bell and Bohm, 1975; King and Richards, 1968; Kotaki, 2005).

2.3. Seasonality in oocyte resorption in termite queens

To investigate the seasonal changes in oogenesis and oosorption in the ovaries of the queens, we collected termite queens from April to September, the reproductive season of this termite species (Matsuura et al., 2007). In February and March, queens were also collected by digging out rotten wood buried in the underground soils to determine ovarian activity in the winter season. We dissected 189 queens from 21 colonies (Table S1) on the day of sample collection and recorded ovarian activity based on our oocyte categorization: pre-vitellogenic, vitellogenic, and resorbed oocytes (see Results section).

2.4. Laboratory experiment to study the oocyte resorption in termite queens

In order to determine the factor that influences the rate of irregularly shaped oocytes (which are the resorbed oocytes, see Results section), we conducted a laboratory experiment. In this experiment, we used a total of 29 queens from three colonies (10 from GB160627, 9 from YO160705, and 10 from MI160727, respectively, Table S1) collected in late June and July, when the colony-level egg production peaked in the termite (Matsuura et al., 2007). After recording the body weight (initial body weight), 14 and 15 queens were transferred into experimental nests (30-mm Petri dish lined with a moist unwoven cloth, Fig. S1) with 10 and 100 workers, respectively. Specifically, 5 queens from GB160627, 4 from YO160705, and 5 from MI160727 were used for the 10-worker condition, and five queens from each colony were for the 100-worker condition. Note that prior to this experiment, we tried rearing queens without the workers and found that almost all queens died within five days (data not shown). Therefore, we considered 10 attending workers as practicable and used this as the smallest colony size in this study. Experimental nests were kept in the dark at 25 °C for five days, after which, body weight of queens and the number of eggs oviposited were measured. Queens were immediately dissected and the type of basal oocyte (undeveloped, pre-vitellogenic, vitellogenic, and resorbed oocytes; see Results section) in each ovariole were recorded. When empty follicles were in the basal region of the ovarioles, we treated the second oocytes from basal ones as basal oocytes. As the initial state of the ovaries (queens at day 0), 6 queens from GB160627, 4 from YO160705, and 10 from MI160727 were dissected on the day of sample collection and the proportion of each oocyte type was recorded. These queens were also included in the assessment of seasonality (Table S1).

2.5. Statistical analysis

To examine the effect of colony and body size on the number of ovarioles in the ovaries of the queens, we used a generalized linear model (GLM) with a Poisson error distribution and log link function, followed by Tukey's HSD post hoc test. In this analysis, the colony, head width, and their interaction were treated as fixed effects. The size of basal oocytes was compared using a linear mixed model (LMM), wherein the oocyte categories (pre-vitellogenic, vitellogenic, and resorbed; see Result section) were included as a fixed effect and individuals nested in colonies as a random effect. Tukey's HSD test was used as a post hoc test. For the seasonality in ovarian activity of termite queens, we analyzed the proportion of each type of oocyte (pre-vitellogenic, vitellogenic, and resorbed) using a generalized linear mixed effect model (GLMM) with binomial distribution. In this model, months were treated as a fixed effect, and years and individuals nested in colonies were included as random effects. We excluded ovarioles with undeveloped oocytes in the basal regions when we calculated the proportion of oocyte types, because in some queens, we could not distinguish activated ovarioles with undeveloped oocytes from non-active (virgin) ovarioles. In the laboratory experiment, to compare the proportions of each type of oocyte in the ovaries between the three types of queens (queens at day 0, with 10 workers, and with 100 workers), GLMM with binomial errors, and a logit link function, followed by Tukey's HSD post hoc test were used. In this analysis, the types of queens were treated as a fixed effect and the original colony was included as a random effect. Simultaneously, by using GLMM with a Poisson error distribution and log link function, we compared the number of eggs oviposited during the 5 days of the experiment, between queens kept with 10 workers and with 100 workers. We also compared the body weight change using LMM. In these models, the original colonies were included as a random effect. All analyses were conducted using the "car" (Fox and Weisberg, 2011), "lme4" (Bates et al., 2015), and "multcomp" (Hothorn et al., 2008) packages in R v3.5.1 (R Core Team, 2018).

