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Developmental and Waste Reduction Plasticity of Three Black Soldier Fly Strains (Diptera: Stratiomyidae) Raised on Different Livestock Manures

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ABSTRACT Black soldier flies, *Hermetia illucens* L., are distributed throughout the temperate and tropic regions of the world and are known an established method for sustainably managing animal wastes. Colonies used to conduct research on the black soldier fly within the past 20 yr have predominately been established from eggs or larvae received from a colony originated from Bacon County, GA. Consequently, little is known about the phenotypic plasticity (i.e., development and waste conversion) across strains from different regions. This study compared the development of three strains of the black soldier fly (Texas; Guangzhou, China; and Wuhan, China) and their ability to reduce dry matter and associated nutrients in swine, dairy, and chicken manure. The Wuhan strain appeared to be more fit. Larvae from Wuhan needed 17.7–29.9% less time to reach the prepupal stage than those from Guangzhou or Texas, respectively. Larvae from Wuhan weighed 14.4–37.0% more than those from Guanghzhou or Texas, respectively. Larvae from the Wuhan strain reduced dry matter 46.0% (swine), 40.1% (dairy), and 48.4% (chicken) more than the Guangzhou strain and 6.9, 7.2, and 7.9% more than the Texas strain. This study demonstrates that phenotypic plasticity (e.g., development and waste conversion) varies across populations of black soldier flies and should be taken into account when selecting and establishing a population as a waste management agent in a given region of the world.

KEY WORDS black soldier fly strain, phenotypic plasticity, life cycle trait, animal waste, waste conversion

The black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is distributed throughout the tropics and temperate regions of the world and is active in the southeastern United States from April to October (Sheppard et al. 1994). Adults mate and then lay eggs in cracks and crevices near larval habitats (Booth and Sheppard 1984). Adults are not synanthropic (Furman et al. 1959). Consequently, they are typically not viewed as a pest. Adults do not need to feed, surviving on the large fat body stored from the larval stage (Tomberlin and Sheppard 2002). The larvae of the black soldier fly are voracious consumers of decaying organic matter, such as rotting fruits, vegetables, dead animals, and associated manures (Sheppard et al. 1994).

Black soldier fly larvae are beneficial arthropods in confined animal facilities. The presence of their larvae in animal wastes results in house fly, *Musca domestica* L., production reduced by 100% (Sheppard 1983), and associated nitrogen content by 62% (Sheppard et al. 1994, Myers et al. 2008). Black soldier fly larvae also suppress *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Enteritidis in contaminated chicken manure (Erickson et al. 2004). Larvae dispersing from the waste can also be used as feed for a variety of livestock and poultry (Sheppard et al. 1994).

Several laboratory studies have examined the developmental biology of the black soldier fly (Furman et al. 1959; May 1961; Tomberlin et al. 2002, 2009). May (1961) reports the occurrence and the life history of the black soldier fly in New Zealand. Sheppard et al. (2002) developed a methodology for mass rearing the black soldier fly in colony. Furthermore, Tomberlin et al. (2002) observed life history traits of black soldier flies on three different artificial diets. A subsequent study described the development of the black soldier fly at different temperatures (Tomberlin et al. 2009). Other studies on the black soldier fly include the enhancement of colony methods for its mass production (Diener et al. 2009; Zhang et al. 2010; Holmes et al. 2012, 2013), using probiotics to enhance development (Yu et al. 2011), production of biofuel (Li et al. 2011), or a survey of bacteria associated with its life stages (Zheng et al. 2013). However, for the most part,

