The Developmental Changes of Immature African and Four Lines of European Honey Bee Workers

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Abstract

Developmental stages were compared between European honey bee (EHB) and local African honey bee (AHB) workers, to provide updated information since the AHB migrated into N. America. The weight and dimensions of each life stage of the immature bee, egg to adult, were recorded. European queens from known stock were selected for this experiment. The queens were the SMR (suppressed mite reproduction, now called Varroa Sensitive Hygiene or VSH) and Russian (RU) lines from the USDA-ARS Honey Bee Breeding, Genetics, and Physiological Research (Baton Rouge, LA), Hygienic queens (HY), Italian queens from Big Island Queens, Hawaii (BI), and local African bees (AHB) found near the lab (Tucson, AZ). The most noticeable differences were the weight and developmental time in the AHB compared to the EHB which are similar to those reported for AHB in South America in 1979. A table recording the changes in size and weight of each stage was created that includes a description and timing of melanization. A photographic chart showing the differences was also made.

Keywords: Africanized Honey Bee, Life stages, Development

Introduction

The African honey bee (AHB) (*Apis mellifera scutellata*) has colonized many regions of the New World and replaced European honey bee (EHB) subspecies (*A. mellifera ligustica*) in most of the environments in which it has migrated. Currently, AHB has been positively identified in many southern regions of the U.S. (see *http://www.ars.usda.gov/Research/docs.htm?docid=11059 &page=6*). The differences between AHB and EHB behaviors [1] and physiology [2] are well documented. While detailed observations have been made on the difference between AHB and EHB queen development [3, 4], information comparing the physical development of EHB with AHB workers is limited

[5] and may be out of date. We examined workers of four EHB lines and local AHB during the spring of 2004 and recorded the weight and dimensions of each life stage. The purpose of this study was to determine the developmental rate changes in AHB and EHB workers during each life stage, to compare our measurements to AHB data prior to their migration into N. America, and to create a photographic chart to serve as a visual reference.

Materials and Methods

Bee stock: We used four different lines of EHB: Russian, Hygienic, an Italian line from Hawaii and the SMR (Suppressed mite reproduction), now called varroa-sensitive hygiene (VSH). All colonies were started by introducing a mated queen from each genetic line into a bee package. RU and SMR queens were obtained from the USDA-ARS Honey Bee Breeding, Genetics, and Physiological Research (Baton Rouge, LA). Queens from the SMR line were instrumentally inseminated with drones from the same stock. Queens from the Russian line (RU) were open-mated in isolation (Marsh Island, LA) with Russian drones. Hygienic queens (HY) were instrumentally inseminated with hygienic drones (Glenn Apiaries, CA). The Italian open-mated queens were obtained from Big Island Queens, Captain Cook, Hawaii (B I). The African queens were collected from swarms in Tucson, AZ. The African genotype was confirmed using mtDNA and morphometric analyses [6, 7]. Colonies were maintained at the Carl Hayden Bee Research Center (CHBRC, Tucson AZ).

Larvae and pupae collection: Two weeks prior to collecting samples, all test colonies were fed natural pollen patties and 1:1 sugar syrup (65% High Fructose Corn Syrup) to stimulate brood rearing. To obtain brood of known ages, each queen was confined for 12 hours in a frame cage made of zinc queen excluder and allowed to lay eggs freely for twelve hours (overnight) on the frame. After confinement, the queen was released and the egg-filled frame placed back in the cage and returned to the colony. Frames of known-age brood were removed from colonies and transported to the laboratory. During sampling, the laboratory was maintained at 28.3-31.1ºC and 35% RH to simulate broodnest conditions. Brood was extracted from cells by using grafting tools or gentle pulling with forceps, after pushing away the wax cell walls and caps.

Physical measurements: Five individuals from each of the queen lines and each age were sampled. Individuals were weighed using a Sartorius CP2P Electronic Micro Precision Balance (Gottingen, Germany). Length and width measurements of each individual were obtained using a clear metric ruler mounted in a Petri dish. Representative photographs of each stage were taken with a Leica DC 100 Microscope (Switzerland) with an ImageAccess Module TWAIN Camera Control Digital Color Camera that interfaced with the computer.