3. Results

3.1. Anatomical structures of the ovaries in R. speratus queens

We found that queens in this species had 104.21 ± 12.76 (mean ± SD) ovarioles in total, constituting their left and right ovaries (Table 1). The number of ovarioles significantly varied among colonies, while the effect of head width was not significant (LMM with type II Wald chi-square test, colony: $\chi^2 = 13.896$, df = 4, $p < 0.01$, head width: $\chi^2 = 1.366$, df = 1, $p = 0.242$, interaction: $\chi^2 = 1.065$, df = 4, $p = 0.900$).

3.2. Cytological features of oocytes in the ovaries

We classified oocytes into four types: undeveloped oocytes, pre-vitellogenic oocytes, vitellogenic oocytes, and irregularly shaped oocytes (Fig. 3A). We often observed several vitellogenic oocytes were ovulated and distinguished them as "eggs in the oviduct". In this categorization, undeveloped oocytes did not have any yolk spheres, and the size was almost the same as that of oocytes in the germarium region of the ovarioles. Ovarioles that have undeveloped oocytes in the basal regions were considered inactive. Pre-vitellogenic oocytes had prominent germinal vesicles, did not have obvious yolk spheres, and were larger than undeveloped oocytes. Vitellogenic oocytes had large amounts of yolk spheres, but their germinal vesicles were not visible. Irregularly shaped oocytes exhibited a globose shape, and their yolk sphere size was not uniform. This type of oocyte was clearly distinguished from the corpus luteum, which is an empty follicle after ovulation, because of the presence of yolk spheres. The volume of oocytes was significantly

Table 1
The number of ovarioles. Total number of left and right ovaries and the head width of the sampled queens, which was measured as a proxy for body size, are given.

		# ovarioles		Head width (mm)	
Colony code*	# dissected queens	mean	SD	mean	SD
OO160505	8	103.63	7.35	1.01	0.02
IW160519	10	109.50	10.60	1.01	0.02
MO160519	10	116.10	6.76	1.02	0.02
NA160530	9	94.33	7.23	0.98	0.02
WA160618	10	96.40	15.24	0.96	0.03
Total	47	104.21	12.76	1.00	0.03

*Numbers in "Colony code" indicate the dates when colonies were collected. For example, colony OO160505 was collected on May 5, 2016.

