



# Breeding Biology of *Reticulitermes aculabialis* (Isoptera: Rhinotermitidae)

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## ABSTRACT

Eusocial insects have a diverse mechanism of reproduction to know the termite genealogy, and we examined the *Reticulitermes aculabialis* breeding mechanism. This well-known species was reared in an artificial environment under darkness at Northwest University, Xian, China, from May 2018 to June 2019. After the inaugural colonies foundation, imagoes started egg-laying during 30-40 days. The hatching ratio increased gradually during the time. The femal+male (FM) worker reproductive (ergatoids) colonies were reported significantly ( $p < 0.005$ ) in egg-laying and chambers making than primary reproductives (imagoes). The morphological measurement shows that the swarmer alates were significantly ( $p < 0.005$ ) extended along with wings, mean live weight of the queen was significantly heavier ( $p < 0.005$ ) than workers but not statistically different mature reproductive queen. The sex ratio of workers was found significantly dominant ( $p < 0.005$ ) among individuals in the colony and busy to find food sources, caring for young termites and eggs, constructing galleries, and helping the soldiers to defend the colony from predators, increases the efficiency of the effectiveness, protection from pathogens and parasites.

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## Authors' Contribution

Haroon, CY and YXL conceived and designed the study and wrote the main manuscript. HXZ and QL performed the study and analyzed the data. XS reviewed the paper. LXX supervised the project.

## Key words

Breeding biology of termites, Eusociality, Fecundity, King termite, Queen termite, Reproduction rates, *Reticulitermes aculabialis*, Subterranean termite colonies

## INTRODUCTION

Sociality or eusociality, mainly an arthropod phenomenon in the parental colony, with several cohorts of their offspring differentiated into behaviourally and morphologically (Boomsma, 2009). Two species of African mole-rats are the only exemptions from the vertebrate. Otherwise, eusociality is present in such myriad arthropods, including snapping shrimps, gall-forming aphids and thrips, bark beetle, seven stinging lineages (aculeate) wasps (including all ants, four bee groups, one crabronid wasp and most Vespidae) and the 3,100 termite species (Engel *et al.*, 2016).

Eusocial insects maintain and intensify their colonies through reproductive castes, the propagative population and species copulatory selectivity in the breeding

system of termites (Perdereau *et al.*, 2013; Ab Majid *et al.*, 2018; Hellemans *et al.*, 2019; Vargo, 2019). Their life history and reproductive system tend to be highly adaptive, occurring in lower termites (Vargo *et al.*, 2013; Wu *et al.*, 2014). Developmental concept to compare reproductive variability in the ants and termites historically hindered by the number and complexity of fertile, sterile and anatomical intermediates, the existence of nomenclature differences and semanticized disputes (Thorne and Traniello, 2003).

The termite reproductive system is typically monogamous, and the colony productivity moderately weakened during the inaugural establishment (Eyer *et al.*, 2018; Kante *et al.*, 2018; Perdereau *et al.*, 2019). Adequate neotenic appears in the colony when primary king and queen die and take over the reproductive function to nurture the colonies (Kobayashi and Miyaguni, 2016). Scientists aim for social development to record the eccentricity in the social insect succession plexus and delineate its first considerations. Therefore, the structure of colonies during breeding collectively coherent in social insects by primary social workers related to group living and external ecological pressures (Waidele *et al.*, 2019). Extremities on the capacity to disperse queens to explored autonomous territories are the key factors in the development of polygyny within subterranean termite

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colonies by encouraging queens to built-up settlements or remain in the natal nest (Kitto, 1997; Weil, 2010; Otani *et al.*, 2019; Vargo, 2019).

Theoretical studies support the theory of environmental and observational limitations showing that the quantity of queens inside territories is significant in areas with higher population densities and reduced nesting limitations (Kitto, 1997; Weil, 2010; Hoffmann *et al.*, 2012). Eusociality has evolved most of the time in aculeate wasps, hymenopteran sex-determining mechanism of haplodiploidy significantly increases sibling fitness inclusively, although this is disputed (Grimaldi and Engel, 2005). In general, the closely related genetic trait of nestmates is a standard in eusocial insects, the main exception being termites, via inbreeding or some sort of parthenogenesis (Hoffmann *et al.*, 2012; Engel *et al.*, 2016; Otani *et al.*, 2019; Vargo, 2019; Haroon *et al.*, 2020).

