ORIGINAL RESEARCH ARTICLE

Temperature affects *Aethina tumida* (Coleoptera: Nitidulidae) Development

Lilia I de Guzman and Amanda M Frake

USDA-ARS, Honey Bee Breeding, Genetics and Physiology Laboratory, 1157 Ben Hur Road, Baton Rouge, Louisiana 70820-5502, USA.

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* Corresponding author. Email: ldeguzman@ars.usda.gov

Summary

The effects of temperature on several life history parameters of small hive beetles (SHB), *Aethina tumida*, were investigated under laboratory conditions. Our results showed that the development, body size and weight of SHB were dependent on temperature. Egg incubation was about two days at higher temperature (34°C) and three days at lower (room) temperature (24–28°C). Exposure of larvae to lower temperature resulted in a 15-day extension to their development to adult emergence with a mean of 36.31 ± 0.08 days as opposed to 20.68 ± 0.08 days at higher temperature (34°C). At lower temperature, the developmental time (first instar to adult emergence) of males was about one-half day longer (36.63 ± 0.12 days) than that of females (36.02 ± 0.15 days). Higher temperature supported larger (length = 6.30 ± 0.07mm, width = 3.48 ± 0.02mm) and heavier (12.95 ± 0.22mg) adult females than did the lower temperature (length = 5.30 ± 0.04mm, width = 3.39 ± 0.02mm, weight = 11.40 ± 0.20mg). Weight and width similarities between males exposed to higher temperature (weight =11.53 ± 0.14 mg, width = 3.43 ± 0.02mm) and females reared under room temperature (weight = 11.40 ± 0.20mg, width = 3.39 ± 0.02mm) were also observed. From this study, we can deduce that the abundance and impact of SHB on honey bee colonies may be influenced by their rate of development in different thermic regimes. A new technique for rearing individual SHB is also described.

Keywords: *Aethina tumida*, small hive beetle, beetle development, temperature

Introduction

The small hive beetle (SHB), *Aethina tumida* (Coleoptera: Nitidulidae) is native to sub-Saharan Africa and was first described by Murray (1867). In the United States, SHB was first detected in Florida in 1998 (Elzen *et al*. 1999) although beetles collected from honey bee colonies in South Carolina in 1996 were later identified as SHB (Hood, 2000). Subsequently, it has spread to more than 30 other states, most of which are east of the Mississippi River (Neumann and Elzen, 2004). A SHB population is now established in Australia (Gillespie *et al*. 2003). SHB has been detected in Egypt (Mostafa and Williams, 2002), Portugal (Ritter, 2004) and Canada (Clay, 2006), but no established population has been reported. DNA analyses showed that SHB in the U.S. is genetically similar to those of the African types (Evans *et al*. 2000).

Temperature is among many environmental factors that affect the establishment of tropical insects in temperate countries (Renault *et al*. 2003). Hence, the rapid spread and abundance of SHB in its current distribution may have been influenced by temperature. SHB is more prevalent in the warmer regions of South Africa (Lundie, 1940). A survey conducted by Pettis and Shimanuki (2000) showed that SHB continued to reproduce during winter (February) in Florida but no reproduction was detected in South Carolina and Georgia during this time. No reproduction was also observed by Lundie (1940) during cooler months in South Africa. There have been limited studies conducted on the life history of SHB, all of which showed inconsistent estimates on developmental time. Lundie (1940), the first researcher to study SHB biology, claimed a wide variation (31–80 days) in total developmental period, without reporting temperatures. Schmolke (1974) estimated a life cycle of 32 days at 30°C. Schmolke also reported slower development of larvae and pupae under room temperature but the values were not recorded. Using similar temperature ranges Neumann, *et al*. (2001) recorded a developmental time of 49 days at 17–24°C, while 41 days was established by Mürrle and Neumann (2004) at 18–25°C. Recently, a total developmental period of 24–46 days was established by Haque and Levot (2005) at 29°C. This discrepancy may have resulted from differences in temperature used during the beetles' development. The effect of temperature on the development of several insect species has been studied. For example, exposure of the desert locusts, *Schistocerca gregaria*, to higher temperature shortened their development, and also increased food consumption and weight gain during the first week of adult

emergence (Gündüz and Gülel, 2002). A similar trend was observed in the nitidulid beetle, *Haptoncus ocularis* (Tsukada *et al.* 2005). To date, no study has been conducted to determine the effect of temperature on the life history of the nitidulid beetle, *A. tumida*. Therefore, this study was conducted to establish the effects of temperature on the rate of development, body size and weight of SHB which may provide insight into their population dynamics. A new technique of rearing individual beetles is also described.