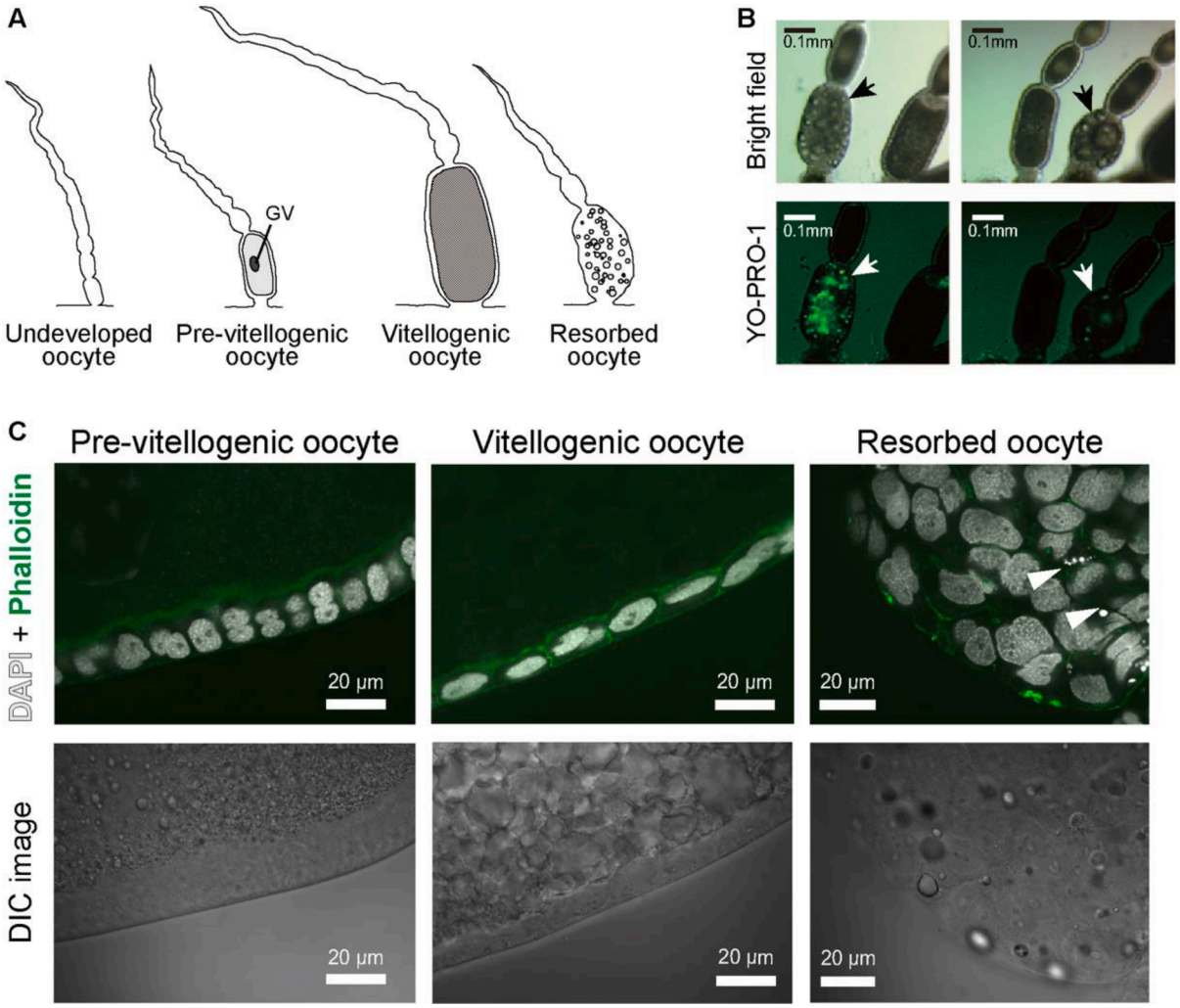

Fig. 3. Oocyte categories and their cytological features. **(A)** Illustrations of each oocyte. GV: germinal vesicle. **(B)** Nuclei in follicle cells surrounding irregular shaped oocytes (resorbed oocytes, white arrow) were stained by YO-PRO-1, which has membrane permeability in apoptotic cells. **(C)** Morphology of follicle cells of each type of oocyte following DAPI-Phalloidin staining and under differential interference contrast (DIC) microscopy. DNA and F-actin were stained by DAPI (gray) and Phalloidin (green), respectively. Pre-vitellogenic and vitellogenic oocytes have cuboidal and flat follicle cells, respectively. Nuclei of follicle cells surrounding resorbed oocytes were strongly deformed and partially fragmented (white arrowheads). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

