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Research Article
Mating Success and Reproductive Behavior of Black Soldier Fly *Hermetia illucens* L. (Diptera, Stratiomyidae) in Tropics

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Abstract

Background and Objective: The Black Soldier Fly (BSF) is known as an alternative source of protein for feed. The limiting factor of sustainable production is the ability to produce a sustainable number of eggs. This study was aimed to understand the BSF mating behavior in tropical areas which necessary to suffice its mass production. **Materials and Methods:** The BSF mating activities, kept in outdoor screen cages in the dry season, were done during the breeding period for every 14 days, from 06:00 to 18:00. Data were analyzed according to descriptive statistical parameters. **Results:** The fly mating activities commence at the age of two up to eight days in good weather when the temperature begins to rise to 27-29°C, approximately from 09:00 to 14:00. Peak mating activities occurred when the intensity of the sun is at its highest between 11:00-12:00 and two to three days after emergence, with a mean duration of mating is 36 min. The sequence of BSF mating behavior is a stereotype, but leaking behavior appeared during copulation. Egg oviposition began on the 4th day after emergence, peaking at 13:00-14:00 and lasting until 17:00. The oviposition period ranged from four to ten days with a number of oviposited eggs ranged from 569 to 650 eggs per female. **Conclusion:** The BSF can perform optimal mating and reproductive behavior when maintained semi-outdoor conditions under direct and full sunlight in tropics. Albeit, the critical part of BSF mating behavior was during attempted copulation.

Key words: Black soldier fly, mating sequence, reproductive behavior, tropical areas

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Black Soldier Fly (BSF) is a tropical fly and cosmopolitan species with great economic benefit and environmental potential impact for the processing of various organic waste and byproducts into insect-based products[1,2]. The BSF larvae can consume varied types of biodegradable materials including food waste, animal manure and some indigestible organic waste[3-6]. The BSF larvae can reduce accumulations of organic waste by up to 50% in a short time, which makes it ideal for optimal reduction of environmental pollution[7] while the biomass produced, by this process, applicable as a source of protein for animal feed[8]; fatty acids for biofuel[9]; minerals[10] and chitin and chitosan[11] for production of various types of bio-based products. This species also can be reared in the container which made them applicable for mass production[12]. Thus, the continuous availability of BSF populations for organic waste treatment and bio-industrial installations is very important. The sustainability of the population is strongly determined by the success of mating and reproduction[13] which influenced by the nutrient storage during the larval period[14,15].

During the rearing period, the larval stages are quite easy to maintain but obtaining successful mating is challenging since special conditions are required and influenced by many factors[16]. Several studies have shown that mating success and reproductive behavior in BSF are influenced by abiotic and biotic factors, such as light intensity[6,17,18], relative humidity[19], temperature[20], cage size and space[16,18] and the density of adult flies during mating[21].

Notwithstanding there are several industrial level BSF productions in Indonesia, but most of the largest industries and most advanced studies on BSF are conducted at the temperate region where BSF is not active during winter months which creates the necessity for indoor rearing under artificial light sources[6]. Unlike the temperate region, Indonesia is a tropical country located on the equator with yearly sunlight of up to 60.000-80.000 lux[22] and solar irradiation[23] ranging between 4.6-7.2 kWh/m^2. This means that Indonesia has sunlight available throughout the year and can strongly support BSF rearing activities since mating can take place throughout the year, thus making BSF reproduction is more efficient and sustainable. Previous studies on the mating behavior of BSF have shown that males are attracted to the "calling" behavior of females in the same resting area and that mating takes place on land with male and female body formations aligned in opposite directions[24] and other finding reported that pair of BSF can mate in the air or while flying[25]. The BSF mating behavior and courtship are similar to other stratiomyid species; for example, the same-sex male interaction and behavioral signals in females which can be followed by making a bodily formation with a female in opposite directions for copulation[25]. Following mating, the female fly oviposits their eggs and occurs over a limited period due to the physiological changes of both the males and females over time, which affects reproductive performance and population growth[26]. The aim of this study was to presents a unique observation from previous research; namely, regarding the sequence of mating success and reproductive behavior of BSF in semi-outdoor conditions that represent tropical areas.

MATERIALS AND METHODS

Study area: The research was conducted for three months during the dry season, from June-August 2019, at two different places with 10 km apart in distance in Bandung city, West Java, Indonesia. The first site under semi-outdoor conditions in a screen house which was located in the integrated gardens of the Faculty of Science and Technology of UIN Sunan Gunung Djati Bandung (6°55' 51.089", E 107°43' 4.670") at an altitude of 679 m. A second screen house was established on the fifth floor (the rooftop) of the building of School of Life Sciences and Technology (SLST), Institut Teknologi Bandung (S 6°53' 34.548", E 107°36' 39.6324") at an altitude of 770 m.

Mating and oviposition behavior of black soldier fly: The BSF eggs were obtained from adult populations of colonies maintained by Laboratory of Entomology, SLST-ITB and kept in 60×60×60 cm cages with 1.5 mm mesh screen nylon netting which were placed inside screen houses with direct sunlight. Eggs were kept in plastic boxes (50×50×15 cm) containing commercial chicken feed (brand HI-PRO-VITE) which had been mixed with water as a medium for hatching eggs as well as larval rearing. The medium was successively added daily to maintain the adequate moisture content of about 70% for larval development[27]. Chicken feed was applied ad libitum until larval development reached at least 50% of larval reached the prepupa stage, characterized by the black larval cuticle. Uniform-sized pupae were collected, placed in containers and put in cages for safekeeping until they emerged as adult BSF to observe mating and reproductive behavior.