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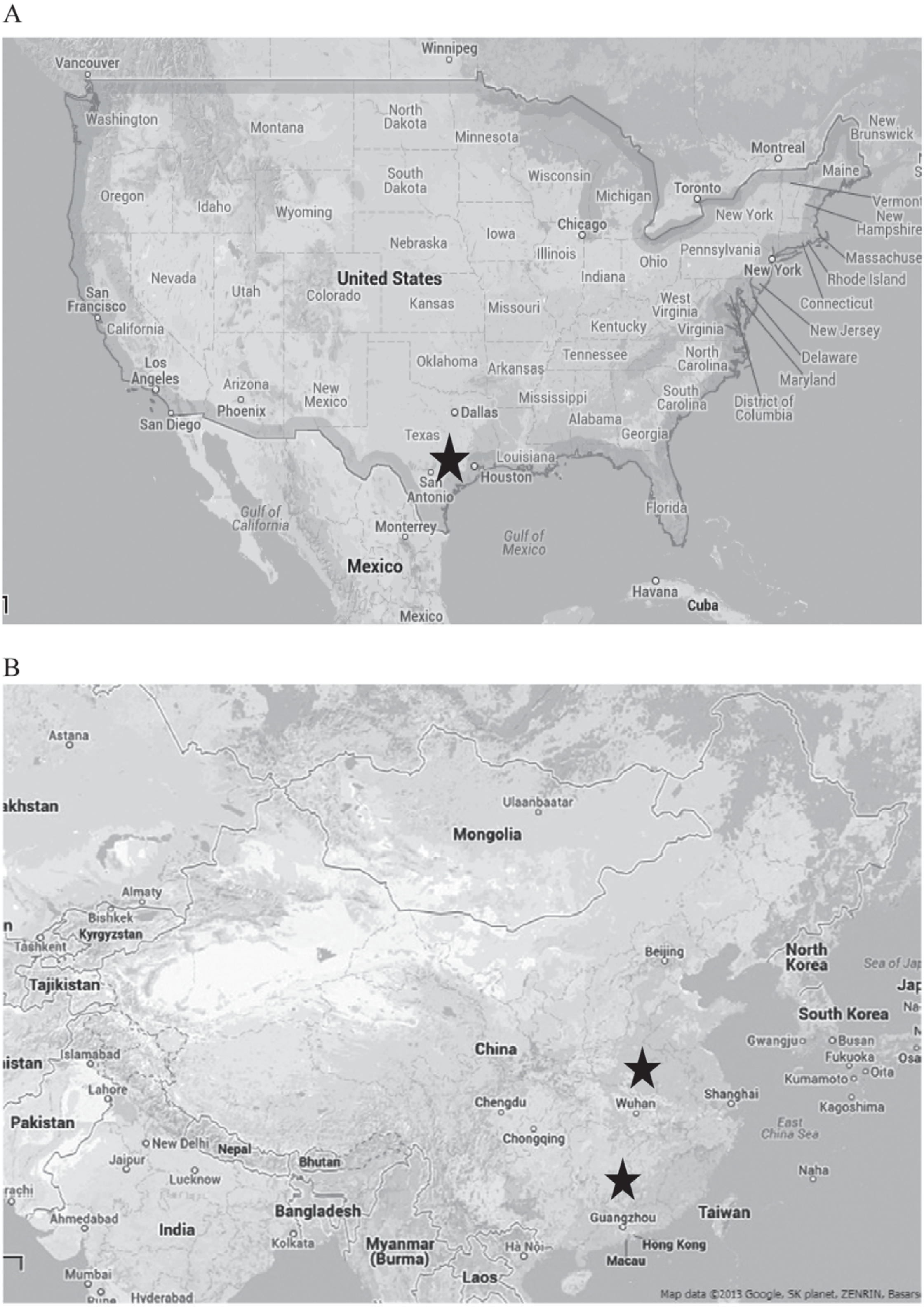

Fig. 1. Maps indicating sites where black soldier fly populations originated in (A) China and (B) the United States (http://maps.google.com.hk/maps?hl=en&tab=il).