different among these oocyte types (LMM with a type II Wald chi-square test, $\chi^2 = 256.77$, $df = 3$, $p < 0.001$, Fig. S2). Vitellogenic oocytes were significantly larger than pre-vitellogenic and irregularly shaped oocytes (Tukey's HSD test, $p < 0.001$). Some vitellogenic oocytes showed similar size to eggs in oviduct, although there was a significant difference in the mean volume (Tukey's HSD test, $p = 0.015$, Fig. S2). The size of the resorbed oocytes and pre-vitellogenic oocytes did not differ significantly (Tukey's HSD test, $p = 0.798$). Irregularly shaped oocytes were always observed in the basal oocytes. Among these four oocyte types, only irregularly shaped oocytes were positively stained by YO-PRO1, which can enter apoptotic cells and emit green fluorescence (Fig. 3B). Analysis by CLMS revealed the cytological features of pre-vitellogenic, vitellogenic, and irregularly shaped oocytes (Fig. 3C). Follicle cells in pre-vitellogenic oocytes were cuboidal and possessed large spherical nuclei. In vitellogenic oocytes, the cells were flat-shaped and formed very thin follicle cell layers. In both cases, follicle cells were binuclear and exhibited a prominent nucleolus in the nucleus. Follicle cells in irregularly shaped oocytes showed irregular morphology, and their nuclei were strongly deformed. Fragmentation of follicle cell nuclei was also observed. These cytological features correspond well with previous observations of oocyte resorption in other insects (Bell and Bohm, 1975; King and Richards, 1968; Kotaki, 2005); thus, hereafter, we refer to irregularly shaped oocytes as "resorbed oocytes". Resorbed oocytes in termite queens were translucent, unlike the grasshopper, *Romalea microptera*, wherein the resorption bodies show orange color (Sundberg et al., 2001). Note that pre-vitellogenic oocytes and vitellogenic oocytes in this study correspond to the oocyte growth stage (stage II) and oocyte vitellogenesis stage (stage III) in previous studies (Su et al., 2014; 2015), respectively.

3.3. Seasonality in the ovarian activity, especially in oocyte resorption in termite queens

In total, 189 queens from 21 colonies were collected between April and September for two years and were dissected on the day of collection (Fig. 4). We found that the queens started yolk accumulation (vitellogenesis) from May, the swarming season of this termite species, and ceased it before September. From May to August, the proportion of vitellogenic oocytes did not significantly vary (GLMM with a type II Wald chi-square test, $\chi^2 = 3.98$, $df = 3$, $p = 0.263$). Pre-vitellogenic oocytes were observed across all seasons, while all active ovarioles were occupied by this type of oocyte in April, and September, and in the

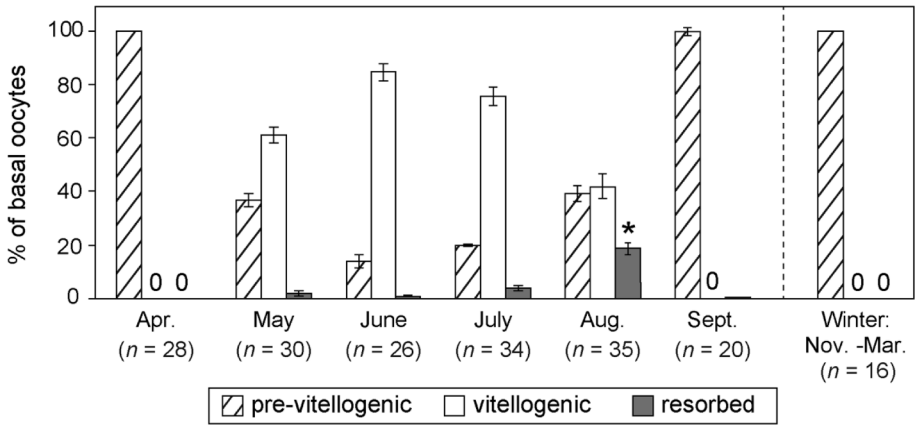

Fig. 4. Seasonal changes in the proportion of each type of oocyte—pre-vitellogenic, vitellogenic, and resorbed oocytes—in the basal region of ovarioles. Pre-vitellogenic oocytes were observed throughout the year. Vitellogenic oocytes were observed starting from May, the swarming season in this termite, while from September to April, which included the winter season, queens did not have vitellogenic oocytes in their ovaries. From May to September, the proportion of resorbed oocytes varied significantly (GLMM with a type II Wald chi-square test, $^{*}p < 0.001$) and was highest in August (Tukey's HSD, $p < 0.01$).

winter season. Resorbed oocytes were observed from May to September and the proportion of these oocytes was significantly different between these months (GLMM with a type II Wald chi-square test, $\chi^2 = 30.12$, $df = 4$, $p < 0.001$). The proportion of resorbed oocytes was significantly higher in August than in other months (Tukey's HSD, $p < 0.01$, Fig. 4). From May to August, queens in most colonies had fully developed ovaries, that is, basal area of almost all ovarioles was occupied with one of vitellogenic, pre-vitellogenic or resorbed oocytes. On the other hand, up to half of the ovarioles of most queens in the colonies NI170611, IW170720 and IW170721 were obviously virgin ovarioles, which were finely filamentous.

