Appl Entomol Zool (2016) 51:161–166
DOI 10.1007/s13355-015-0376-1

TECHNICAL NOTE

Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: low-cost and year-round rearing

Satoshi Nakamura[1] · Ryoko T. Ichiki[1] · Masami Shimoda[2] · Shinsuke Morioka[1]

Received: 7 September 2015 / Accepted: 16 October 2015 / Published online: 12 November 2015

Abstract The large-scale rearing of the black soldier fly, *Hermetia illucens* (L.), to obtain fertilized eggs is not conducive to the collection of information on oviposition by and survival of adult flies. To obtain this information, we raised 100 adults each within small cages (27 × 27 × 27 cm) in the laboratory with either supplementary light-emitting diode (LED) lighting or 2 h of sunlight per day. We obtained fertilized eggs in both light treatments. Although sunlight enhanced the proportion of fertilized eggs, the patterns of oviposition were similar in both treatments, and there were no significant differences in the total numbers of egg clutches and oviposition periods. To examine effects on adult longevity, we fed newly emerged adults sugar and water, water only, or nothing. Sugar increased longevity to 3 times in males and 2 times in females compared to water alone. This small-scale rearing method would help to maintain cultures of the black soldier fly throughout the year in the laboratory at low cost.

Keywords Rearing method · Oviposition · Longevity · Sugar supply

Introduction

The black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is a good candidate for treating organic wastes or livestock manure. It can feed on a wide variety of organic matter, from fruits and vegetables to animal remains and manure (James 1935; May 1961), and can reduce manure accumulation by up to 56 % (Sheppard 1983). At the same time, the larvae and pupae can provide valuable feed for a variety of animals, including chickens (Hale 1973), swine (Newton et al. 1977), fish (Bondari and Sheppard 1981, 1987; St-Hilaire et al. 2007), and even predatory mites (Nguyen et al. 2015). Prepupae contain 44 % dry matter and are composed of 42 % protein and 35 % fat, including essential amino acids and fatty acids (Hale 1973). The major obstacles associated with the production of larvae to treat organic wastes or to feed animals involve scaling up the production capacity and insufficient knowledge of the fly's biology necessary to produce large amounts of eggs (Čičková et al. 2015).

Although there are some reports on rearing black soldier fly adults to obtain fertilized eggs (Sheppard et al. 2002; Tomberlin and Sheppard 2002; Tingle et al. 1975; Zhang et al. 2010), all of those studies used large cages (>1 m on all sides) holding 750–1000 flies in a greenhouse or outdoors. Such methods can make it costly to maintain suitable temperatures throughout the year (Sheppard et al. 2002), and the large scale makes it inconvenient to conduct precise experiments. As there is little information on the biology of the black soldier fly, a small-scale method of rearing in the laboratory is needed.

Here, we obtained fertilized eggs in small cages in the laboratory and compared ovipositional and survival curves of adults between methods using supplemental artificial lighting or sunlight, since light sources are reportedly a key factor for fertilizing eggs (Tingle et al. 1975; Tomberlin and Sheppard 2002; Zhang et al. 2010). We also compared adult longevities among feeding conditions.