Observations of mating and reproductive behavior of BSF were conducted by continuous behavioral sampling using up to 50 pairs of newly-emerged adult BSF. Next, this newly adult

BSF were kept in a small cage 60×60×60 cm, placed on a screen 4×4×4 m at the first location and 3×3×3 m at the second location, respectively and reared with five replications of each cage for a total of more than 500 pairs of BSF. Observation of mating behavior cycle was carried out every day for 14 days from 06:00 to 18:00, for 30 min periods at a one-hour interval during daylight hours each day. The adult rearing cage was equipped with a water source and an ovitrap placed near a container filled with decaying organic medium (mixed vegetable and fruit waste) as a stimulant for the females to lay eggs. The ovitrap was made of 4 woods or pieces of cardboard (length 20 cm, width 5 cm, height 1 cm) with gaps or small spaces. Ovitraps were replaced daily and the numbers of eggs were recorded. The parameters observed and calculated include: (a) sequences of mating behavior, (b) daily mating frequency, (c) oviposition behavior, (d) the average number of eggs, (e) the average mass of eggs, (f) the development time for each stage and (g) the longevity of adults. Data were analyzed descriptively on average from five replicates.

Measurement of environmental factors: Measurement data of environmental factors in the screen house were obtained hourly from 06:00 to 18:00 for 14 observational days. The parameters included: average daily temperatures (°C); relative humidity (%), using the HTC-1 digital humid-thermometer; and sunlight intensity (Lux), using Lutron LT (lux LX-101 A) lux meter.

RESULTS

Overall, the environmental factors inside the screen houses during observation are as follows: daily temperature means of 27.9°C with successive ranges of 26.4-27.9°C and hourly temperature means of 27.3°C with successive ranges of 22.9-30.5°C. The highest temperature occurred at the afternoon around 13.00-14.00. Average daily sunlight intensity was 6069.8 lux and fluctuated between 4915.8-7755.8 lux and hourly sunlight intensity means of 6158.6 lux with successive ranges of 436.8-13617.5 lux (Fig. 1). The highest sunlight intensity occurred at noon around 11.00-12.00. Additionally,

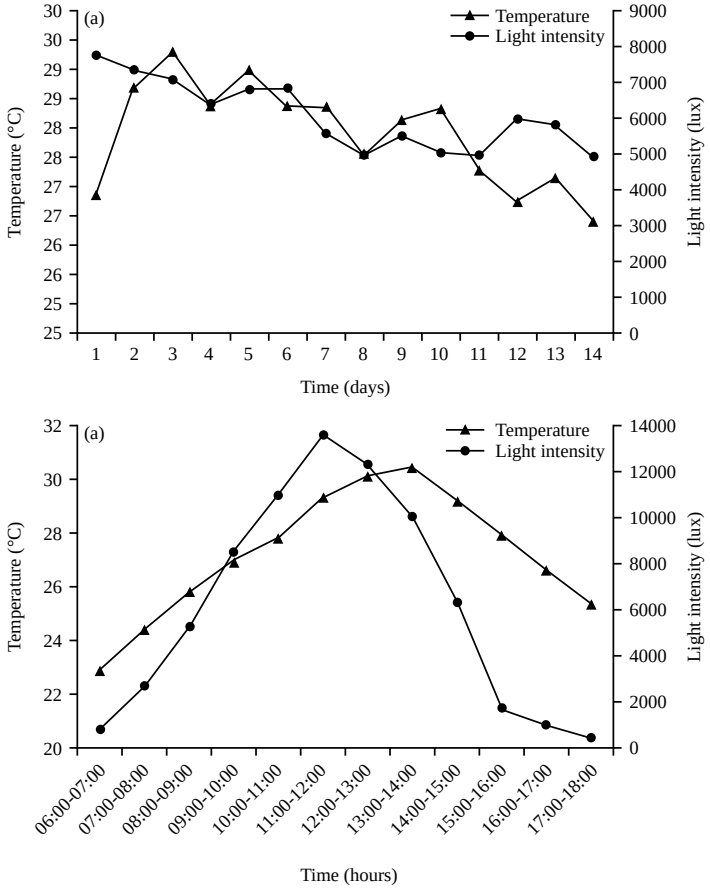

Fig. 1(a-b): (a) Daily temperature and light intensity measurements during observation time and (b) Temperature and light intensity per hour during the observation time