3.4. The effect of attending workers on oocyte resorption in termite queens

Our laboratory experiment (Fig. S1) showed that there was a significant difference in the proportion of each oocyte between the three types of queens, that is, queens at day 0, with 10 workers, and with 100 workers (GLMM with a type II Wald chi-square test; undeveloped, $\chi^2 = 507.1$, $df = 2$, $p < 0.001$, pre-vitellogenic, $\chi^2 = 332.2$ $df = 2$, $p < 0.001$, vitellogenic, $\chi^2 = 1209.4$, $df = 2$, $p < 0.001$, and resorbed, $\chi^2 = 471.4$, $df = 2$, $p < 0.001$). Queens at day 0 had vitellogenic oocytes in three-quarters of their ovarioles (75.6%), while they had a very small number of undeveloped (5.4%) and resorbed oocytes (4.3%). They showed a small number of pre-vitellogenic oocytes in their basal region of the ovarioles (14.6%). On the other hand, queens isolated from the natal nest and kept with 10 workers for a duration of five days exhibited a significantly lower proportion of pre-vitellogenic (5.5%) and vitellogenic oocytes (12.7%), and significantly higher proportions of undeveloped (41.1%) and resorbed oocytes (40.6%) than the field-collected queens (Tukey's HSD, $p < 0.001$, Fig. 5). There was a significant difference in the proportion of resorbed oocytes between conditions; queens attended by 100 workers showed lower proportion of resorbed oocytes (24.1%) than those by 10 workers (Tukey's HSD, $p < 0.001$), while that of vitellogenic oocytes did not differ (Tukey's HSD, $p = 0.169$, Fig. 5). Note that all of queen in these three colonies had fully developed ovaries; all ovarioles were not filamentous and exhibited obvious yellow bodies (strong brown pigmentations), which is assumed to the evidence of active reproduction (Weesner, 1969), in their pedicels. We also found that queens attended by 100 workers oviposited significantly more eggs than those by queens attended by 10 workers (GLMM with a type II Wald chi-square test, $\chi^2 = 72.3$, $df = 1$, $p < 0.001$, Fig. S3A). The body weight of the queens did not significantly differ between conditions (LMM with type II Wald chi-square test, $\chi^2 = 2.36$, $df = 1$, $p = 0.124$). However, the initial weight and the weight after five days differed significantly ($\chi^2 = 156.06$, $df = 1$, $p < 0.001$). The interaction of the number of attending workers and initial/final body weight was not significant ($\chi^2 = 0.14$, $df = 1$, $p = 0.7062$, Fig. S3B).