Social insects colony foundation approaches are eminent by two main categories: splitting colony and alates independent colony foundation. These strategies were prevailing in evolutionary Hymenoptera and Isoptera (Wilson, 1971), requiring sexual reproduction between a male and female. In contrast to these widely accepted perceptions, sexual and asexual colonies in *Reticulitermes speratus* were established through one or two female alates. In addition, fitness outlays and benefits of the different foundation strategies are comparable utilizing three colony foundation success measures: reproductive founder survivorship, female reproductive fecundity, and the rates of first brood development within a newly founded nest (Matsuura and Nishida, 2001). To extrapolate the nuptial flight, the establishment of the new colony and the mating behaviour of termites is strenuously consequential (Hoffmann *et al.*, 2012; Vargo, 2019). Dispersion is dependent on the humidity, temperature, pressure and seasons. After nuptial flight the primary king (PKs) and queen (PQs) imagoes fall off their wings and start tandem behaviour, which is the most critical genetic behaviour of termite during building new colonies (Matsuura and Nishida, 2001; Pervez, 2018).

PKs and PQs of the termites construct the inaugural colonies and mate, where they subsequently produce other members of the colony (Yashiro and Matsuura, 2014; Kuhn *et al.*, 2019; Vargo, 2019). A female individuals colony foundation will remain more costly than a monogamous foundation, even if there are no developmental defects in the parthenogenetic progeny. A female must do all the work in a single female foundation colony, including building the primary cell or bringing up the first brood. The two sexes will share their reproductive and labour outputs in the female-female (FF) foundation colony. Such effects of male less foundation could impact the time a male partner was looking for, the deaths of foundress females and the

profitability of the initial colonies (Matsuura and Nishida, 2001; Vargo, 2019). Tandems behaviour among female and female will continue to search for male individuals to improve fitness. Prolong searches will increase the risk of predation and reduce the nutrient reserves needed for the first breeding. This trade would force individual females to stop mate searcher and make FF foundation work if prolonged searching costs and benefits are balanced. Because of this compromise, individual females would abandon their mate quest. Therefore, it is a reasonable measure and relative fitness of these three colony foundation types spent searching for a male (Matsuura and Nishida, 2001; Waidele *et al.*, 2019). It is estimated that more emphatic genetic diversity among the workers might be beneficial because it befools the deleterious effects of genetically incongruous mating, increases the efficiency of the division of workers in colonies and betters protection against pathogens and parasites (Su *et al.*, 2014; Vargo, 2019).

Termite gonads are placed in females and males in the last segments of the abdomen. Reproductive castes like primary and secondary reproductive have been reported to only fully mature, morphological, and physiological functional sex organs (Su *et al.*, 2015; Brent *et al.*, 2016). The genital cavity, accessory gland, spermatheca, fallopian tube and ramified ovary are part of the queen reproductive system, while it is inked with accessory gland, ejaculatory gland, seminal vesicle, vas deferens and the testis (Raina *et al.*, 2014). Swollen belly of the termite fell by many eggs on tubes, which is the typical sign for the matured ovarium. The ovary consists of several oocyte branches, which in turn are arranged in the ovary tubing. Secondary reproductive female expanded significantly because of matured ovaries in the abdomen. Sperm is transferred and stored in the spermatheca region of the adult female during male and female mating (Saran *et al.*, 2007; Yashiro and Lo, 2019). Sperm is released from spermatheca, to fertilize the ovum in the genital cavity expelled through birth channel (Raina *et al.*, 2014). If the eggs laid are not fertilized or parthenogenetic, they will not be able to grow and breed (Yashiro *et al.*, 2018). Mated females can reserve long-term sperm in spermathecas and use this sperm to fertilize ova in the body (Saran *et al.*, 2007; Dedeine *et al.*, 2016; Dion *et al.*, 2019). Termites demonstrate their eccentric diversity in the breeding system of the colony as the colony ages. Therefore, we investigate their breeding biology and frequency in an artificial environment. In the present study, we compare external morphology and internal gonads developments of the *Reticulitermes aculabialis*, alate(s) and the new kings and queen, and the mature king and mature queen (ergatoid) and neotenic (Johnson *et al.*, 2011). Differences between swarmer alates and reproductive alates and worker and reproductive worker (ergatoid) are quantified by measurements of many exterior features as

well as gonads and ovarians dimensions. Ratios of those measurements are evaluated for predictive utility (used to normalize the predicted differences in size and age of individuals). Swarmer alates and reproductive alates and worker and reproductive worker (ergatoid), gonads and ovarians are associated with age and live weight.

## MATERIALS AND METHODS

### *Swarmer season*

The reproductive male and female swarmers alate(s) naturally erstwhile in a year loom in April, May, and June in Xian, Shaanxi, China. Few swarms usually occur on a hot day after rain. It is conceivably an excellent time to swarm as the soil is soft after rain, and it is effortless for the reproductives (imagoes) to dig in (Liebenberg, 2013).

### *Samples, data collection and colonies foundation*

Termite samples were collected (May 2018) from termite house situated at Northwest University Xian (<http://english.nwu.edu.cn/>), then transferred from the field to the laboratory through plastic boxes (25×18×15 cm) and reared to June 2019. The individuals were subjected for inaugural colonies and make a total of 184 colonies; each colony corresponds to male×female 47.83% (88/184) and female×female 52.17% (96/184) alates. A small colony (5×3.5×2.5 cm) strategy were used for imagoes mating in plastic bottles (Haroon *et al.*, 2020).