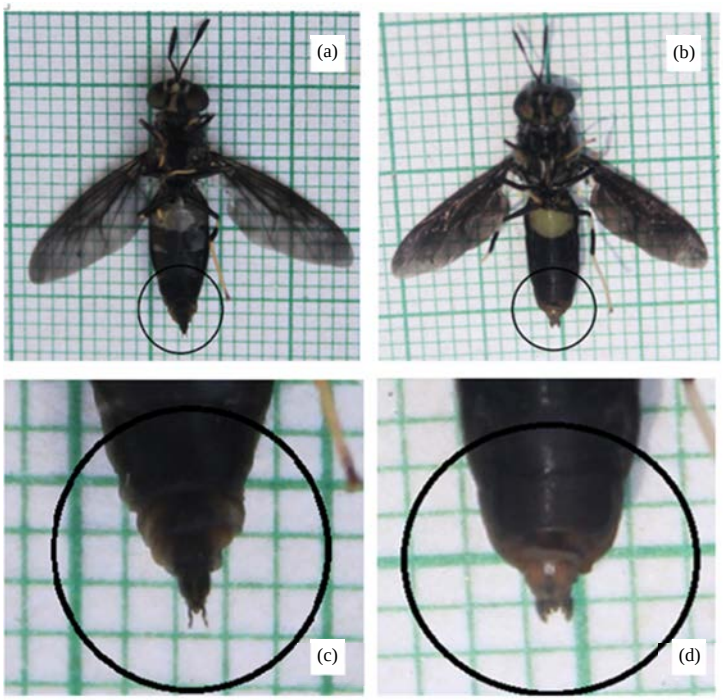

Fig. 2(a-d): Characteristic differences between male and female BSF, (a) female individual, (b) male individual, (c) end of the female abdomen and (d) end of the male abdomen

Fig. (3a-b): BSF lek (aggregation) behavior results in (a) Male groups and (b) Combination of male and female groups

daily relative humidity means of 80.2% with successive ranges of 73.2-85.8% and hourly relative humidity means of 80.2% with successive ranges of 73.1-91.1%.

Mating behavior of the black soldier fly: Black soldier fly gender can be determined by observing the endpoints of the abdominal segment of the adult insect (Fig. 2a-b). Females tend to have sharp abdominal tips, ending with ovipositor lines that have two strands similar to retractile tubular oviducts which function as genital organs as well as organs for laying eggs during the oviposition in the post-mating period (Fig. 2c). Male BSF are characterized by the presence of the aedeagus in the abdominal tip as a male reproductive organ that is equipped with a pair of hooks which serve to grip the female genital organs so that the flies are interlocked during copulation (Fig. 2d).

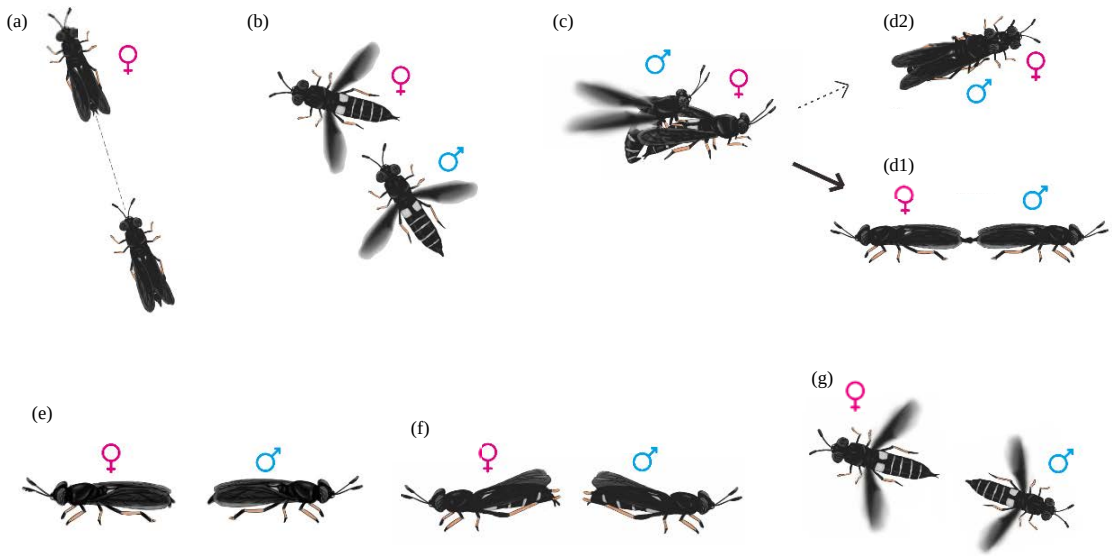

Fig. 4(a-g): BSF mating sequences (personal drawing), (a) Orientation, approaching and following (♂ to ♀), (b) Chasing (in-flight approach) and attempt mounting, (c) ♂ Attempt copulation: wing fanning, abdomen curling male mount the female, tap the female abdomen with the tarsi, ♀ Wings extention, decrease locomotion, (d) D1: Reverse coupling, D2: copulation, genital contact and spermtransfer, antennal waving (♀ and ♂), (e) End copulate/end genital contact, (f) Grooming with the tarsi of its third pairs of legs and (g) ♂ and ♀ move away, walking then flying

On the first day after emerging from its pupa case, the newly adult BSF is very fragile with its wings still folded and the wings will be fully opened 2 a few minutes later. Once the sunrise and the weather warms up, the adult BSF fly immediately and become more active with their dominant activities are walking, resting and grooming which is accomplished by moving the hind legs and front legs towards the head and wings. The day after (the 2nd day), the courtship ritual begins and BSF starts to display the initial activity in a series of mating sequences with males starting to perform lek behavior characterized by a gathering, or males taking part in competitive mating displays, while sometimes females are also involved. Several males flying together (aggregation) in groups which can result in piled-ups with each other, bend the tips of their abdomens one another and opening the hooks on their aedeagi while wing-fanning (Fig. 3). This aggregational behavior was performed either by all-male (same-sex interaction) or by males and females together (mixed-sex interaction) with the number of male individuals higher than females. The aggregation behavior or lek behavior starts with the males chasing each other while flying and then perching on the ground, or all of the stages of aggregate behavior taking place on the ground. Lek behavior increased in the larger rearing cages, with 4-12 individuals in a group exhibiting aggregation, compare with the smaller cages.