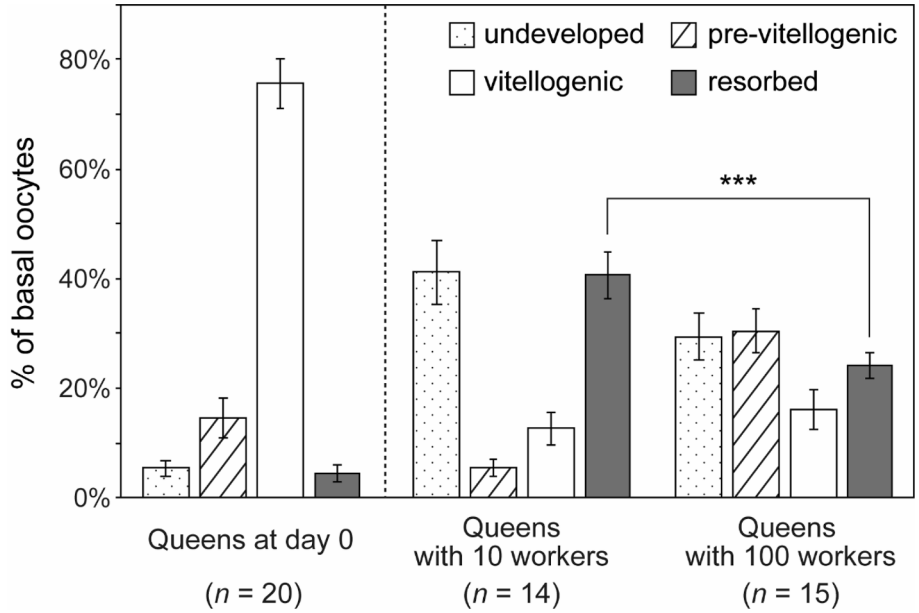

Fig. 5. Laboratory experiment for the analysis of oocyte resorption in termite queens. The proportions of each type of oocyte in queens at day 0, with 10 workers, and with 100 workers. Error bars represent standard error of means. The proportion of resorbed oocytes were significantly greater in queens isolated from their nests for 5 days (Tukey's HSD, $p < 0.001$). Queens with 100 workers exhibited a significantly lower proportion of oocyte resorption than those with 10 workers (Tukey's HSD, $^{***}p < 0.001$). The proportion of vitellogenic oocytes was significantly reduced from the initial state (Tukey's HSD, $p < 0.001$), yet there was no difference between queens with 10 workers and 100 workers (Tukey's HSD, $p = 0.169$).

4. Discussion

Our present study revealed that queens in *R. speratus* frequently resorbed their oocytes within their ovaries, especially at the end of the reproductive season of this species (Fig. 4). Nevertheless, the season itself may not be the only factor that induces oocyte resorption. This is because, even queens collected in June-July, in which almost all (70–80%) of the basal oocytes were categorized as vitellogenic, exhibited high-levels of oocyte resorption (at most 40%) when they were placed under the experimental conditions (Fig. 5). We also demonstrated that an increase in the number of attending workers improved the rate of oocyte resorption (Fig. 5). This result suggested that oocyte resorption could be influenced by social factors, including the number of workers attending to the queens, although the experimental condition with 100 workers was not sufficient to suppress oocyte resorption completely and to promote vitellogenesis. To gain more detailed insights into the effect of social factors on both oocyte resorption and vitellogenesis of queens, the effect of isolation and proportional changes in each oocyte type accompanied to the lapse of time should be examined. In addition, the effect of mating also should be considered in future researches, because termite queens frequently copulate with the king, unlike eusocial Hymenoptera (Hartke and Baer, 2011; Matsuura et al., 2009).

Queen in *R. speratus* exhibited oocyte resorption especially in August (Fig. 4), and our laboratory experiments showed that the smaller colony size, or the smaller number of workers induced higher proportion of resorbed oocytes (Fig. 5). Nevertheless, it is unlikely that the colony size in this species, which has been estimated to be greater than 100,000 in previous study (Tsunoda et al., 1999), would decrease in August, because numerous numbers of hatched larvae in the early summer should grow up and start to work in the late summer. In August, the temperature is the best for worker activity (Fuchikawa et al., 2012; Nakayama et al., 2004), while the number of immature larvae soon after hatching is estimated to be at a maximum in this month (Matsuura et al., 2007). Moreover, the number of eggs is the second highest in this month (Fig. 1, Matsuura et al., 2007). Therefore, alternatively, it is possible that workers take care of immature larvae and eggs more frequently in August, resulting in a reduced number of workers feeding and caring the queen. Future studies should verify this hypothesis and also include behavioral observations that focus on nutritional flow, in termite colonies.