Materials and Methods

Rearing conditions

A small hive beetle culture was established in the laboratory by collecting wild specimens of *A. tumida* adults (≥100) from infested honey bee colonies from an apiary near Baton Rouge (30° 21' 42.56 N, 91° 11' 11.07 W), Louisiana. Eggs were obtained using the glass slide technique initiated by Pettis (personal communication) with some modifications. Adult beetles (n = 50) were placed in a rearing container, a glass slide staining dish (10.5 x 8.5 x 7cm) outfitted with a slide rack. Beetles were fed at least ten honey bee pupae, 2g of pollen and 2tsp of honey. Four moistened cotton balls were also added to provide increased humidity. Slides were prepared for beetle oviposition by separating two slides with cover slips at each end of the slides to produce a space for egg laying. The slides were held together by taping both ends with clear tape. Beetles also laid eggs in spaces between the dish and the slide rack and in the cotton balls.

Upon hatching, larvae were transferred into a Plexiglas container (length 15cm x width 15cm x height 16cm) supplied with honey bee pupae, honey and pollen *ad libitum*. Upon reaching the wandering phase, larvae were put in a separate Plexiglas container which was about three quarters full of moist potting soil (Baccto®, sterilized in an oven at 260°C for 30min) for pupation. The lid of the container had a hole (diameter = 6cm) covered with a fine mesh screen to allow ventilation. Upon emergence, adult beetles were maintained in a 1-pint mason jar (with a screen mesh on the lid) supplied with honey bee pupae, honey and pollen *ad libitum*. All rearing containers were provided with moistened cotton balls for humidity.

Effect of temperature on egg eclosion

To obtain synchronized eggs, three rearing containers were prepared as described above. Each container containing at least 50 adult beetles (nonsexed) was placed in an incubator at 34°C. This temperature is within the optimal range (30–35°C) for brood rearing in honey bees (Winston, 1987). At that time, estimation of egg incubation began. After 20 h, slides with eggs were removed and separately placed in six rearing containers with one slide of eggs per container. Three containers each with two moist cotton balls were kept in an incubator at 34°C and three at room temperature (24–28°C). To determine the percentage of egg hatching through time, containers were examined for the presence of larvae twice a day under a dissecting microscope. All larvae were counted and removed from the containers. The proportion of eggs hatching through time was analyzed using Fisher's Exact Test (SAS Institute 2001, Version 8.2).

Determining the development of *A. tumida* as affected by temperature

The effects of temperature on several developmental parameters of *A. tumida* were also evaluated in an incubator at 34°C and at room temperature (24–28°C). Laboratory-reared adults (n = 50) (nonsexed) from above were randomly selected and placed in a rearing container with three egg-laying slides. The rearing container was then placed in an incubator (34°C) overnight. After 20 h, the rearing container held slides full of eggs. Adult beetles were removed and the containers were returned to the incubator for eggs to hatch. Egg-hatching was monitored every hour to determine the age of newly emerged larvae.

First instar larvae (≤5 h-old) were used in this study. Larvae were reared individually using Eppendorf® vials (1.5 ml) each containing one honey bee pupa. Each vial was cleaned daily using Q-tips® and given a fresh pupa. A moistened cotton wad was used to close each vial and prevent desiccation. During the first day, we found that a few larvae were able to escape. Thereafter, a piece of parafilm was used to seal the edges and to reinforce the stability of the cotton caps. All Eppendorf vials were numbered and placed in partitioned trays. A total of four trays each containing 50 Eppendorf vials were prepared. Two trays were placed in an incubator (34°C, hereafter referred to as high temperature) and the other two were kept at room or low temperatures (24–28°C). Vials were examined daily under a dissecting microscope to determine the length of time required for the SHB larvae to moult from one instar to the next. The cotton caps were moistened daily for all vials kept in the incubator while every three days or when needed for the vials left at room temperature. Larvae were considered to have reached the wandering phase and thus ready to pupate when feeding ceased. The larvae either tried to find their way out or began to pupate in the cotton cap. At this time, about 1.20g of moist potting soil (Baccto®) was placed in each vial for pupation. The majority of the larvae dug pupation cavities next to the translucent wall of the vials. The development of SHB was followed daily in these tubes. When pupae became teneral adults, cotton wads were removed and replaced with regular caps. Using a dissecting needle, about five holes were made in the caps for ventilation. The removal of the cotton wad provided a space for the emerging adults.