Based on the observations of mating behavior inside the rearing cages, the sequence of successful mating behavior in the BSF is as follows (Fig. 4), orientation, male approaching and following a female while flying (Fig. 4a), chasing, the male chases, intercepts and captures the female in mid-air. Both may descend to the ground or remain on the vertical side with the male's legs gripping the female dorsal thorax area (Fig. 4b), copulation attempt, the male vibrate its wings (wing-fanning) while bending the tip of its abdomen, exposing the aedeagus, opening its hooks and trying to connect with the tip of the female abdomen. The receptive female will stop moving, open both wings and receive the male aedeagus. Non-receptive females will engage in rejection behavior by wagging their wings, which releases the male grip, continue locomotion and try to fly away to avoid the male. This part is the most challenging behavior since it may not continue to the next step even though the male struggles hardly to grip the female in an attempt to perform copulation (Fig. 4c), copulation, the connection both sexes genital until sperm successfully transfer from the male reproductive tract to the female reproductive tract ((Fig. 4d). During copulation, both antennae of male and female are moving, though the female body remains static. The copulation model for BSF in this study occurs in two forms: (1) reverse coupling (Fig. 4d1), this occurs when the male genitals are successfully connected

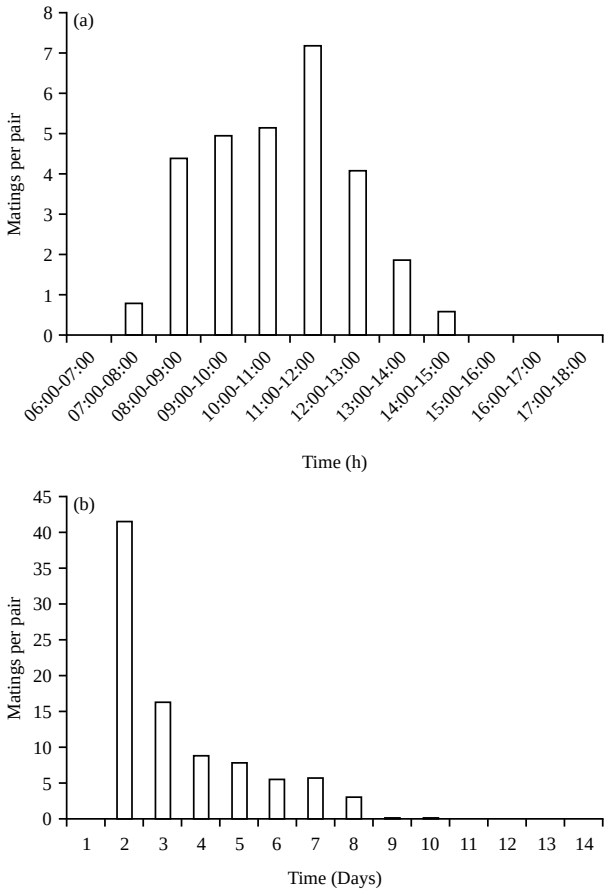

Fig. 5(a-b): BSF daily mating activity, (a) Mean number of mating pairs per hour and (B) ean number of mating pairs per day

Fig. 6(a-b): Oviposition behavior of BSF females, (a) Eggs were placed in gaps in the ovitrap and (b) Eggs were placed outside the ovitrap (nylon netting of the cage)

to the female genitals; the male will rotate 180° its body so that pairing occurs in opposite directions; (2) mounting (Fig. 4d2), where the male is close to the dorsal part of the female, its legs attached to both the thorax and abdomen with the aedeagus remaining connected to the tip of the female abdomen. This form is rare and occurs in only 10% of all mating pairs. The BSF mean duration of copulation is 36 min, ranged from 28 to 48 min, Fig. 4e) end copulation, characterized by the loss of contact between both male and female genital organs, particularly when the male retracts its aedeagus at the end of copulation session (Fig. 4e), grooming, when both male and female use their tarsi of the hind legs and stroke the abdomen tips (Fig. 4f), move away; when the male and female walking rather than flying and move away from each other (Fig. 4g).

Most failure in these sequences of mating behavior occurred at stage C and this condition forced the male start the attempt behavior from beginning. Competition among males are also seen, once a male had successfully copulate with a female, other male try to seize the female and this may occur several time.

In this study, the BSF mating activities took place when the weather was clear and sunny with the sun begins to warm up at around 08:00 and lasting until 14:00 (Fig. 5a). The peak mating activity occurred at 11:00-12:00, which was indicated by the highest frequency of copulating pairs. The average longevity of the adult BSF is 12-14 days, while the mating period occurs on the 2nd day and lasts until the 8th day when adult flies exhibit a declining tendency towards mating. During the observation period, the average total number of matings that occurred from five replications was 89 pairs (Fig. 5b).