Not only the nutritional status, but also hormonal titers in queens might be affected by seasonal factors and the number of attending workers. From the endocrine point of view, juvenile hormone (JH) is known to play an important role in the suppression of oocyte resorption (Bell and Bohm, 1975; Kotaki et al., 2016). For instance, in the stink bug, *Plautia stali*, wherein starvation is one of factors inducing oocyte resorption, both the implantation of JH-producing glands including corpus allatum and the treatment with JH could nullify the inducing effect of food deprivation (Kotaki, 2003; Kotaki et al., 2016). Furthermore, the removal of the JH-producing glands induced oocyte resorption even in well-fed females (Kotaki et al., 2016). There are several studies addressing the JH titers in termite reproductives (Elliott and Stay, 2007; Maekawa et al., 2010; Saiki et al., 2015), however, seasonal changes in JH titers of queens remains unknown. Investigation of the relationship among ovarian status and JH titers in queens, under various (socio)environmental conditions will provide fundamental insight into the mechanism underlying oocyte resorption in termite queens.

We found seasonality in vitellogenesis within queens' ovaries (Fig. 4); vitellogenesis began in May, the swarming season in this species (Takematsu, 1999) and continued until August (Fig. 1C). In August, or at the end of the reproductive season, the ratio of vitellogenic oocytes decreased and oocyte resorption was frequently observed. All queens ceased yolk deposition in September, and from then on up to April, which included the winter season, the queens had only pre-vitellogenic oocytes in their activated ovarioles (Fig. 4). This seasonal pattern is similar to those reported in a previous study in which egg piles were observed from late May and no eggs were observed from mid-October to early May (Matsuura et al., 2007, Fig. 1C).

Although August is one of the months showing the greatest colony activity in *R. speratus* (Fuchikawa et al., 2012), queens partially resorbed their oocytes while accumulating yolk in other oocytes. Considering that the hatching period of this termite was approximately 35 days at 25 °C (Matsuura and Kobayashi, 2007), it is reasonable that queens cease oviposition in August, allowing all the eggs to hatch by October. Our data also demonstrated that resorbed oocytes were much smaller than vitellogenic oocytes (Fig. S2), suggesting that queens resorb immature oocytes (pre-vitellogenic or early vitellogenic oocytes), rather than mature vitellogenic oocytes, which were large enough to be ovulated. In fact, Watson (1972) observed that relatively small vitellogenic oocytes were resorbed in founding queens of a harvester termite, *H. mossambicus*. A similar strategy has also been used in the aphid, *Megoura viciae*, wherein viviparous females resorbed the smallest embryos, while the largest embryos in each ovariole continued to mature (Ward and Dixon, 1982). Selective resorption of immature oocytes allows termite queens to reallocate the retrieved resources into other developing oocytes and to use up their internal resources effectively. To address this possibility, more detailed studies focusing on the process of oocyte resorption and its selectivity are required.

Although degeneration of oocytes in termites has been discovered under laboratory conditions (Elliott and Stay, 2007; Watson, 1972), our study provides the first evidence of oocyte resorption in field-collected and actively reproducing termite queens. Grandi and Chicca (1999) and Su et al. (2014) studied the oogenesis of *Reticulitermes* termites in detail. Based on these studies, we categorized oocytes in the ovaries of the queens into undeveloped, pre-vitellogenic, and vitellogenic oocytes, taking into account the size, presence or absence of vitellogenesis (yolk deposition), and the morphology of follicular cells (Fig. 3A, C). In addition to the above types of oocytes, we described "resorbed oocytes", in which follicular cells undergo apoptosis (Fig. 3B, C). Our oocyte categorization, including resorbed oocytes, will promote further field-based research focusing on the reproductive biology of termites.

Taken together, this study provides a unique insight into the reproductive biology of social insects. We have described for the first time the cytological features of oocyte resorption in termites and raised the possibility that queens' oocyte resorption may be regulated by socio-environmental changes rather than abiotic changes related to seasons. Future research should include more detailed experiments and behavioral analysis to reveal the functional significance and underlying mechanisms of oocyte resorption in termite queens.

CRediT authorship contribution statement

Tomonari Nozaki: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Writing - original draft, Writing - review & editing. **Kenji Matsuura:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2021.104242.

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