Analysis

Averages of weights and volumes were compared for each life stage among the different bee lines using analysis of variance. Pairwise comparisons were made among means using the Tukey LSD [8]. Measurements were used to calculate volume using the formula for a spheroid (V= 4/3 pabc).

Results

Egg Stage Days 1-3: Eggs laid by HY queens weighed significantly more than those from AHB and BI on Days 1 and 2. By Day 3 however, there were no differences i n weights of eggs among the bee lines (see Fig. 1, Table 1). The volume of the eggs from AHB and RU on Days 1 and 2 was smaller than the other lines but by Day 3 all lines were equivalent in volume (Table 2).

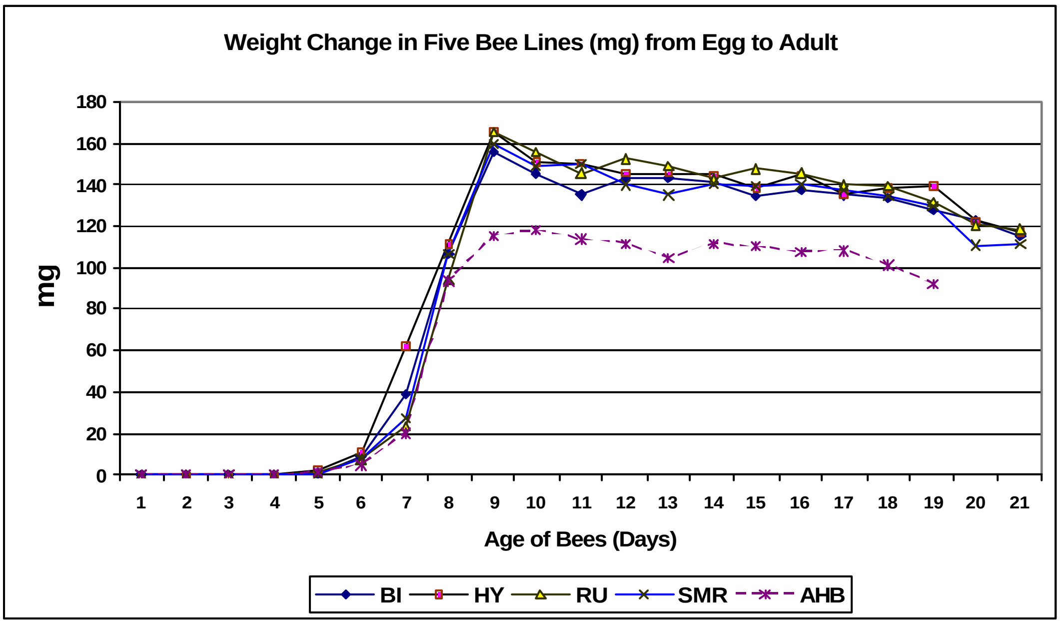

Fig.1. Weight changes from egg to adult in four EHB lines and an AHB strain (dashed line) of honey bees (in mg).

Larval Stage Days 4-9: On Day 4, the egg ecloses and the larva is then fed brood food by the nurse bees [9]. From Day 4 to Day 8, the larvae grow rapidly until Day 9 when they begin to spin cocoons and begin pupation. The BI Day 4 larvae were significantly lighter than the other lines and the RU larvae significantly heavier (see Fig. 1, Table 1). There was no significant difference among the lines in the body volumes of the larvae on Day 4. During the next four days (Days 5-8), the larva moults about once a day, shedding its exoskeleton to accommodate its rapidly growing body [9]. AHB Day 6 larvae (larva 3) were lighter than BI and HY (mean weights: 4.69mg, 8.78mg, 10.95mg, respectively. While the weights were similar among the RU and European lines, the volume of the HY larvae was greater than the other lines. By Day 8, just before the larvae are capped and ready to spin the cocoons, the average weight increased 10 to 20-fold and volume 10 to 33-fold (AHB and HY respectively) in just two days. After the period of rapid growth, days 6-8, there were no significant differences in weight or volume among the bee lines.