excluding Furman et al. (1959) and May (1961), most research with the black soldier fly was accomplished with colonies initiated from eggs or larvae originating from a single colony from Bacon County, GA (Sheppard et al. 2002). However, if the black soldier fly is to be used globally for reducing wastes and producing alternate protein sources for use as feed for aquaculture (Bondari and Sheppard 1981, 1987; St. Hilaire et al. 2007; Sealey et al. 2011), poultry (Sheppard 1983), and livestock (Sheppard et al. 1994), an understanding of its phenotypic plasticity (i.e., development and waste conversion) among strains from different regions is needed. Recognizing these fundamental differences in strains may have global implications, as such data could result in informed decisions when selecting a strain for initiating a facility to reduce animal wastes and produce feed for livestock and poultry. The goal of this study was to examine the variation in targeted biological traits of three different strains. Secondly, the ability of the three different soldier fly strains to reduce manure and associated nutrients in different animal manures was examined.

Materials and Methods

The Black Soldier Fly Colonies. The experiment was performed using three strains of black soldier fly. One colony (Texas strain) was provided by the Texas Agrilife Experiment Station, Stephenville, TX, which originated from a colony developed at the Coastal Plain Experiment Station, University of Georgia, Tifton, GA (Sheppard et al. 2002). The colony (Texas strain) was maintained in greenhouse in Wuhan, China, for 1 yr before use in this study. The second colony (Guangzhou strain) was provided by the Guangdong Insect Institution, Guangdong, China. The Guangzhou colony was also maintained in greenhouse in Wuhan, China, for 1 yr before use in this study. The third colony (Wuhan strain) was established at the State Key Laboratory of Agricultural Microbiology of Huazhong Agricultural University, Wuhan, China. The Wuhan strain was also maintained in a greenhouse for 1 yr before its use in this study. Wuhan, China, and Bacon County, TX, are situated in temperate regions whereas Guangzhou is considered tropical (Fig. 1).

Methods for rearing black soldier flies and collecting eggs were adapted from Sheppard et al. (2002). In nature, black soldier flies oviposit in dry cracks and crevices around moist decomposing organic matter (Booth and Sheppard 1984). To initiate the colonies from the Chinese locations, one or more 0.5-liter plastic containers filled with fresh chicken manure saturated with water were placed at a poultry farm during the summer. Corrugated cardboard was taped to the inside of the containers ≈5 cm above the waste, as black soldier flies will lay their eggs in it. Once ≈50 black soldier fly egg masses were present in the cardboard, the containers were removed and monitored for larval activity. Resulting larvae were taken to a greenhouse maintained at 28°C, 75% relative humidity (RH), and under natural lighting conditions, that is, a photoperiod of 16:8 (L:D) h. Larvae were reared on chicken manure mixed with water (60–70% moisture). Resulting adults were used to maintain the colony.

Table 1. Comparison of black soldier fly egg traits for 30 clutches of eggs from three strains of black soldier flies reared at 28°C and 75% RH

Strains	Egg development (d) ± SEM
Wuhan, China	4.64 ± 0.29A[a]
Guangzhou, China	5.40 ± 1.06A
Texas	2.80 ± 0.41B

[a] Significant ($P \leq 0.05$; LSD) difference indicated by different letters within columns. All data were analyzed using SPSS Statistical (SPSS 2010).

Manure Types. Manure was collected from a commercial poultry, dairy, and swine farms located in Huazhong Agricultural University, Wuhan, China. There were no known uses of pesticides applied to the manures. The animals in the farm were fed a standard total mixed ration diet. For each manure type, 100 g samples were placed in three glass containers (250-ml beaker; sterile). These "predigested" (before conversion) samples and the total "digested" (after conversion) manure volume from each treatment, which will be discussed below in Experiment Design, were analyzed for dry matter and nitrogen. Percentage dry matter was determined using the gravimetric method. Nitrogen was measured using the Kjeldahl nitrogen method (Jung et al. 2003).