### *Intercolonial interactions*

In order to inspect conflicts among colonies and *R. aculabialis* species, we made pairwise tests of female and male alates in the laboratory. We calculated the intercourses between colonies of intra-specific competition with their shared natural food and nesting resources. Additionally, the protocol for colonial exchanges, *Cedrus deodara* debris feed in each colony as nesting material (Xing *et al.*, 2013). The laboratory colonies (integral folks) were reared from 14 months enclosing upto 250 individuals in (14.5×12.5×1.9 cm) transparent glass-covered petri dishes (Haroon *et al.*, 2020). The colonies consisted of 2-5 female neotenic workers, all male and female reproductive workers, and 4-6 secondary reproductive workers and soldiers.

### *Colonial interaction between reproductive male and female workers*

A queen releases alluring pheromone and searching an appropriate nesting spot, where king ensuing tandem activity. Furthermore, during tandem behaviour, males piled up behind the female and eventually retired to the nesting site. The mating may occur in the nest area within hours or weeks of the pair being sealed and continue to mate for a

lifetime like ants (Alleman *et al.*, 2019). After 30-40 days, imagoes start laying eggs at room temperature (25°C).

### *The digging behaviour and egg-laying time*

Finally the permanent colony establishment was subjected in (14.5×12.5×1.9 cm) glass made petri dishes. In the early few days, colony growth has been slow, while after some days, the queen lay more eggs, or the secondary reproductives (ergatoid) often begin to lay eggs (Hopkins, 2003; Wako, 2015) and the response of termite queen will never leave the nest again. It becomes an egg-laying machine; therefore, the ergatoids queen, food and cleanness responsibility drop on workers heads (Wako, 2015). Ergatoids extract their eggs as they have driven; the abdomen of ergatoids will significantly puff from the eggs.

### *Colonial moisture and temperature*

The inaugural colony humidity of *R. aculabialis* (alates) is monotonous for mating and tunnels digging, considering in dripping stipulations, fungus snappily attacks alates. The permanent colony was observed randomly and watered when necessary, but mostly once/week (Ebeling *et al.*, 1959; Gouge *et al.*, 2009; Suiter *et al.*, 2009). The mature colony needs more humidity as inaugural colonies. Therefore, fifteen colonies were left untreated (water and dryness) and did not show a productive breeding system on room temperature (25°C) (Olfert *et al.*, 1993; Quarcoo *et al.*, 2019). Temperature also affects age because eggs and nymphs were found inactive during cold temperatures. Breeding biology of *R. aculabialis* was also reported petty at low temperatures (Xing *et al.*, 2013). There is a constant risk of drying out subterranean termites; that is why they must stay in the soil. Therefore, we revealed that soil has a long-term capacity to hold water and keep the colony moist (Quarcoo *et al.*, 2019).

Whenever termites forage over the ground, they must maintain their relationship to the soil so that workers and soldiers can return and replenish their body moisture regularly, the relationship of the soil to the termites formed by the mud tubes (Howard, 2006; Turbe *et al.*, 2010; Pervez, 2018). If the pipe is damaged, the worker termites will be compelling reconstructed. Accordingly, when the pipe is not reconstructible, dehydration will often result in the termites living above the ground; however, sometimes subterranean termite colonies are structured ground up. Such infestations above ground are found almost exclusively in buildings with excessive humidity problem (Pervez, 2018). The main problems with moisture usually arise from the accumulation of leaves and moisture on a flat roof, leaking pipes in the system, and poor ventilation in dragging regions, where the colony lives on the ground endless (Zungoli and Benson, 2004; Gold *et al.*, 2005).

### Statistical analysis

The measurement and counting results were subjected to analyze statistically using SAS 9.1 (ANOVA, MANOVA, Correlation Analysis) and XLSTAT. Results at the ( $p < 0.000$ ) level were considered significant. PCA and cluster correlation (similarity index) rate was reported at ( $p < 0.005$ ) significantly.

## RESULTS AND DISCUSSION

A total of 184 *R. aculabialis* colonies were subjected and reared at room temperature under darkness. Termite workers probably allure type larvae of termites; like many insects, they do not have callous (hard) exoskeletons. The king and queen are the only fully mature termites in the complete colony; even the other reproductives are neotenic (sexually mature), but considering larval. Mature male and female reproductive workers were lighter ( $0.05/\text{live weight g}$ ) ( $p < 0.005$ ) than mature queen. Typical sterile workers ( $0.042\text{g}$ ) and newly mature reproductive ( $0.043\text{g}$ ) workers have no conflict in weight ( $p < 0.005$ ) (Fig. 1A, B); this may not always be the case. A colony may be a single-family headed by one or two neotenic

and one primary reproductive if the reproductive pair is the offspring of all individuals in the colony. Therefore, neotenic cannot independently locate colonies, for a simple family to lead one or two neotenic would have to replicate for a sufficiently long period to replace any offspring of primary reproductives that they substitute. Such cases are most likely unusual given the relatively long lifespan of many termite workers and soldiers.