Table 1. Weights (mg) of African (AHB), European (EHB) Honey Bees from Egg to Adult. The four European lines are RU (Russian) Italians (BI) from Big Island, Hawaii, a hygienic line (HY) and the mite resistant SMR lines (now called VSH Varroa Sensitive Hygiene). On days 2 and 3, some eggs ruptured during weighing. The column *Bee Stage* refers to observed changes in the EHB lines only. Numbers in parenthesis indicate sample size. Averages of weights were compared for each life stage among the different bee lines using analysis of variance. Means within each row (life stage) followed by a different letter are significantly different at the 0.05 a-level as determined by Tukey's LSD.
** AHB emerged on Day 19.

Stage	Age (days)	n	AHB	BI	HY	RU	SMR
egg	**1**	*5*	0.11a	0.13a (7)	0.15b	0.14ab	0.14ab
egg	**2**	*5*	0.07a (4)	0.11a	0.13b (6)	0.12b (10)	0.10ab
egg	**3**	*5*	0.11	0.10	0.09	0.11 (3)	0.09
larva	**4**	*5*	0.22bc	0.13a	0.21bc	0.30c	0.17ab
larva	**5**	*5*	1.16a	0.54b	2.76c	1.24a	0.93ab
larva	**6**	*10*	4.69a	8.78b	10.95b	7.71ab	7.67ab
larva	**7**	*5*	20.23a (10)	38.92a	62.36b (10)	23.42a	27.49a (10)
larva	**8**	*5*	93.16	106.44	111.76	94.00	106.52
capped & spinning	**9**	*10*	115.02a	156.03b	165.16b	165.6b	159.48 b
spinning	**10**	*5*	117.96a (8)	145.64b	151.06b	155.5b	148.62 b (9)
Prepupa (immobile)	**11**	*5*	113.48a	135.26ab	150.22b	145.06b	149.88 b
white pupa	**12**	*10*	111.69a	145bc (9)	145.41bc	152.01c	139.89 b
white pupa	**13**	*5*	104.88a	143.6bc	145.36b	149.16b	135.16 c
light pink compound eyes	**14**	*5*	111.2a	141.26b	144.52b	142.74b	140.48

							b
dark pink compound eyes	**15**	*5*	109.88a	134.06b	138.54b	147.54c	138.82 b
purple compound eyes & ocelli	**16**	*5*	107.26a	137.18b	144.92b	145.04b	140.08 b
dark purple compound eyes, light brown head & thorax	**17**	*5*	107.78a	135.24b	135.44b	139.7b	137b
Dark purple compound eyes, light brown abdomen	**18**	*5*	101.38a	133.46b	138.76b	139.46b	134.64 b
black compound eyes, light grey wing pads, gray head &thorax, tan/whitish abdomen	**19****	*5*	92.34a	127.42b	138.82c	131.78bc	129.48 b
black antennae, medium grey wing pads	**20**	*5*		122.43a (10)	122.24a	120.44a	110.86 b
imago/emerging	**21**	*5*		115.06	117.16	118.10	111.18

Pupal Stage Days 10-21: Larvae are capped mid-way through Day 9, spinning their cocoons through Day 10 with the prepupa (immobile larva) developing on Day 11. On Day 9, the cells are capped over with wax, marking the end of the larva's feeding stage. By Day 10, the larvae change into prepupae and quickly into the pupal form, defecating gut content early in cocoon construction [9]. From our observations, white pupae appeared on Day 12 and the bee started to darken on Day 14. Melanization followed this sequence: first the compound eyes, then the ocelli (Day 15), next the wing bases (Day 17), then the thorax (Day 18), and lastly, the head and antennae (Day 19-20).

The EHB emerged on Day 21 (see Fig. 2). During the pupal stage, the weight for EHB gradually dropped 35mg on average by the time the teneral adult bee emerged on Day 21. Throughout the pupal stage, AHB weighed less and had lower volumes than the other bee lines. However, development rates were greater for AHB than the other lines. For example, the AHB Day 14 pupae had dark pink compound eyes and ocelli compared with the light pink compound eyes and unpigmented ocelli of the other lines (Fig. 2).