Experimental Design. Eggs were collected from each colony using methods previously described. For each strain, cardboard containing eggs was placed on 80 g Gainesville diet (Hogsette 1992) saturated with water (Tomberlin et al. 2002). Resulting larvae were used in the following experiment. For each strain, 300 6-d-old larvae were placed in each of nine containers (3 liter; plastic). All containers were stored in the greenhouse previously described with the aforementioned environmental parameters.

Each strain was fed three manure types (swine, chicken, and dairy). Nine replicates of each strain on each manure type were established for a total of 27 containers. Manure was provided ad libitum (≈100 g). The date, time, and amount of manure provided were recorded. When 50% of the larvae in a given container reached the dispersal phase (also referred to as the prepupal stage in the literature), the total amount of manure consumption in each treatment was calculated based on a dry matter basis.

To determine dry matter reduction, percent moisture of manure provided to black soldier flies was recorded. The dry matter reduction was $(W1-W2)/W1 \times 100\%$, with W1 being the dry matter provided over the course of the experiment and W2 being the dry matter amount remaining at the conclusion of the experiment. Nitrogen content for dry matter and protein content for black soldier fly larvae was measured using the Kjeldahl nitrogen method (Jung et al. 2003). For N reduction calculation, nitrogen was measured

Table 2. Comparison of black soldier fly final larval wt (g) and development (d) ± SEM when raised on the Gainesville house fly diet (Hogsette 1992) at 28°C, 75% RH, and a photoperiod of 16:8 (L:D) h

Strains	Sex	Final larval wt (g)	Larval development (d)	Sample size
Wuhan, China	F	0.1701 ± 0.0013A[a] (3[b])	20.07 ± 1.48B[a]	151
	M		18.22 ± 1.27A	149
	Combined		19.15 ± 1.37A	300
Guangzhou, China	F	0.1456 ± 0.0078B (3)	23.52 ± 0.86C	153
	M		21.56 ± 1.01B	147
	Combined		22.55 ± 0.93B	300
Texas	F	0.1071 ± 0.0022C (3)	25.62 ± 0.95D	156
	M		24.12 ± 0.56CD	144
	Combined		24.88 ± 0.65C	300

[a] Significant difference ($P \leq 0.05$; LSD) indicated by different letters within columns. All data were analyzed using SPSS Statistical (SPSS 2010).
[b] n = replicate.

before (N1) and after (N2) manures conversion. N reduction was (N1−N2)/N1 × 100%.

Life History Traits. For each replicate, 10 larvae were randomly selected each day, individually weighed on an Adventurer Pro-balance (NHON HOA Company Ltd., Ho Chi Minh City, Vietnam) and returned to their respective containers. The mean larval weight on the date that 50% of the larval of a container reached the dispersal phase defined the final larvae weight. To differentiate the life stages, the prepupae are black, whereas larvae are white (Tomberlin et al. 2002).

Prepupae were removed daily from all treatments and individually weighed using the balance previously mentioned. Prepupae were placed individually in 50-ml cups, covered with breathable gauze (Victory Sanitary Material Factory, Fushun, China) and returned to the greenhouse. Each container was labeled for identification. Time of the prepupal stage was recorded for each individual. Emergent adults were sexed and maintained in their respective cups. Adults were observed daily to determine longevity for each. Adults were provided water by spraying the cups twice daily.

Statistical Analysis. Larval and pupal development times, final larval, prepupal and adult weights, and adult longevity were analyzed using SPSS Statistical (SPSS 2010); the least significant difference (LSD) test was used after a significant F test ($P \leq 0.05$) to separate mean differences for each variable. Percentage data were arcsine transformed before analysis to meet the normalization assumption.

Results and Discussion

Strains from different locations ($F = 11.68$; df = 2; $P = 0.0085$) significantly influenced egg development, with the Texas strain needing less time to reach the larvae stage. The strains from China were not significantly different in time needed for eggs to hatch (Table 1).