Alates and reproductive alates differentiated according to morphological differences, the body length (total length  $10.919\text{ }\mu\text{m}$  and  $4.965\text{ }\mu\text{m}$  actual length) and width ( $1.177\text{ }\mu\text{m}$ ) of reproductive male and female alates were significantly ( $p < 0.000$ ) heavier than a typical male and female alates ( $8.919\text{ }\mu\text{m}$ ). The fertile reproductive termites body escalate on the morphological sign of maturity. The head, eyes and mandibles of alate(s) (male alate HL,  $0.984 \pm 0.003$  and  $0.979 \pm 0.002$  male reproductive alate) (female alate EL:  $0.114 \pm 0.002$  and female reproductive alate  $0.111 \pm 0.002$ ) and (male alate MD:  $0.117 \pm 0.001$  and male reproductive alate  $0.115 \pm 0.001$  and female alate  $0.113 \pm 0.001$  and  $0.112 \pm 0.001$  female reproductive alate) were significantly higher ( $p < 0.001$ ) than reproductives alates (Table I).

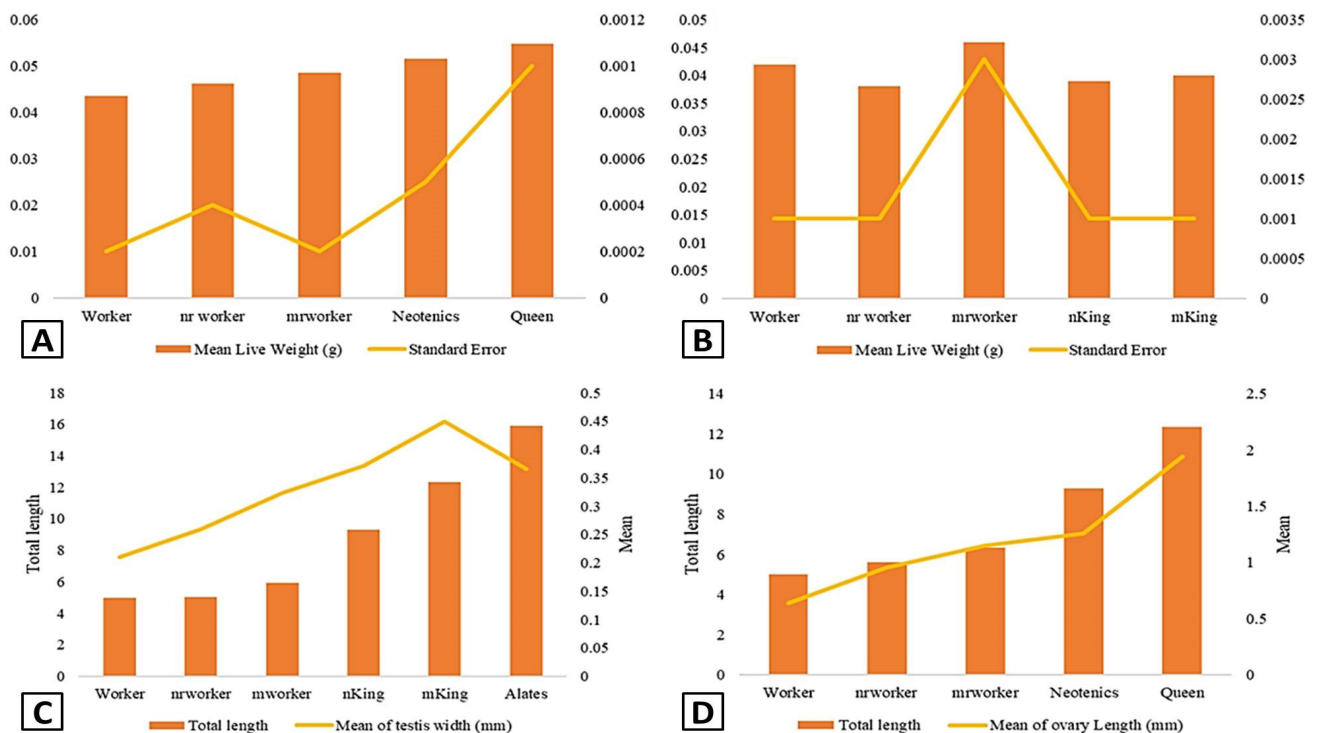


Fig. 1. A, the comparison of mean live weight (g) of the worker, new reproductive worker (nrworker), mature reproductive worker (mrworker), neotenic and queen; B, the comparison of mean live weight (g) of the worker, new reproductive worker (nrworker), mature reproductive worker (mrworker), new king (nKing) and mature king (mKing); C, the comparison between the total length of different species and the mean of the testis; D, the comparison between the total length of different species and mean of ovary length.