✉ Satoshi Nakamura
s.nakamura@affrc.go.jp

[1] Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

[2] National Institute of Agrobiological Sciences, Tsukuba, Japan

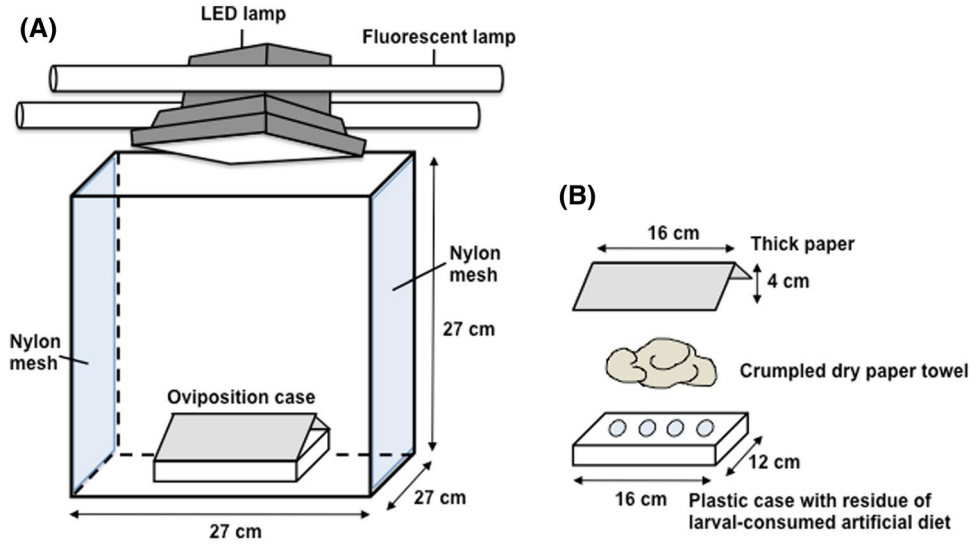

Fig. 1 **a** A wire-framed polyethylene cage with two 40-W fluorescent lamps and a 20-W light-emitting diode (LED) lamp, and **b** an oviposition case

Materials and methods

All experiments were conducted at 25 °C and a relative humidity of 70 ± 5 %, with a 16-h light: 8-h dark photoperiod.

Flies and rearing procedures

The culture was initiated from nine females collected in Tsukuba, Japan (36°03′N, 140°04′E) in October 2013. The females were individually placed in ventilated plastic cages (20 × 23 × 35 cm) containing a plastic case (16 × 12 × 3 cm), which had a lid with four holes 2 cm in diameter that was covered with nylon mesh, and which held residue of a larva-consumed artificial diet (see below) to induce oviposition. Thick paper folded in two (ca. 4 cm high) was placed on the case over a crumpled dry paper towel, which served as an oviposition site. Although many studies (e.g., Sheppard et al. 2002; Tomberlin and Sheppard 2002; Zhang et al. 2010) used corrugated cardboard as an oviposition site, we used a paper towel because it made it easy to estimate sizes of egg clutches, and females had no problem in laying eggs (Fig. 1b).

We collected the eggs and put them in a petri dish (9 cm diam., 2 cm high, sealed firmly to prevent larval escape) with rice bran mixed with water (25 % bran relative to the weight of water) for a week. The hatched larvae were then transferred to a plastic container (20 × 15 × 7 cm) with a ventilated lid and kept on an artificial diet (Table 1) until about 70 % of them turned dark brown (prepupae). This container without its lid was then placed in a larger container (28 × 22 × 10 cm) holding dried coffee grounds as a substitute for soil for the larvae to pupate in. Emerging adults were transferred to a wire-framed polyethylene cage (27 × 27 × 27 cm) that had nylon mesh on two opposing sides (Fig. 1a); no food was provided, but water was sprayed onto the nylon mesh three times a day. The cage had an oviposition case as described above to collect eggs. Artificial light sources were same as used in the oviposition and longevity in group rearing experiment described below, and the cage was exposed to sunlight for 2–3 h per day when the weather was clear.

Table 1 Ingredients of the artificial diet for *Hermetia illucens*

Ingredients	Amount
Wheat bran	100 g
Rabbit and guinea pig diet[a]	60 g
Dried yeast	10 g
Water	240 mL

[a] RC4, Oriental Yeast Co. Ltd., Japan

Oviposition and longevity in group rearing

Newly emerged (<15 h) adults (50 males and 50 females) were released into wire-framed polyethylene cages (27 × 27 × 27 cm) (Fig. 1a), and oviposition and longevity were recorded every day. Each cage had an oviposition case as described above. Oviposited eggs were observed for hatching (fertilized). After preliminary experiments, two 40-W fluorescent lamps (FLR40SEX-W/M/36-HG, NEC, Japan) were set ca. 10 cm above the cages and lit