Newly emerged adults were collected and examined individually under a dissecting microscope to determine sex. By applying very gentle pressure on the abdomen, the male's 8th tergite was easily viewed dorsally while the male genitalia was seen extending at a right angle from the ventral surface (Schmolke, 1974). The long ovipositor of the female extends straight from the tip of the abdomen and can also be distinguished without full extension. Body weight (mg) of newly emerged adults (unfed, since there was no access to a food supply) was recorded using a Mettler analytical balance. The length and width (mm) of newly emerged adults were also recorded using a vernier caliper. Length was determined by measuring individual beetles from anterior to posterior termini, and the widest margin of the pronotum was measured for width (Ellis, *et al.* 2002a). Adults were narcotized with carbon dioxide to facilitate measurements.

The effects of temperature on developmental time, body size, weight of newly-emerged adults, sex ratio and mortality were compared using the Wilcoxon two-sample test. The Kruskal-Wallis test was used to compare differences among sex/temperature combinations (SAS Institute 2001, Version 8.2).

Results

Effect of temperature on egg eclosion

Nearly all eggs hatched within two days when they were exposed to high temperature (*Table 1*). An additional day was needed to achieve about 98% hatching for those eggs kept at room temperature.

Development of *A.turmida* as affected by temperature

The duration of each developmental stage of SHB was greatly affected by temperature (*Table 2*). Three larval instars were recorded; the third instar (feeding plus non-feeding stages) took the longest time. The developmental period from first instar to adult emergence was significantly ($P < 0.0001$) shorter at higher temperature (20.69 ± 0.08 days) than at lower temperature (36.31 ± 0.10 days). This was consistent for both sexes. However, females had significantly shorter developmental periods than males at room temperature. Further, SHB spent more than 75% of their developmental time in the soil, but about three days longer at room temperature (79.28 ± 0.14%) than at high temperature (75.81 ± 0.09%) ($P < 0.0001$).

Our results also showed that temperature had a significant effect on size and weight of newly emerged adults (*Table 3*). Exposure of larvae to high temperature during their development resulted in the longest females ($P < 0.0001$). Although males reared in the incubator were significantly shorter than the females from the same treatment, they were significantly longer than both females and males reared at room temperature. Exposure of larvae to a higher temperature also resulted in a significantly ($P < 0.0001$) wider body than those beetles reared at room temperature. However, males reared in the incubator and females reared in room temperature were similar in size. The same trend was observed for the weight of newly emerged SHB. Females exposed to higher temperature were the heaviest ($P < 0.0001$) while males reared in room temperature weighed the least. Males and females reared at high and lower temperatures, respectively, had similar weights. There were no effects of temperature on the sex ratio of the adults ($P = 0.644$) (*Table 2*), and on mortality ($P = 0.429$). Death of beetles (high temperature = 10%, lower temperature = 7%) was observed only during pupation.

Table 1. Number of Aethina tumida eggs that hatched through time (h).

Temp	Rearing box no.	Number of eggs hatched through time (h)*									Non viable**	Total eggs
		24	26	30	51	56	71	77	100	124		
Incubator (34°C)	1	0	36	189	700	2	1	0	0	0	4	932
	2	0	17	28	358	3	0	1	0	0	1	408
	3	0	83	169	405	1	0	1	0	0	2	661
	total eggs	0	136	386	1436	6	1	2	0	0	7	2001
	% hatched		6.80%	26.09%	99.20%	99.50%	99.55%	99.65%	99.65%	99.65%	0.35%	
Room (24–28°C)	1	0	1	42	390	124	222	8	2	2	2	793
	2	0	5	121	488	59	122	14	0	0	1	810
	3	0	0	29	398	65	238	5	3	0	1	739
	Total	0	6	192	1276	248	582	27	5	2	4	2342
	% hatched		0.26%	8.45%	62.94%	73.53%	98.38%	99.53%	99.74%	99.83%	0.17%	

*Time started when rearing containers were placed in the incubator for egg-laying.

**Non-viable eggs were those that either turned brown or dried up.

Table 2. Average developmental time in days (mean ± SE) for Aethina tumida reared in an incubator and at room temperature.