BSF oviposition behavior: At First, BSF females explored the area around the ovitrap to find an appropriate location and then laid their eggs in groups inside gaps in the ovitrap by extending their ovipositor (Fig. 6a). However, some females laid eggs outside the ovitrap in the nylon netting of the cage (Fig. 6b).

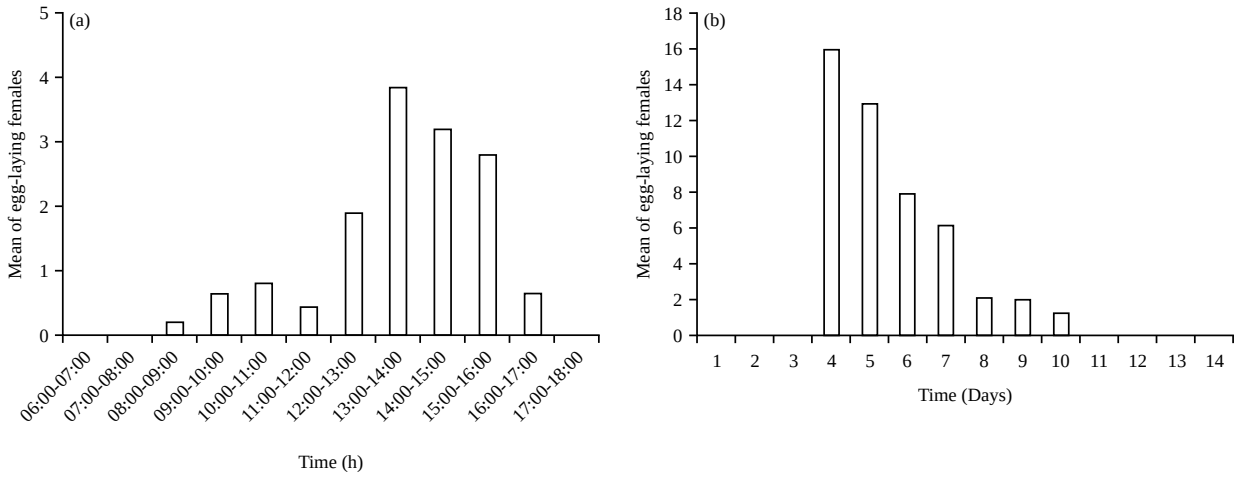

Fig. 7(a-b): Daily female BSF oviposition activities (a) Mean of egg-laying females in terms of hours (b) Mean of egg-laying females in terms of days

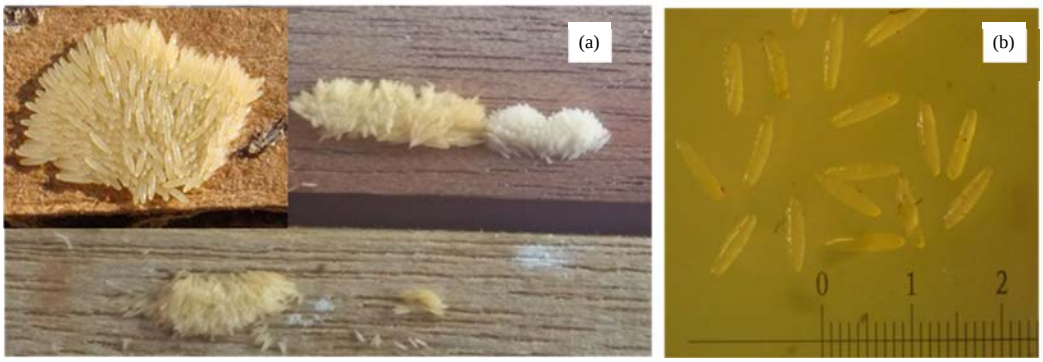

Fig. 8(a-b): BSF eggs (a) Egg clusters placed inside the ovitrap and (b) Observation of individual eggs under the microscope

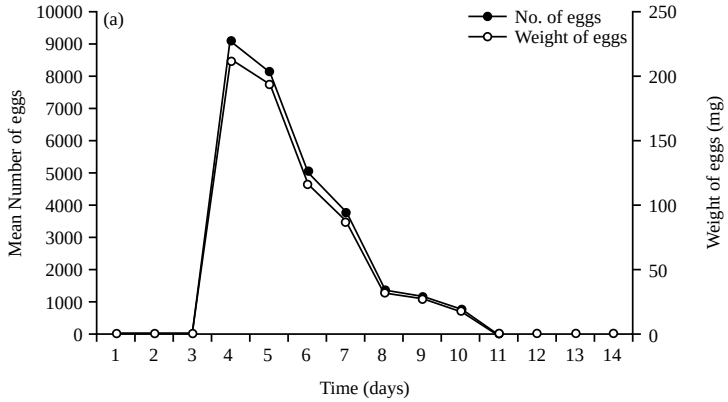

Fig. 9: Daily number of BSF eggs and weight of eggs.