On Day 15, the compound eye of the AHB was light purple and the body was white. In the other lines, the compound eyes were medium pink and the ocelli were dark pink. The AHB compound eye was dark purple and the thorax was starting to tan on Day 16 while the thorax was still white in the other lines. The AHB pupal head darkened to brown on Day 18 and the bee molted on Day 19 to a striped, winged imago that chewed out of the cell by the end of the day, about 48 hours before the EHB (see Figs. 2-3).

Table 2. Volumes of African (AHB), European (EHB) Honey Bees from Egg to Adult. Big Island Italians (BI), a hygienic line (HY), SMR and Russian (RU) lines are also represented. The column *Bee Stage* refers to observed changes in the EHB lines only. Numbers in parenthesis indicate sample size. Averages of volumes were compared for each life stage

among the different bee lines using analysis of variance. Means within each row (life stage) followed by a different letter are significantly different at the 0.05 a-level as determined by Tukey's LSD. ** AHB emerged on Day 19.

Stage	Age (days)	n	AHB	BI	HY	RU	SMR
egg	**1**	*5*	0.08a	0.15bc	0.16b	0.07a	0.15bc
egg	**2**	*5*	0.21a (4)	0.09b	0.14b	0.07b (10)	0.19a
egg	**3**	*5*	0.07	0.08 (4)	0.07	0.07 (3)	0.07
larva	**4**	*5*	0.26	0.15	0.16	0.15	0.25
larva	**5**	*5*	1.83a	0.39b	2.36c	1.37a	0.75b
larva	**6**	*10*	2.88a	5.74a	11.71b	5.74a	3.22a
larva	**7**	*5*	21.13a (10)	20.10a	63.1b (10)	41.41b	22.32ac (10)
larva	**8**	*5*	97.00	103.57	119.27	97.00	115.92
capped & spinning	**9**	*10*	136.24a (9)	167.05ab	207.25b	209.79b	183.45b
spinning	**10**	*5*	164.31a (8)	209.60ab	221.20b	226.05b	220.37b (9)
Prepupa (immobile)	**11**	*5*	137.77a	176.21b	226.05c	224.47c	193.63b
white pupa	**12**	*10*	133.92a	176.20b (9)	172.47b (6)	152.14ab (7)	165.99b (8)
white pupa	**13**	*5*	88.18a	182.84b	117.12a	147.44b	99.64a
light pink compound eyes	**14**	*5*	104.25a	157.0b	158.31b	158.31b	153.65b
dark pink compound eyes	**15**	*5*	137.39a	154.38ab	160.93b	175.70b	163.59b
purple compound eyes & ocelli	**16**	*5*	96.61a	155.69b	139.10b	151.03b	155.69b
dark purple compound eyes, light brown head & thorax	**17**	*5*	101.89a	157.0b	154.38b	158.31b	157.0b
Dark purple compound eyes, light brown abdomen	**18**	*5*	131.88a	157.0ab	163.59b	160.93b	158.31b
black compound eyes, light grey wing pads, gray head &thorax, tan/whitish abdomen	**19 ****	*5*	162.23	143.08	155.69	159.62	154.38
black antennae, medium grey wing pads	**20**	*5*		183.17a	170.08b	170.08b	162.23b
imago/emerging	**21**	*5*		167.47a	185.78b	196.25c	193.63bc

All lines lost weight (dropping 35mg in the EHB and 23mg in the AHB) and volume during the pupal stage. In general, the AHB were the smallest and lightest throughout this stage (see Tables 1-2). Newly emerged AHB workers weighed less overall than the EHB lines. On Day 21 when the teneral EHB bees emerged, the lightest bee was the BI.