Temp	Sex (n)	Larva				Pupa	Teneral adult to emergence from soil	First instar to adult emergence
		First instar	Second instar	Third instar				
				Feeding^{ns}	Non-feeding*			
Incubator (34°C)	Female (n=39)	$1.00 \pm 0.00_b$	$1.03 \pm 0.03_b$	2.87 ± 0.05	$5.30 \pm 0.10_b$	$5.33 \pm 0.09_b$	$4.83 \pm 0.12_c$	$20.49 \pm 0.10_c$
	Male (n=41)	$1.00 \pm 0.00_b$	$1.02 \pm 0.02_b$	2.98 ± 0.02	$5.27 \pm 0.08_b$	$5.48 \pm 0.09_b$	$5.24 \pm 0.09_b$	$20.88 \pm 0.11_c$
Room (24–28°C)	Female (n=46)	$2.68 \pm 0.08_a$	$1.75 \pm 0.07_a$	3.12 ± 0.12	$9.36 \pm 0.11_a$	$10.80 \pm 0.10_a$	$8.46 \pm 0.20_a$	$36.02 \pm 0.15_b$
	Male (n=41)	$2.63 \pm 0.08_a$	$1.80 \pm 0.07_a$	3.11 ± 0.12	$9.57 \pm 0.11_a$	$10.90 \pm 0.10_a$	$8.62 \pm 0.16_a$	$36.63 \pm 0.12_a$

*Non-feeding includes the mobile (wandering phase) and immobile stages.
Means within columns followed by the same letter are not significantly different (P > 0.05).
ns = not significant.

Table 3. Average body length, width and weight (mean ± SE) of newly-emerged adults of Aethina tumida reared individually inside the incubator and at room temperature.

Temperature	sex	length (mm)	Width (mm)	Weight(mg)
Incubator (34°C)	Female (n=39)	$6.30 \pm 0.07_a$	$3.48 \pm 0.02_a$	$12.95 \pm 0.22_a$
	Male (n=40)	$6.00 \pm 0.07_b$	$3.43 \pm 0.02_{ab}$	$11.53 \pm 0.14_b$
Room (24–28°C)	Female (n=46)	$5.30 \pm 0.04_c$	$3.39 \pm 0.02_{bc}$	$11.40 \pm 0.20_b$
	Male (n=41)	$5.29 \pm 0.04_c$	$3.33 \pm 0.02_c$	$10.02 \pm 0.22_c$

Means within columns followed by the same letter are not significantly different (P > 0.05).

Discussion

The ability to rear individual beetles not only allowed isolation of beetles for systematic observation but also prevented overcrowding and thus, food competition among larvae, which can potentially affect growth and size of beetles. Newly emerged beetles were also prevented from feeding and mating. This procedure may be useful when beetles are needed for nutritional, mating behavior, or other studies.

Our result showed that the development of *A. tumida* was dependent upon temperature. A high percentage (78%) of egg-hatching was observed by Lundie (1940) between 2 to 3 days at an unreported temperature. Schmolke (1974) reported that at 30°C, egg incubation period is about 41 h since about 52% of the eggs hatched during this time and 89% hatched within 48 h. We observed nearly 100% egg-hatching within 51 h at high temperature. Recently, Haque and Levot (2005) reported an egg incubation period of 1–2 days at 29°C. In this study, we cannot ascertain whether or not the beetles laid eggs immediately after the rearing containers were put in the incubator. Nevertheless, we assumed that the egg incubation period of SHB was about two days at higher temperature. Another day was needed to achieve 98.4% hatching at lower temperature. These observations suggest that egg incubation is accelerated by higher temperature.

Exposure of SHB larvae to 34°C also accelerated their development. From egg to adult emergence, a total developmental time of about 23 days was observed. This duration was about nine days shorter than the life cycle of 32 days reported by Schmolke (1974) at 30°C. An extension in developmental time of more than two weeks (39 days) was observed when SHB were exposed to lower temperatures (24–28°C). This observation was about 10 and

three days less than the developmental time observed by Neumann *et al*. (2001) and Mürrle and Neumann (2004) who used lower temperature ranges of 17–24°C and 18–25°C, respectively. At 29°C, Haque and Levot (2005) reported a total developmental time of 24–46 days, the lowest value of which was similar to our findings at 34°C. The longest developmental period was about 80 days reported by Lundie (1940) at unreported temperatures. The discrepancy of our estimates from those in the literature was probably due to differences in the temperature during SHB development. Nevertheless, our results and those of others seem to suggest that a change in temperature can make a significant impact on SHB abundance, since reduced development time will result in increased generations per year.