Female oviposition activities began on the fourth day after emerging from the pupa or two days after mating, starting at 08:00 to 17.00 and peaking at 13:00-14:00 (Fig. 7a). The oviposition period ranged from four to ten days with an approximate average number of 48 egg-laying females (Fig. 7b).

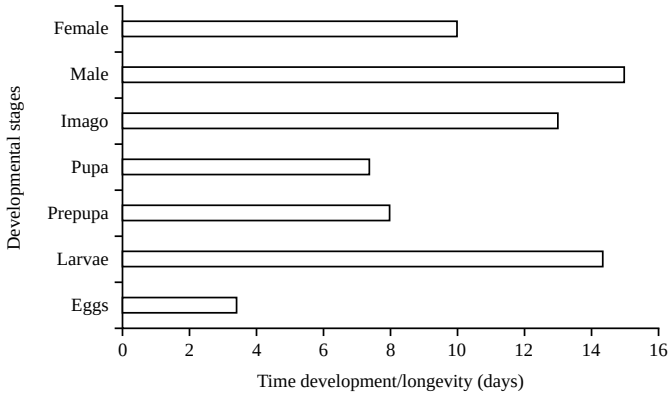

Fig. 10: The average development time of each stage of BSF reared on chicken feed as the larval medium in the semi-outdoor screen house

Eggs from each female were placed in clusters and each consisted of 500-900 eggs. Eggs are oval with 1 mm long and are in pale white turning to yellowish as incubation time continues (Fig. 8). The eggs hatch between 4-7 days of incubation period, depending on ambient temperatures. The total number of eggs found during the observation period was 29,396 eggs with a total weight of 227.7 mg. The highest number of eggs laid daily on the ovitrap was obtained on the fourth day after emergence (Fig. 9) or two days after peak mating activity when most of the female oviposited their eggs. BSF average development time from the egg stage to adulthood was approximately 55 days. The larval period requires the longest development time compared to other stages and males have greater longevity than females (Fig. 10).

DISCUSSION

This study found that the BSF adult mating started 2 days after eclosion in the morning when the weather warms up and peaks from 11:00-12:00 when daily sunlight intensity is at its highest. This result corresponded with previous studies that found that BSF starts their mating activities at the age of two days relies on sunlight to detect the presence of females which require optimum light intensity either from sunlight or artificial light sources in indoor rearing[6,14,18,28]. The intensity of light, at 200 µmol m^{-2} sec^{-1} (10,800 lux) of artificial light, played a major role in determining when BSF mate[14]. Furthermore, BSF mating success can be dramatically increased by exposure to light, particularly under the rich wavelengths near 440 and/or 540 nm and having irradiance with an appreciable fraction equivalent to the intensity of full sunlight[17].

During the observation period, lek behavior or males gathering for competitive mating displays, as well as aggregations of males and females during the mating period were more frequently observed in larger cages than the small one. This BSF lekking behavior in the form of all-male aggregations or a combination of mixed-sex interaction that overlap during courtship in the mating period has never been reported previously. Aggregational behavior has been observed in other Stratiomyidae species, such as in *Hermetia comstocki* males that aggregate on the agava tree (*Agava palmeri*) and rest individually on the upper surface of the leaves. Lek behavior is defined as territorial behavior exhibited by some resting males and returning males after successful mating. Males utilized the lekking area while waiting for females and defend the area from other males[29] while increases the possibility of meeting the females as locations with high-male densities may attract females that are ready to mate[30]. Lek behavior usually plays a role in attracting potential mates and is critical for mating[28].

However, there are no differences between the BSF mating sequence behavior in small cages (1×1×1 m in size) and in large cages (3×3×3 m3 in size) except that lek behavior and mating display showed more frequently in larger cage. During courtship, the males approach and chase females, catching them while flying or using interceptive behavior. Afterward, both males and females descend and try to copulate. Copulation occurs while they are descending to the ground or while on the ground. Generally, BSF mating behavior as observed in this study is the same as previously reported[13,23,25,28]. Copulation in BSF is dominated by opposing positions (90% of total mating attempts) and small numbers of mounting. This study found, regardless the copulation type, the copulation duration lasted, on average, for 36 min.

The copulation attempt performed by the male fly on females is the most complex BSF behavior and determines mating success. This behavior is characterized by the male

starting to perform wing-fanning while mounting the female. The duration of wing-fanning reflected mating success as successful mating males tends to have a shorter duration of wing-fanning than unsuccessful males which agreed to Giunti *et al.*[25] who conducted the study in an artificial environment. It seems that wing fanning is a key behavior for the female to assess the quality of male[13,25].

The result of this study also showed the importance of the size of the cages for successful mating and egg production which may be related to adult fly[21,29,30]. Bigger spaces allowed less adult density which allows males to produce large numbers of lekking areas[31] and detect the signal from females as shown by the study by Giunti *et al.*[25] that males of BSF were sometimes mistaken males as females when they unable to detect the mating signals from females.

After copulation, the female will enter the oviposition period over the next two days. In this study, on the fourth day of observation, females had begun laying eggs in the ovitrap placed near decaying organic waste in the rearing cage.