between 0600 and 2200 JST. One 20-W light-emitting diode (LED) lamp (400–800 nm with peaks at 451 and 555 nm; JLM-LTG20 W, Jinxing Rantoon Co. Ltd., China) was also suspended ca. 5 cm above each of five replicate cages and was lit between 0900 and 1500 (Fig. 1). The light intensity of the LED lamp was 790 μmol m^{-2} s^{-1} just under the top wall of the cage and 47 μmol m^{-2} s^{-1} on the floor. The emission spectra and the photon flux density were measured with a spectrometer (HSU-100S, Asahi Spectra, Japan). This was called the "LED" treatment. This cage was replicated 5 times. Another five cages were exposed to sunlight between 1000 and 1200 for the first 15 days. This was called the "SUN" treatment. All other conditions were the same between treatments. The intensity of the sunlight depended on the weather, but the cages received sunlight for 14 days of the first 15 days. Photosynthetic photon flux is typically around 2000 and 600 μmol m^{-2} s^{-1} at noon on fine and cloudy days, respectively. No food was provided, but ca. 10 mL of distilled water were sprayed onto the nylon mesh at 0900, 1300, and 1700 every day. We estimated the number of egg clutches from their slight color differences and locations on the paper, although some clutches were clustered. To estimate the number of eggs per clutch by appearance, we individually soaked different sizes of clutches ($n = 6$) in 70 % ethanol to separate the eggs, photographed them under a microscope, and then counted the eggs in the photographic prints. We used these results to estimate the numbers of eggs in clutches of various sizes. Although females can produce 206–639 eggs per clutch (Tomberlin et al. 2002), we categorized clutches into ≤300, 301–600, 601–900, or 901–1200 eggs per clutch for comparison between light treatments.

Longevity under different food conditions

We compared adult longevity of both sexes among treatments with sugar and water, water only, and nothing added as food. Sugar was supplied as a sugar cube. Distilled water was provided in an 18 mL plastic case containing cotton. Newly emerged (<12 h) adults were individually kept in a ventilated plastic cup (12 cm diam., 10 cm high), and their survival was recorded every day. Two 40-W fluorescent lamps were set ca. 25 cm above the cages and were lit between 0600 and 2200. Each treatment was replicated 15 times.

Statistical analyses

Differences in oviposition and longevity between the lighting treatments were tested by one-way ANOVA. In the case of hatchability, angular transformation was applied before one-way ANOVA. Differences in longevity among the food conditions were tested by one-way ANOVA and then compared by Tukey–Kramer HSD post hoc test. These analyses were conducted in JMP software v. 7.0.1 (SAS Institute Inc., Cary, NC, USA). The accepted level of significance was 5 % in all analyses.

Results

Oviposition and longevity in group rearing

We obtained fertilized eggs in the small cages under both lighting treatments (Fig. 2). The patterns of oviposition were similar in both treatments (Fig. 2); the periods of pre-oviposition and oviposition were not significantly different (Table 2). Oviposition peaked on day 6 (6 days after emergence) in LED and on day 7 in SUN, and the daily number of clutches showed similar patterns. Fertilized eggs were collected until day 10 in LED and day 12 in SUN (Fig. 2). Additional eggs were laid after that for 3 days in both treatments, but none hatched. The mean numbers of clutches per female were not significantly different between the treatments (Table 2). However, hatchability and the number of hatched clutches per female were significantly larger in SUN than in LED. On the other hand, the numbers of total and hatched eggs per female were not significantly different between treatments. Few flies died during the first week, and then the survival curves decreased gradually in both (Fig. 2). Males lived longer than females (Table 2, LED $p = 0.0489$; SUN $p = 0.0002$). Only male longevity differed significantly between the treatments (Table 2).

Longevity under different food conditions

Although males lived an average of 9.1 days with no added food and 20.9 days with only water, the difference was not significant (Table 3). Females showed the same tendency. When sugar and water were provided, males lived 73.1 days—much longer than females (47.6 days).