Lundie (1940) observed a great variation in body size of adult SHB. However, no difference in body size was reported by Schmolke (1974). Ellis *et al*. (2002a) claimed that females are generally bigger and heavier than males. In this study, we observed that temperature influenced body size and weight of newly emerged SHB. Higher temperature resulted in larger and heavier adult females. We also observed similarities in body weight and width between adult males exposed to high temperature and adult females reared under low temperature. These observations corroborate the findings of Ellis *et al*. (2002a) indicating size similarities of males and females collected from different apiaries in South Carolina and Georgia. However, the size similarities between the sexes observed by Ellis *et al*. (2002a) can be attributed to sampling techniques. Ellis *et al*. (2002a) measured beetles that were collected from infested colonies in different locations in South Carolina and Georgia at three different sampling months. Thus, their samples included well-fed (had open access to pollen, brood and honey) and mated beetles (gravid females have extended abdomens) whilst we measured newly emerged beetles (unfed and unmated). Further, differences in temperature between the two states may have affected Ellis et al's data collected at different months. At 29°C, Haque and Levot (2005) fed SHB larvae with a diet of pollen, honey and yeast and produced adults with an average weight of 149 mg. This value is within the range (81-151 mg) of the weight of newly emerged worker bees (Winston, 1987). It is to a large extent higher than our findings and those of Ellis, *et al*. (2002a). We also observed no difference in the proportion of female to male SHB, which did not agree with the findings of Neumann *et al*. (2001) and Ellis *et al*. (2002a, b). This disagreement may be due to a smaller sample size in this study than in the previous studies.

Decreased developmental time of insects exposed at higher temperature regimes has been documented in several studies such as on the carrot weevil (Woodson and Edelson, 1988), desert locust (Gündüz and Gülel, 2002), and nitidulid beetle, *Haptoncus ocularis* (Tsukada *et al*. 2005). In the desert locust, this accelerated development (including weight gain) was attributed to increased food consumption at high temperature (Gündüz and Gülel, 2002). In this study, we cannot ascertain whether or not increased food consumption and assimilation by larvae at higher temperature caused faster development, and increased body size and weight of adult SHB. Although we noticed a higher daily food consumption of larvae exposed at higher temperature than those at lower temperature, no quantification of food consumption was conducted.

In contrast to the four larval instars reported by Haque and Levot (2005), only three instars were evident in this study. This inconsistency may be due to some methodological differences. In this study, larvae were reared individually, and ecdysis was systematically monitored by examining vials under a dissecting microscope for the presence of exuviae. Haque and Levot (2005) failed to provide details as to what they actually did. We also found that SHB spent much of their developmental life (>75%) in the soil. Therefore, timing of soil treatment when an abundance of pupating beetles are in the soil is critical for the success of this control method. Nevertheless, judicious spraying of chemicals in the soil is needed to prevent the demise of entomopathogenic nematodes that may be present in the soil. Entomopathogenic nematodes have been reported to be infective against larval SHB in the laboratory (Cabanillas and Elzen, 2006), and have been found naturally infesting young adults of SHB in the soil (de Guzman *et al*. submitted). Entomopathogenic fungi are also known to cause mortality of SHB (Ellis *et al*. 2004; Mürrle *et al*. 2006).

Other biological observations were also made. Using their mandibles and through peristaltic movement of their bodies, wandering larvae pushed their way through the soil. The larvae smoothened their tunnels and pupation cavities using a saliva-like secretion. This secretion may be used by SHB for several purposes: a) softening the soil as larvae moved in search of a suitable place for pupation, b) serve as a cement-like support preventing pupation cavities from collapsing, c) slow down entry of rainwater into cavities, d) prevent rapid escape of moisture from the cavities, and e) inhibit microbial growth inside the cavities. SHB larvae also moved constantly through the soil until they reached the immobile stage. At this stage, the larvae became shorter and stouter. Teneral adults spent several days in their cells, and gradually moved up through the soil sometimes accompanied by defecation (white faeces). This period is probably critical for SHB to attain sexual maturity since females were observed to have mated and laid eggs after a day of emergence (personal observation). We also observed that some newly emerged adults went back underneath the soil. While being entrapped in the Eppendorf vials, these adults had limited movement and no access to food. This inactivity and lack of food may have forced adult beetles to move down (and congregate for those adults reared in the Plexiglas pupation container) below the soil surface. This 'sit and wait' strategy was used by isopods to conserve energy when food is unavailable (Hervant and Renault, 2002), and was also observed in a polyphagous beetle, *Alphitobius diaperinus* (Renault *et al*. 2003),

SHB is also known to survive winter in the honey bee cluster (Pettis and Shimanuki, 2000). This ability is shown by their occurrence even in colder parts of the US and its recent discovery in Canada (Clay, 2006). However, a survey conducted by Pettis and Shimanuki (2000) showed that SHB continued to reproduce during winter (February) in Florida but no reproduction was detected in South Carolina and Georgia during this time. In South Africa, Lundie (1940) obtained five complete generations despite the lack of reproduction during the cool months. From this study, we can hypothesize that the establishment of SHB population and their impact on honey bee colonies may be influenced by their rate of development in different thermic regimes. Colder climates will inhibit SHB population development. However, during warmer months population development may accelerate and reach levels that damage colonies.

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