**Table I.- Morphological differences between swarmer alates and reproductive alates (Mean±SEM).**

	Male alates	Male reproductive alates	Female alates	Female reproductive alates
EL	0.114±0.001	0.115±0.002	0.114±0.002	0.111±0.002
HL	0.984±0.003	0.979±0.002	0.977±0.001	0.974±0.001
HW	1.117±0.005	1.002±0.001	1.001±2.341	1.001±2.341
LL	0.623±0.001	0.62±0.002	0.618±0.001	0.617±0.001
LW	0.102±0.165	0.058±0.001	0.057±0.001	0.056±0.001
MD	0.117±0.001	0.115±0.001	0.113±0.001	0.112±0.001
PL	0.865±0.004	0.862±0.004	0.859±0.004	0.858±0.004
PWW	1.006±0.003	1.002±0.002	1.001±0.000	1.001±2.341
PWN	0.219±0.004	0.219±0.004	0.218±0.004	0.219±0.004
TDS	0.035±0.002	0.419±0.003		
TDL	0.044±0.003	0.425±0.002		
OL			1.547±0.005	1.549±0.005
OWM			0.496±0.002	0.498±0.002
OWW			0.998±0.001	0.999±0.000

EL, eye length; HL, head length; HW, head width; LL, labrum length; LW, labrum width; MD, mandible length; PL, postmentum length; PWW, postmentum width at widest point; PWN, postmentum width at narrowest point; TDS, testis diameter smallest; TDL, testis diameter largest; OL, ovary length; OWM, ovary width midpoint; OWW, ovary width widest point.

**Table II.- Morphological differences between worker and reproductive worker (Mean±SEM).**

	Male worker	Male reproductive worker	Female worker	Female reproductive worker
EL	0.115±0.000	0.112±0.000	0.117±0.000	0.112±0.000
HL	0.978±0.000	0.976±0.001	0.979±0.000	0.976±0.000
HW	1.007±0.000	1.005±0.000	1.009±0.000	1.003±0.000
LL	0.623±0.000	0.621±0.000	0.626±0.000	0.622±0.000
LW	0.061±0.000	0.059±0.000	0.063±0.000	0.057±0.000
MD	0.117±0.000	0.114±0.000	0.118±0.000	0.115±0.000
PL	0.864±0.001	0.862±0.001	0.865±0.001	0.861±0.001
PWW	1.008±0.000	1.006±0.000	1.008±0.000	1.001±0.000
PWN	0.225±0.001	0.223±0.001	0.226±0.000	0.223±0.000
TDS	0.422±0.001	0.425±0.001		
TDL	0.423±0.000	0.427±0.000		
OL			1.551±0.002	1.555±0.001
OWM			0.505±0.000	0.508±0.000
OWW			0.508±0.000	0.511±0.000

For abbreviations, see Table I.

Morphologically ergatoid reproductives body length (9mm) and width (4.20mm) were significantly ( $p<0.000$ ) higher than typical male and female workers (length 5mm and 2.65mm width). The ergatoid reproductive actual body larger than other castes, and workers feed (trophallaxis) to the mature king and queen. Worker head, eyes and mandibles (male worker HL,  $0.978\pm0.000$  and  $0.976\pm0.001$  of male reproductive worker; female worker EL:  $0.115\pm0.000$  and  $0.112\pm0.000$  of the female reproductive worker; male worker MD:  $0.117\pm0.000$  and  $0.114\pm0.000$  male reproductive worker whereas the female worker MD:  $0.118\pm0.000$  and  $0.115\pm0.000$  female reproductive worker) were significantly higher ( $p<0.000$ ) than reproductives male and female workers and alates. The male reproductive testis (male worker TDS:  $0.422\pm0.001$  and  $0.425\pm0.001$  male reproductive worker; male worker TDL:  $0.423\pm0.000$  and  $0.427\pm0.000$  male reproductive worker) reported significantly ( $p<0.000$ ) than (male alate TDS:  $0.035\pm0.002$  and  $0.419\pm0.003$  male reproductive alate; male alate TDL:  $0.044\pm0.003$  and  $0.425\pm0.002$  male reproductive alate) and the ovaries of female reproductive (female worker OL:  $1.551\pm0.002$  and  $1.555\pm0.001$  female reproductive worker; female worker OWM:  $0.505\pm0.000$  and  $0.508\pm0.000$  female reproductive worker; female worker OWW:  $0.508\pm0.000$  and  $0.511\pm0.000$  female reproductive worker) reported significantly ( $p<0.000$ ) than female and reproductive alate(s) (female alate OL:  $1.547\pm0.005$  and  $1.549\pm0.005$  female reproductive alate; female alate OWM:  $0.496\pm0.002$  and  $0.498\pm0.002$  female reproductive alate; female alate OWW:  $0.998\pm0.001$  and  $0.999\pm0.000$  female reproductive alate) (Table II).