Discussion

Laboratory rearing of the black soldier fly is difficult (Čičková et al. 2015), since the flies have a complex mating behavior (Tomberlin and Sheppard 2001). All previous studies that obtained fertilized eggs used a large cage (ranging in size from 1.8 × 1.2 × 1.5 m to 2 × 2 × 4 m) holding 750–1000 adults in the field or in a greenhouse (Sheppard et al. 2002; Tomberlin and Sheppard 2002; Tingle et al. 1975; Zhang et al. 2010). Tingle et al. (1975) could not achieve mating or egg collection in two small cages (53 × 91 × 53 cm and 38 × 46 × 38 cm) in a greenhouse

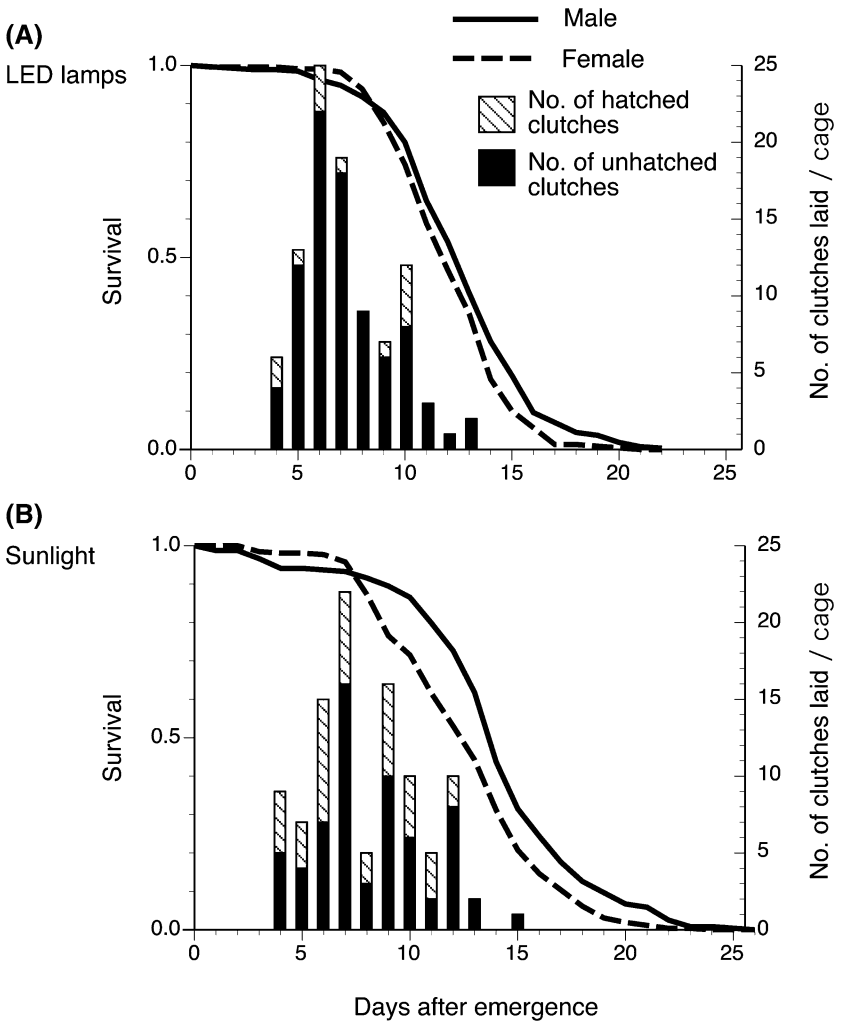

Fig. 2 Survival curves and numbers of clutches laid of *Hermetia illucens* under artificial lighting supplemented with **a** LED lamps ($n = 5$) and **b** 2 h of sunlight per day ($n = 5$)

Table 2 Life-history parameters of adult *Hermetia illucens* under different light sources