Under natural conditions, BSF females lay eggs near moist and decaying organic matter[32]. In rearing cages, the ovitrap that is generally used is made of cardboard or wood material which provides gaps or spaces for laying eggs[18,21]. Eggs were placed near potential food sources in sturdy and hidden gaps so that the eggs were protected from predation. The BSF females laid eggs close to those of other females and then died after oviposition[14]. In this study, it was found that BSF females laid eggs not only in the ovitrap but also outside the ovitrap on an exposed open area. Eggs were found around the organic medium, which was intended as a food source for when the eggs hatch into larvae and on the lateral wall of the cage made of nylon netting. This oviposition behavior has not been reported in previous studies. About 78% of the eggs laid on the nylon netting were fertile and had an incubation period of 3-7 days, which did not differ from eggs laid in the ovitrap and in agreement with previous studies on the incubation period of BSF[29,33].

This study showed that BSF females lay more eggs in the afternoon when the intensity of the sunlight begins to decrease. Furthermore, the oviposition period ranged from 4 to 10 days, similar to results obtained by Tomberlin *et al.*[14] who reported an oviposition peak after four days of emergence under sunlight. However, our results differ from several previous studies that had reported: (1) an oviposition peak after 17 days under sunlight, while oviposition peaked after 13 days under artificial light sources (quartz iodine lamps, 350-2500 nm)[6], (2) an oviposition peak at 10 days under artificial light sources (fluorescent tubes 300-650 nm) and oviposition peak under LED (365-515 nm) which occurred after 16 days[31]. It seems other environmental factors create a cascading effect on the oviposition behavior of BSF such as temperature[34,35], humidity and nycthermal cycles[36] which should be considered in design a sustainable BSF egg production system. From all these reports, the use of different light sources can be predicted to affect the different results of BSF oviposition period and this study reported acceleration of oviposition period under the sunlight exposure in tropic.

CONCLUSION

The mating sequence in this study has an alteration form from the stereotype behavior of BSF, particularly with the frequent inverse copulation position and lek behavior. From this study, which covers the results of courtship performance, sexual and oviposition behavior as well as the number of eggs produced, it can be concluded that BSF can perform optimal mating and reproductive activities in maintained semi-outdoor conditions under direct and full sunlight in tropics. Furthermore, the results obtained can be used as representative of BSF reared in environmental conditions similar to tropical areas.

SIGNIFICANCE STATEMENT

This study revealed the mating behavior of the black soldier fly. This study will help the researchers to develop an understanding of variables that may influence egg production which is the foundation of the sustainable production of black soldier fly larvae as an alternative of a relatively environmentally friendly, protein and lipid.

ACKNOWLEDGMENT

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REFERENCES

1. St-Hilaire, S., K. Cranfill, M.A. Mcguire, E.E. Mosley and J.K. Tomberlin *et al.*, 2007. Fish offal recycling by the Black Soldier Fly produces a foodstuff high in omega-3 fatty acids. J. World Aquacult. Soc., 38: 309-313.
2. Marshall, S. A., N.E. Woodley and M. Hauser, 2015. The historical spread of the lack soldier fly, *Hermetia illucens* (L.) (Diptera, Stratiomyidae, Hermetiinae) and its establishment in Canada. J. ent. Soc. Ont., 146: 51-54.