Sex-based data were determined for individuals successfully reaching the adult stage. The larval development was significantly different among the three strains ($F = 23.71$; df = 2; $P = 0.0014$). The larvae from Wuhan needed 17.7 and 29.9% less time to reach the prepupal stage than those from Guangzhou and Texas, respectively. Final larvae weight was significantly different across strains ($F = 134.23$; df = 2; $P < 0.0001$). The larvae from Wuhan weighed 14.4 and 37.0% more than those from Guanghzhou and Texas, respectively. There are significant differences between male and female larvae in the Wuhan strain and the Guangzhou strain. The female larvae need more time to reach the prepupal stage than the male larvae. However, there were no significant differences between the male and female development time for the Texas strain (Table 2).

Prepupal weight was significantly different among the three strains ($F = 36.84$; df = 2; $P = 0.0004$). Male

Table 3. Comparison of black soldier fly prepupal weight (g) and development (d) SEM when raised on the Gainesville house fly diet (Hogsette 1992) at 28°C, 75% RH, and a photoperiod of 16:8 (L:D) h

Strains	Sex	Prepupal wt (g)	Sample size	Pupal development (d)	Sample size
Texas	F	0.0884 ± 0.0051B[a]	156	10.70 ± 0.82A	156
	M	0.0633 ± 0.0024A	144	10.28 ± 0.80A	144
	Combined	0.0766 ± 0.0049A	300	10.49 ± 0.81A	300
Guangzhou, China	F	0.1040 ± 0.0103C	153	12.22 ± 0.59B	153
	M	0.0715 ± 0.0068A	147	12.13 ± 0.27B	147
	Combined	0.0876 ± 0.0037B	300	12.18 ± 0.27B	300
Wuhan, China	F	0.1202 ± 0.0051D	151	13.48 ± 0.74B	151
	M	0.0999 ± 0.0075C	149	13.51 ± 1.28B	149
	Combined	0.1100 ± 0.0058C	300	13.50 ± 1.01B	300

[a] Significant difference ($P \leq 0.05$; LSD) indicated by different letters within columns. All data were analyzed using SPSS Statistical (SPSS 2010).

Table 4. Comparison of black soldier fly adult weight (g) and longevity (d) ± SEM when raised on the Gainesville house fly diet (Hogsette 1992) at 28°C, 75% RH, and a photoperiod of 16:8 (L:D) h

Strains	Sex	Adult wt (g)	Sample size	Adult longevity (d)	Sample size
Texas	F	0.0333 ± 0.0039B[a]	156	10.09 ± 0.23A	156
	M	0.0243 ± 0.0035A	144	10.13 ± 0.56A	144
	Combined	0.0288 ± 0.0031A	300	10.11 ± 0.20A	300
Guangzhou, China	F	0.0467 ± 0.0036CD	153	12.68 ± 0.23B	153
	M	0.0435 ± 0.0044C	147	12.73 ± 0.90B	147
	Combined	0.0451 ± 0.0039B	300	12.71 ± 0.38B	300
Wuhan, China	F	0.0583 ± 0.0049E	151	13.17 ± 0.86B	151
	M	0.0507 ± 0.0011D	149	13.20 ± 0.61B	149
	Combined	0.0541 ± 0.0036C	300	13.18 ± 0.72B	300

[a] Significant difference ($P \leq 0.05$; LSD) indicated by different letters within columns. All data were analyzed using SPSS Statistical (SPSS 2010).

and female prepupae from Wuhan weighed 26.5–36.6% more than those from the Texas strain and weighed 13.5–28.4% more than those from the Guangzhou strain. The prepupae from the Guangzhou strain weighed less than the Wuhan strain but more than the Texas strain. The female prepupae weighed more than male prepupae among the three strains. Pupal developmental time differed significantly across strains ($F = 11.70$; df = 2; $P = 0.0085$). Pupae from the Texas strain needed significantly less time to reach the adult stage than those from the other strains. No significant differences were determined between the Guangzhou strain and Wuhan strain (Table 3).