Life-history parameter[b]	Supplemental light source[a]		ANOVA		
	LED lamp	2 h sunlight	*F*	*p*	n^b
Pre-oviposition period (days)	4.6 ± 0.3	4.4 ± 0.3	0.1818	0.6811	5
Oviposition period (days)	7.6 ± 0.8	9.4 ± 0.8	2.8929	0.1274	5
No. of clutches/female	0.43 ± 0.04	0.39 ± 0.04	0.4901	0.5037	5
No. of hatched clutches/female	0.05 ± 0.03	0.15 ± 0.03	5.9168	0.041	5
Hatchability (%)[c]	11.2 ± 9.1	39.5 ± 6.3	8.0806	0.0217	5
No. of eggs/female	289.0 ± 27.0	240.2 ± 31.6	1.3842	0.2732	5
No. of hatched eggs/female	43.7 ± 35.8	84.4 ± 19.0	1.0101	0.3443	5
Male longevity (days)[d]	12.8 ± 0.2 a	14.1 ± 0.3 a	13.7761	0.0002	250
Female longevity (days)[d]	12.3 ± 0.2 b	12.7 ± 0.2 b	2.3561	0.1254	250

[a] ±SEM

[b] The values for longevity indicate the number of individuals and the values for other life-history parameters show the number of cages tested

[c] Number of hatched clutches/total number of clutches

[d] Means followed by different letters within each column are significantly different between sexes at the 5 % level by ANOVA

Table 3 Longevity of adult *Hermetia illucens* under different food conditions

	Male longevity (days ± SEM)	Female longevity (days ± SEM)	*F*	*p*	*n*
Sugar + water	73.1 ± 7.1 a	47.6 ± 7.1 a	6.5559	0.0161	15
Water only	20.9 ± 1.2 b	21.5 ± 1.2 b	0.0921	0.7638	15
None	9.1 ± 0.7 b	10.8 ± 0.7 b	3.0140	0.0935	15

Means followed by different letters within each column are significantly different at the 5 % level by Tukey–Kramer HSD post hoc test

with an unstated number of adults. Here, we achieved fertilized eggs in a 27 × 27 × 27 cm cage, which was only 1/800 of the volume of the cage used by Sheppard et al. (2002) and 1/165 that of Zhang et al. (2010). However, the adult density in our experiment was approximately 108 times that of Sheppard et al. (2002) and 25 times that of Zhang et al. (2010). We thus speculate that high density is an important factor in achieving mating and fertilized eggs in a limited volume.

Sunlight is an important factor in achieving mating: higher light intensity promoted mating (Tomberlin and Sheppard 2002) and shade or cloud inhibited it (Tingle et al. 1975). No mating occurred when the light intensity was <63 μmol m^{-2} s^{-1}, and the mating rate was 75 % when it was >200 μmol m^{-2} s^{-1} (Tomberlin and Sheppard 2002).

Nevertheless, we obtained fertilized eggs without sunlight, using fluorescent lighting supplemented with only a 20-W LED lamp with a light intensity range of 47–790 μmol m^{-2} s^{-1}. Zhang et al. (2010) achieved mating and fertilized eggs of black soldier flies in a 1.8 × 1.2 × 1.5 m cage under a 500-W quartz-iodine lamp with an intensity of 135 μmol m^{-2} s^{-1} at 50 cm below the bulb, but not under a 450-W rare-earth lamp with a light intensity of 160 μmol m^{-2} s^{-1}. The quartz-iodine lamp had a spectrum between 350 and 2500 nm, and the rare-earth lamp had a spectrum between 350 and 450 nm. They concluded that wavelengths between 450 and 700 nm were crucial, since insects typically cannot see wavelengths longer than 700 nm (Briscoe and Chittka 2001). As the LED lamp has a wavelength range of 400–800 nm, our results support the suggestion of Zhang et al. (2010) that wavelengths between 450 and 700 nm influence the mating behavior of the black soldier fly.

Although the LED lamps promoted fertilization, sunlight promoted greater fertility and hatchability (Table 2). There was no significant difference in the total numbers of egg clutches per female or oviposition periods (Table 2). Therefore, additional research on the characteristics of light sources, such as light intensity, spectral range, and duration of exposure, is needed to understand mating behavior better.