The mean testis with (mm) comparison among individuals indicates that testis width directly proportional to the body length. Therefore, we concluded that the reproductive alate(s) soldiers testis width (0.45mm) were reported significantly ( $p<0.0001$ ) than other castes. While the swarmer alates have a compact body (help in nuptial flight) with broad and elongated wings. Consequently, the testis width was reported shorter than imgeos and equal to the new king (nKing) (Fig. 1C).

The morphological measurement shows the mean ovary length (2.2mm) significantly ( $p<0.0001$ ) more considerable and broader mature and swarmer alate(s) (Fig. 1D). If the inaugural colony queen dies, neotenic-producing colonies will replace primary reproductive; as a result, the variations in the breeding biology in the colony are reported smoothly. Colonies of these species either do not live long enough to produce neotenic and probably the inbreed colonies are inferior to the outbreeding colonies. Similarly, the mKing, mean of testis with (0.34mm) was reported significantly ( $p<0.004$ ) as compared to workers, which mostly affects the number of gonads during longevity through the life span (Fig. 2A-L).

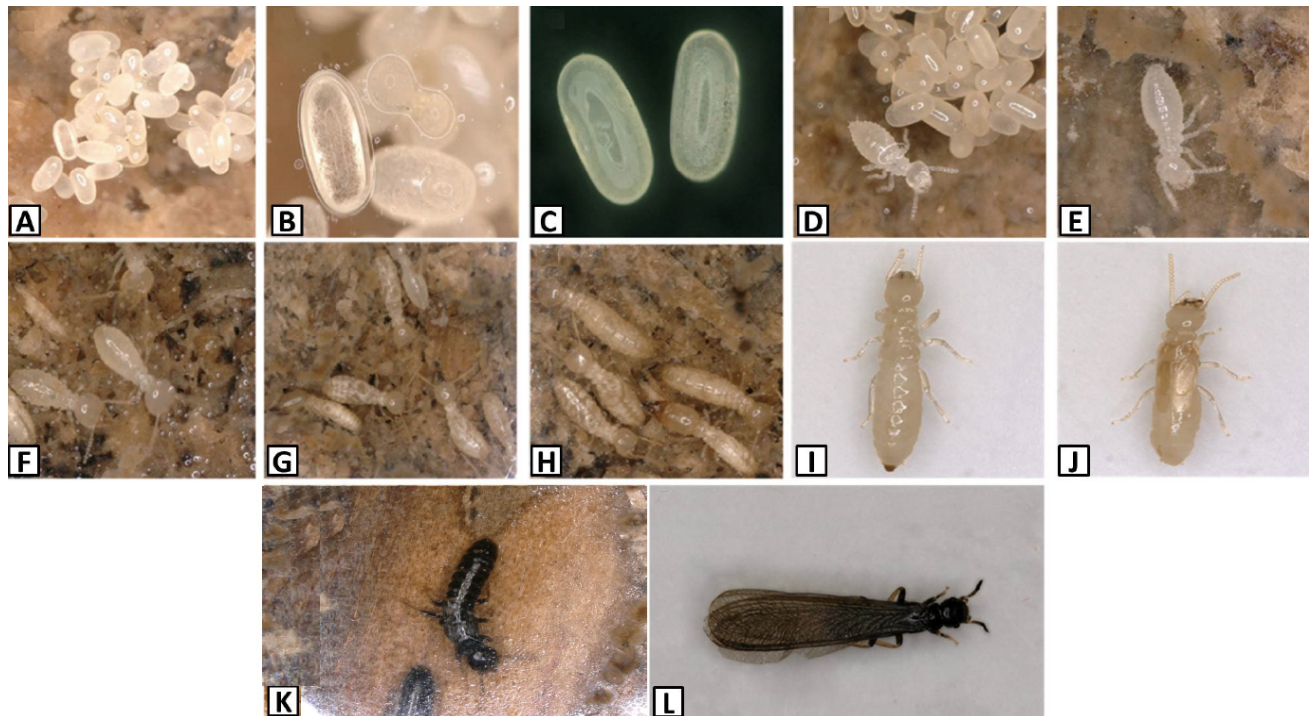


Fig. 2. The life cycle of *R. aculabialis*: A, cluster of termite eggs; B, cell division in termite eggs; C, the primitive streak; D, 1<sup>st</sup> instar nymph; E, 2<sup>nd</sup> instar nymph; F, 3<sup>rd</sup> instar nymph; G, 4<sup>th</sup> instar nymph; H, primary reproductive; I, neotenics; J, mature neotenics; K, newly reproductive male and female Alates; L, swarmer alate.