3. Wang, H., K. Rehman, W. Feng, D. Yang and R. Rehman *et al*., 2020. Physicochemical structure of chitin in the developing stages of black soldier fly. Int. J. Biol. Macromol., 149: 901-907.
4. Kinasih, I., R.E. Putra, A.D. Permana, F.F. Gusmara, M.Y. Nurhadi and R.A. Anitasari, 2018. Growth performance of black soldier fly larvae (*Hermetia illucens*) fed on some plant based organic wastes. HAYATI J. Biosci., 25: 79-84.
5. Surendra, K.C., R. Olivier, J.K. Tomberlin, R. Jha and S.K. Khanal, 2016. Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renew. Energy, 98: 197-202.
6. Zhang, J., L. Huang, J. He, J.K. Tomberlin, J. Li *et al*., 2010. An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. J. Insect Sci., 10: 1-7.
7. Myers, H.M., J.K.Tomberlin, B.D. Lambert and D. Kattes, 2008. Development of black soldier fly (Diptera: Stratiomyidae) larvae fed dairy manure. J. Environ. Entomol., 37: 11-15.
   Diener, S., C. Zurbrügg and K. Tockner, 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Manag. Res., 27: 603-610.
8. Li, Q., L. Zheng, N. Qiu, H. Cai, J.K. Tomberlin and Z. Yu, 2011. Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. Waste Manage., 31: 1316-1320.
9. Sprangers, T., M. Ottoboni, C. Klootwijk, A. Ovyn and S. Deboosere *et al*., 2016. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. J. Sci. Food Agric., 97: 2594-2600.
10. Smets, R., B. Verbinnen, I. Van De Voorde, G. Aerts, J. Claes and M. Van Der Borght, 2020. Sequential extraction and characterisation of lipids, proteins and chitin from Black Soldier Fly (*Hermetia illucens*) larvae, prepupae and pupae. Waste Biomass Valori., 10.1007/s12649-019-00924-2
11. Park, K., K. Wontae, K. Eunsun, K. Kyu-won and C. Ji-young *et al*., 2016. Oviposition site preference in black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in artificial rearing system. Int. J. Industrial Entomol., 33: 54-58.
12. Wicker-Thomas, C., 2007. Pheromonal communication involved in courtship behavior in Diptera. J. Insect Physiol., 53: 1089-1100.
13. Tomberlin, J.K., D. C. Sheppard and J.A Joyce, 2002. Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Ann. Entomol. Soc. Am., 95: 379-386.
14. Gobbi, P., A. Martinez-Sanchez and S. Rojo, 2013. The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). Eur. J. Entomol., 110: 461-468.
15. Sheppard, D.C., J.K. Tomberlin, J.A Joyce, B.C Kiser and S.M. Sumner, 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). J. Med. Entomol., 39: 695-698.
16. Schneider, J.C., 2019. Effects of light intensity on mating of the black soldier fly (*Hermetia illucens*, Diptera: Stratiomyidae). J. Insects Food Feed, 6: 111-119.
17. Heussler, C.D., A. Walter, H. Oberkofler, H. Insam, W. Arthofer, B.C. Schlick-Steiner and F.M. Steine, 2018. Influence of three artificial light sources on oviposition and half-life of the Black Soldier Fly, Hermetia illucens (Diptera: Stratiomyidae): Improving small-scale indoor rearing. PLoS ONE, Vol. 13 10.1371/journal.pone.0197896
18. Holmes, L.A., S.L. Vanlaerhoven and J.K. Tomberlin, 2012. Relative humidity effects on the life history of *Hermetia illucens* (Diptera: Stratiomyidae). Environ. Entomol., 41: 971-978.
19. Harnden, L.M. and J.K. Tomberlin, 2016. Effects of temperature and diet on black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), development. Forensic Sci. Int., 266: 109-116.
20. Nakamura, S., R.T. Ichiki, M. Shimoda and S. Morioka, 2016. Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: low-cost and year-round rearing. Appl. Entomol. Zool., 51: 161-166.
21. Ahmed, A.Z., K. Sopian, Z.Z. Abidin and M.Y.H. Othman, 2002. The availability of daylight from tropical skies a case study of Malaysia. Renewable Energy, 25: 21-30.
22. Rumbayan, M., A. Abudureyimu and K. Nagasaka, 2012. Mapping of solar energy potential in Indonesia using artificial neural network and geographical information system. Renew. Sustain. Energy Rev., 16: 1437-1449.
23. Tingle, F.C., E.R. Mitchell and W.W. Copeland, 1975. The soldier fly, *Hermetia illucens* in poultry houses in north central Florida. J. Ga. Entomol. Soc., 10: 179-183.
24. Giunti G., O. Campolo, F. Laudani and V. Palmeri, 2018. Male courtship behaviour and potential for female mate choice in the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae). Entomologia, 38: 29-46.
25. Carmel, I., U. Tram and Y. Heifetz, 2016. Mating induces developmental changes in the insect female reproductive tract. Curr. Opin. Insect Sci., 13: 106-113.
26. Cheng, J.Y.K., S.L.H. Chiu and I.M.C. Lo, 2017. Effects of moisture content of food waste on residue separation, larval growth and larval survival in black soldier fly bioconversion. Waste Manag., 67: 315-323.
27. Tomberlin, J.K. and D. Sheppard, 2001. Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). Fla. Entomol., 84: 729-730.
28. Diclaro II, J. W. and P.E. Kaufman, 2009. Black soldier fly *Hermetia illucens* Linnaeus (Insecta: Diptera: Stratiomyidae). EENY-461, Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

29. Alcock, J., 2008. A large male competitive advantage in a lekking fly, *Hermetia comstocki* Williston (Diptera: Stratiomyidae). Psyche: A J. Entomol., 97: 267-279.
30. Oonincx, D.G.A.B., N. Volk, J.J.E. Diehl, J.J.A. Van Loon and G. Belušič, 2016. Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction. J. Insect Physiol., 95: 133-139.
31. Zheng, L., T.L. Crippen, B. Singh, A.M. Tarone, S. Dowd *et al*., 2013. A survey of bacterial diversity from successive life stages of black soldier fly (Diptera: Stratiomyidae) by using 16S rDNA pyrosequencing. J. Med. Entomol., 50: 647-658.
32. Booth, D.C. and C. Sheppard, 1984. Oviposition of the black soldier fly, *Hermetia illucens* (Diptera, Stratiomyidae): eggs, masses, timing and site characteristics. J. Environ. Entomol., 13: 421-423.
33. Shumo, M., F.M. Khamis, C.M. Tanga, K.K.M. Fiaboe, S. Subramanian, 2019. Influence of temperature on selected life-history traits of black soldier fly (*Hermetia illucens*) reared on two common urban organic waste streams in Kenya. Animals, 10.3390%2Fani9030079
34. Chia, S.Y., C.M Tanga, F.M. Khamis, S.A. Mohamed, D. Salifu *et al*., 2018. Threshold temperatures and thermal requirements of black soldier fly *Hermetia illucens*: Implications for mass production. PLoS ONE, Vol. 13. 10.1371/journal.pone.0206097
35. Hoc, B., G. Noel, J. Carpentier, F. Francis and R.C. Megido, 2019. Optimization of black soldier fly (*Hermetia illucens*) artificial reproduction. PLoS ONE, Vol. 14. 10.1371/journal.pone.0216160