Adult weight differed significantly across strains ($F = 34.74$; df = 2; $P = 0.0005$). Adults resulting from the Wuhan strain weighed 16.6 and 46.7% more than those resulting from the Guanghzhou strain and Texas strain, respectively. Adults from the Texas strain weighed significantly less than those from the other two strains. Adults from the Guangzhou strain weighed significantly less than those from the Wuhan strain. The adults of the Wuhan strain weighed most, and males weighed significantly less than females among the three strains. Strains significantly influenced male and female adult longevity. The Wuhan strain and the Guangzhou strain live significantly longer than the Texas strain, but no significant differences were found between the Guangzhou strain and the Wuhan strain (Table 4).

Parental effects on offspring morphology, development, and population dynamics have been shown for other species. Temperature and photoperiod are considered to be key variables responsible for parental influence on resulting offspring life histories. For example, rearing *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) larvae under identical conditions on artificial diet for two generations significantly reduced variation in diapause among populations. Diapause in *C. rosaceana* was influenced by temperature, photoperiod, and parental environment (Hunter and McNeil 2000). This variation across strains of black soldier flies was observed in this study. Adult black soldier flies were collected from each of the locations in China and used to initiate respective colonies. The larval development of flies from the Wuhan strain was 5–6 d less than the Texas strain and 3–4 d less than the Guangzhou strain. In addition, the larvae from the Wuhan strain weighed 37% more than the Texas strain and 14.4% more than the Guangzhou strain.

Final larval dry weight recorded for each strain was significantly different ($F = 1776.89$; df = 2; $P < 0.0001$). The dry weight of larvae from Wuhan strain was significantly more than that from Guangzhou strain and Texas strain in swine manure and chicken manure. The dry weight of larvae from Wuhan strain was not significantly more than that from Guangzhou strain, and both were significantly more than that from Texas strain in dairy manure. The dry weight of larvae in chicken manure was about three times of that in dairy manure and 2.4 times of that in swine manure in Texas strain. Results were similar in other strains. The larvae fed with dairy manure were lowest in content of protein, and the content of protein in larvae fed with chicken manure is more than the other two. However, when the same animal manure is digested by the three different strains of larvae, there were no significant differences across treatments in the protein content of larvae (Table 5).

Prepupae are ≈40% protein and 30% fat (Sheppard et al. 1994), which makes for a suitable source of food

Table 5. The output of the larvae and the content of their protein ($n = 3$) for three strains of black soldier flies reared on three manure types at 28°C, 75% RH, and a photoperiod of 16:8 (L:D) h

Strains	Swine manure		Chicken manure		Dairy manure	
	Larvae dry wt (g)	Protein (%)	Larvae dry wt (g)	Protein (%)	Larvae dry wt (g)	Protein (%)
Texas	53.66 ± 0.72C[a]	32.27 ± 0.23B	69.17 ± 0.18C	34.60 ± 0.20A	22.43 ± 0.64B	33.53 ± 0.30B
Guangzhou, China	59.03 ± 0.48B	33.16 ± 0.29AB	73.20 ± 0.21B	34.23 ± 0.41A	31.30 ± 0.64A	34.77 ± 0.43A
Wuhan, China	65.47 ± 0.88A	33.03 ± 0.30AB	76.63 ± 0.30A	34.80 ± 0.46A	31.20 ± 1.19A	34.13 ± 0.32AB

[a] Significant difference ($P \leq 0.05$; LSD) indicated by different letters within columns. All data were analyzed using SPSS Statistical (SPSS 2010).