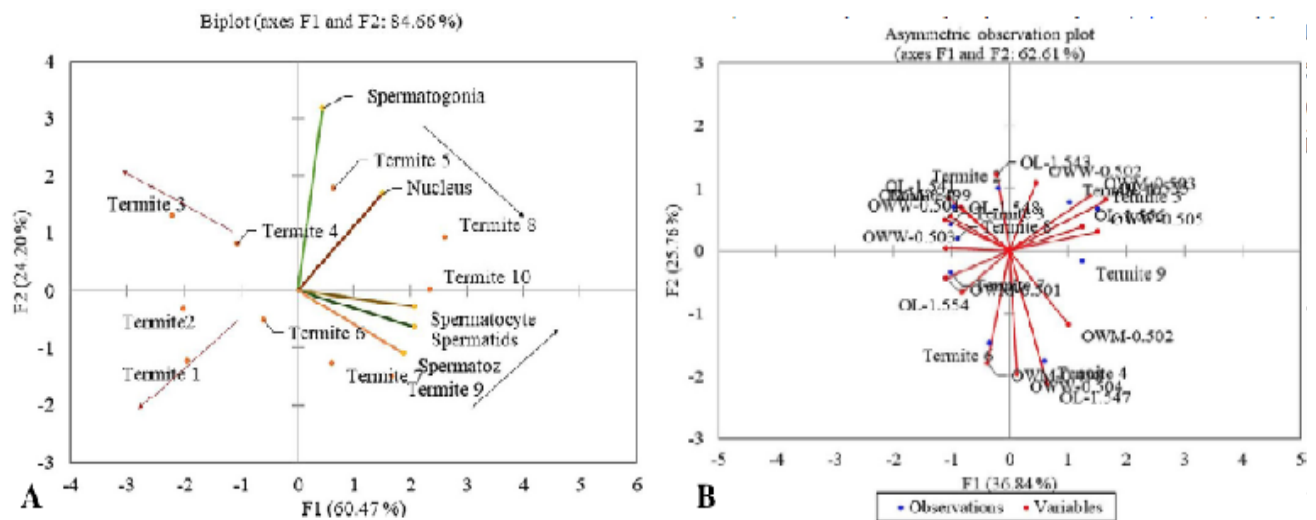


Fig. 3. Principal component analysis (PCA) dimension (F1 and F2) represent the horizontal (F1) and vertical (F2) factor analysis of primary and secondary male (A) and female (B) reproductive gonads.

The testis mean of primary male reproductive alate(s) and worker was reported less fertile than the secondary male reproductive termite worker, which is fully mature due to ageing. The arrows (black in colours) indicate the increase of the secondary male reproductive worker and

the red arrows indicate the decrease of the size of the primary male reproductive worker (Fig. 3A). Although, the fecundity ratio increases during ageing of the queen. Similarly, ovary length and width of the mature queen reported significantly ( $p < 0.005$ ). There were no statistical

differences between the newly reproductive queen and the mature queen (Fig. 3B).

The correlation similarity index between species is shown in Figure 4, they were divided into two main groups male and female reproductive workers (alate(s), prQueen, seQueen, nQueen and mQueen) and non-reproductive male and female workers (1<sup>st</sup>-5<sup>th</sup> larvae). Reproductive workers are closely related to each other in the morphological similarity index and non-reproductive as well. Longevity increases from the non-reproductive toward reproductive workers (queen).

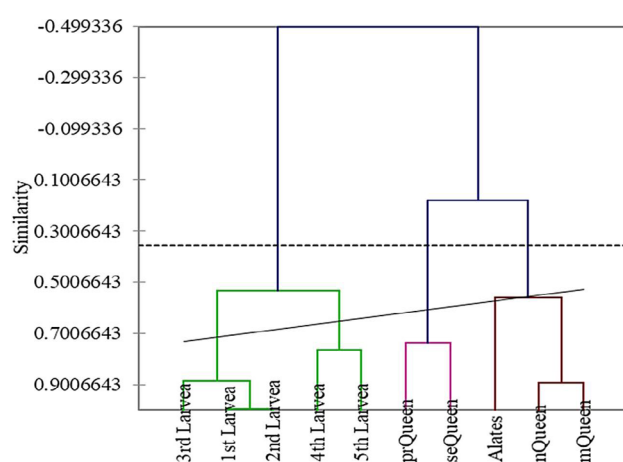


Fig. 4. The similarity, proximity matrix (Pearson correlation coefficient). The bar indicates the longevity.

The morphological parameters of reproductive castes were reported during the study, including eggs, 1<sup>st</sup>-5<sup>th</sup> instar (nymphs) larvae, primary reproductive workers, secondary reproductive workers, mature king and queen. The results show that the queen and king have a unique fatty body that mainly helped in longevity and fecundity.