Adult longevity did not differ significantly between sexes in the water-only and unfed treatments (Table 3). However, males lived significantly longer than females on sugar and water and in the group oviposition experiment, although we dont know why. Tomberlin et al. (2002) found that females dissected 3 days after oviposition contained no visible fat or developing ovaries, and speculated that females mate once and oviposit once in their lifetime on account of their short adult lifespan and reliance on fat reserves acquired during the larval stage. However, they did not examine ovaries and oviposition when females were provided with sugar, as would be available in the wild. In our experiment, adult longevity increased greatly with sugar supply (Table 3). Thus, it will be necessary to study how many times the flies mate and oviposit in their lifetime when adequately nourished in order to understand their biology and to enhance their mass production.

Although we successfully obtained fertilized eggs in a small cage and have maintained a black soldier fly colony for more than 22 months in the laboratory, only 11.2 % of clutches were fertilized under LED, and only 39.5 % under sunlight. Therefore, we need to examine how to increase the rate of fertilization for more efficient rearing in the laboratory.

Acknowledgments We thank Aki Konishi for her assistance in the experiments. Research activities for this article were funded by the Japan International Research Center for Agricultural Sciences.

References

Bondari K, Sheppard DC (1981) Soldier fly larvae as feed in commercial fish production. Aquaculture 24:103–109

Bondari K, Sheppard DC (1987) Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus puctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). Aquac Fish Manage 18:209–220

Briscoe AD, Chittka L (2001) The evolution of color vision in insects. Annu Rev Entomol 46:471–510

Čičková H, Newton GL, Lacy RC, Kozánek M (2015) The use of fly larvae for organic waste treatment. Waste Manage 35:68–80

Hale OM (1973) Dried *Hermetia illucens* larvae (Diptera: Stratiomyidae) as a feed additive for poultry. J Georgia Entomol Soc 8:16–20

James MT (1935) The genus *Hermetia* in the USA (Diptera: Stratiomyidae). Bull Brooklyn Entomol Soc 30:165–170

May BM (1961) The occurrence in New Zealand and the life-history of the soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae). NZ J Sci 4:55–65

Newton GL, Booram CV, Barker RW, Hale OM (1977) Dried *Hermetia illucens* larvae meal as a supplement for swine. J Anim Sci 44:396–399

Nguyen DT, Bouguet V, Spranghers T, Vangansbeke D, De Clercq P (2015) Beneficial effect of supplementing an artificial diet for *Amblyseius swirskii* with *Hermetia illucens* haemolymph. J Appl Entomol 139:342–351

Sheppard DC (1983) House fly and lesser house fly control utilizing the black soldier fly in manure management systems for caged laying hens. Environ Entomol 12:1439–1442

Sheppard DC, Tomberlin JK, Joyce JA, Kiser BC, Sumner SM (2002) Rearing methods for the black soldier fly (Diptera: Stratiomyidae). J Med Entomol 39:695–698

St-Hilaire S, Cranfill K, MacGuire MA, Mosley EE, Tomberlin JK, Newton L, Sealey W, Sheppard C, Irving S (2007) Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. J World Aquac Soc 38:309–313

Tingle FC, Mitchell ER, Copeland WW (1975) The soldier fly, *Hermetia illucens* in poultry houses in North Central Florida. J Georgia Entomol Soc 10:179–183

Tomberlin JK, Sheppard DC (2001) Lekking behavior of the black soldier fly (Diptera: Stratiomyidae). Florida Entomol 84:729–730

Tomberlin JK, Sheppard DC (2002) Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. J Entomol Sci 37:345–352

Tomberlin JK, Sheppard DC, Joyce JA (2002) Selected life-history traits of black soldier fly (Diptera: Stratiomyidae) reared on three artificial diets. Ann Entomol Soc Am 95:379–386

Zhang J, Huang L, He J, Tomberlin JK, Li J, Lei C, Sun M, Liu Z, Yu Z (2010) An artificial light source influences mating and oviposition of black soldier flies, *Hermetia illucens*. J Insect Sci 10:202