Table 6. Mass reduction and N reduction of animal manure (n = 3) transformed by three strains of black soldier flies reared at 28°C, 75% RH, and a photoperiod of 16:8 (L:D) h

Strains	Swine manure		Chicken manure		Dairy manure	
	Dry matter reduction (%)	N reduction (%)	Dry matter reduction (%)	N reduction (%)	Dry matter reduction (%)	N reduction (%)
Wuhan, China	53.4 ± 0.3A[a]	47.9 ± 0.1B	61.7 ± 0.2A	51.8 ± 0.4B	57.8 ± 0.7A	50.6 ± 0.3B
Guangzhou, China	28.8 ± 0.2C	49.9 ± 0.3A	31.8 ± 0.3C	56.0 ± 0.6A	34.6 ± 0.3C	53.2 ± 0.5A
Texas	49.7 ± 0.4B	22.1 ± 0.5C	56.8 ± 0.4B	24.8 ± 0.4C	53.6 ± 0.2B	25.8 ± 0.2C

[a] Significant difference ($P \leq 0.05$; LSD) indicated by different letters within columns. All data were analyzed using SPSS Statistical (SPSS 2010).

for animals (Newton et al. 1977; Bondari and Sheppard 1981, 1987). The Wuhan strain needed less time to harvest, and at the same time, resulting prepupae were typically bigger than those from the Texas or Guangzhou strains. Although the fat and protein content are not known for these Chinese strains, the Wuhan strain did produce larger prepupae, which could be indicative of greater resource utilization. The protein content of the resulting prepupae was significantly different across treatments as expected. For example, when just examining protein content of prepupae from each population resulting from a diet of swine manure, those from Wuhan contained 30% more protein than those from Texas and 10% more than those from Guangzhou. Furthermore, similar results were also recorded for prepupae resulting from a diet of chicken or dairy manure.

Dry matter and N reduction in waste for the three black soldier strains in the three different animal manures are presented in Table 6. Larvae from the Wuhan strain reduced dry matter 46.0% (swine), 40.1% (dairy), and 48.4% (chicken) more than the Guangzhou strain and 6.9, 7.2, and 7.9% more than the Texas strain. The Wuhan strain was superior in reducing manure dry matter than the other two strains. The same trend occurred when measuring percent N in the prepupae. Black soldier fly larvae were able to reduce N by 22–56% across treatments. N reduction was greatest in the chicken manure and least in dairy manure. The larvae from the Guangdong strain exhibited the greatest N reduction and those from the Texas had the least. N reduction of chicken manure was the most while the N reduction of dairy manure was the least because chicken feed has more nutrition than that in dairy feed. There is also more nutrition in chicken manure. There are more fiber and poor nutrition in the dairy manure (Table 6).

This study provides information on the life history of three different black soldier fly strains and the ability of their larvae to reduce dry matter and associated nutrients in three different animal manures. Wuhan strain showed outstanding traits in the life history and ability of larvae to reduce dry matter. This study demonstrates a tremendous amount of phenotypic plasticity among the three different black soldier fly strains. Some research indicated the phenotypic plasticity could be because of climatic variation (e.g., temperature and rainfall) (Werner 2003, Chown et al. 2007). The three strains were collected from different climate regions. Wuhan is in temperate region. The average rainfall each year in Wuhan is 1,100 mm, and average temperature is 0.4°C in January and 28.7°C in July and average temperature 17.6°C in whole year. Texas strain originated from Stephenville, TX, a subtropic region. The average rainfall each year in Stephenville is 1,270 mm, and average temperature is 4°C in January and 26°C in July and average temperature 18.5°C in whole year. Guangzhou is in tropic region. The average rainfall each year in Guangzhou is 1,982 mm, and average temperature is 13°C in January and 28°C in July and 21.7°C annually. These data suggest that individuals interested in using the black soldier fly for waste management attempt to develop a colony with a local strain. Using strains from other regions might prove less effective in waste reduction and could present a risk of colony collapse. Minimally, if an individual has to rely on a sample from another region, it is better that the colony should be maintained for a few generations to allow the strain to adapt and become a more effective waste management agent.

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