Kobayashi and Miyaguni (2016) studied the facultative parthenogenesis in *Neotermes koshunensis* termite and reported that the founders male×female (FM) colony had slightly higher survival rates as compared to those of the female×female (FF) inhabitants. Focusing on the colonies where one or both of the founders survived to the end of the experiments, FM colonies produced more eggs and larvae than FF colonies. Although the first egg was found for colony species simultaneously (10<sup>th</sup> day), the first hatch was recorded for the FM colonies on the 60<sup>th</sup> day and the FF colonies on the 70<sup>th</sup> day. The estimated hatching rate for FM colonies was  $0.88 \pm 0.05$ , approximately half  $0.52 \pm 0.07$  for FF colonies. Whereas, from the present study, *R. aculabialis* laid egg after 37 days of the colony foundation. We subjected a total of 184 colonies, and these colonies were consisting of FM 47.83%

(88/184) and FF 52.17% (96/184) alates. The reproductive rates were significantly higher in FM ( $p < 0.000$ ) colonies rather than FF colonies. The replacement of queens in *R. aculabialis* is not accidental; the queen often disappears while the primary king remains alive and active, and the parthenogenetic production of female neotenics is evidence that the queen anticipates her succession.

Furthermore, the parthenogenetic nymphs were almost systematically present with physiologically reproductive primary queens. However, there are two singularities in the dynamics of asexual queen succession (AQS) in *C. tuberosus*: first, the main queen was found more commonly than neotenic in field-collected colonies (41% vs 29%), including in mature colonies (62% vs 21%); second, all *C. tuberosus* neotenics were small and non-physogastric. The approach with assumption that AQS boosts the colony development rate, which would otherwise restrict the maximum egg-laying capacity of the main queen while spreading her genes and avoiding inbreeding expenses (Fournier *et al.*, 2016). Likewise, from the present study, the founded colonise was entailed FM (47.83%) and FF (52.17%), respectively. The fecundity capacity was significantly reported in FM colonies, while the FF colonies have an inadequate number of the egg-laying ability of the new reproductive queen. The survival rate of parental pairs (imagoes) reported poorly because, after mating and egg-laying, the parental pairs found dead, while some alates were survived more than eight months.

Su *et al.* (2015) reported fundamental differences in *R. labralis* reproductive ability of sperm transfers to queens and stored in the spermatheca (Wu *et al.*, 2014). The standard methods to understand a structural differentiation of sperm, testis, accessory glands and spermatheca are considered morphological observations and morphometric analysis in *Reticulitermes* species. Therefore, for the description of the morphological comparison of testes, sperm, vas deferens, accessory gland of *R. aculabialis*, we considered dissection and slice formation to this study useful. The mid-intersegmental (7<sup>th</sup>-8<sup>th</sup>) membrane testicles dissected by Wu *et al.* (2014) and Laranjo *et al.* (2018) divided the testis into an apical mid-cross-section, mid and lower section according to cells function. The top zone has spermatogonial cells that rest in the plasmosoma close the nucleus with the intensely stained body. The same results were also reported in the current study. Reproductive king queen was significantly ( $p < 0.000$ ) found as mature castes.

Moreover, mature reproductive king and queen survival rate was found significantly ( $p < 0.000$ ). The kin selection is the overarching principle of social behaviours such as evolved and practical; individuals will choose to support the offspring of a close relative rather than reproduce themselves (Strassmann and Queller, 2010).

Therefore, biologists were working under the endeavour of family selection to understand how social insects societies operate and why social behaviours have evolved as they have done. The inspiration for studying these animals comes from parallels that have been observed between insects and human societies. Social insects form integrated and collaborative communities with a relatively simple mind, with a clear division of labour that emulates human organization.

## CONCLUSION

*Reticulitermes aculabialis* colonies were found intensely populated under the experimental observation. Therefore, from the present research, we concluded that the gonads and ovaries ratio of secondary reproductive (worker) king and the queen was found (male reproductive worker TDS  $0.425 \pm 0.001$  and TDL  $0.427 \pm 0.000$ ; ovaries of female reproductive OL  $1.555 \pm 0.001$ , OWM  $0.508 \pm 0.000$  and OWW  $0.511 \pm 0.000$ ) significantly ( $p < 0.000$ ) than imagoes and helpful for longevity, fecundity and reproduction. According to the observed phenomenon, female and male (FM) colonies produce the maximum number of egg, this variability in breeding systems has increased rapidly, evolutionary and ecological factors such as temperature, population density, pathogenic stress, nesting habits, food resources (wood, soil, fungi) and phylogenetic relationships have increased. However, the variability in breeding systems is prevalent in social insects, providing a desirable community for insight into the strengths that shape the breeding systems in social insects.

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### Statement of conflict of interest

The authors have declared no conflict of interests.

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