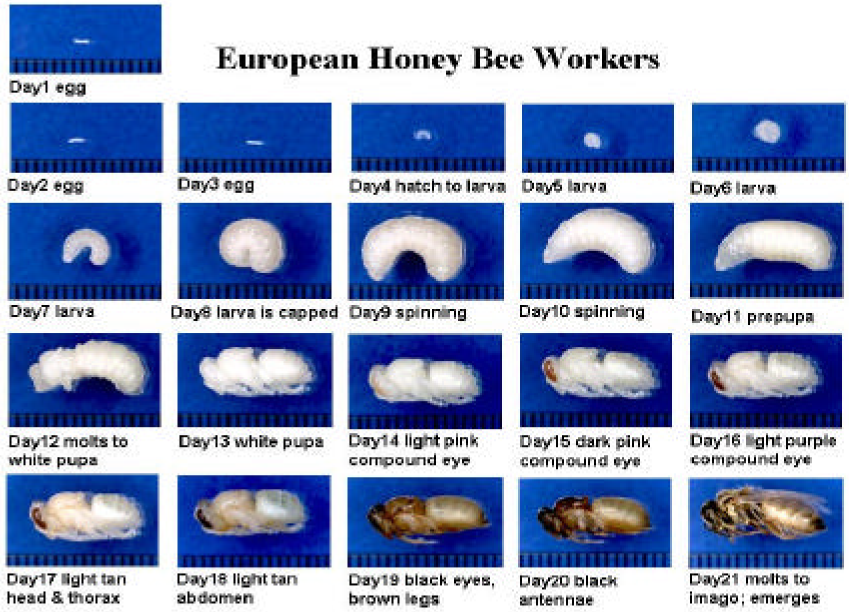

Fig. 2. Photographic record of the development of the European (BI) worker bees.

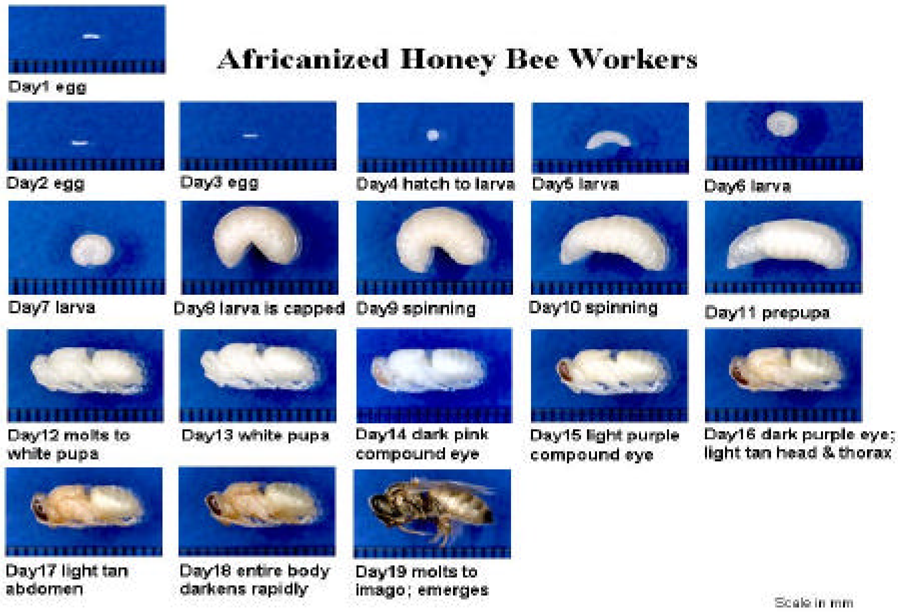

Fig. 3. Photographic record of the development of the AHB worker bees.
of the workers are from Days 1 to 21. Scale in mm.

Discussion

Regarding the physical characteristics of the bee lines, as expected, the most noticeable differences were the weight and developmental time in the AHB workers compared with the EHB. The AHB were lighter and had shorter development times than all the EHB lines. These data are similar to those reported by Winston et al. (1979) for AHB in South America. Apparently, AHB size and development time has not changed significantly as the bee has migrated and become established northward into the U.S.

Shorter development time is advantageous for the AHB in several ways. First, AHB are able to build up large populations faster than the other lines (http://gears.tucson.ars.ag.gov/beepop/), which usually results in more swarming. A faster development time also may confer some protection against the varroa mite, since it does not allow the youngest immature mites enough time to fully develop before the adult bee emerges. In addition, repeated swarming interrupts the mite's life cycle which would result in fewer founder mites finding brood in which to deposit eggs.

With the growing concern about the spread of the AHB, this short paper provides visual and physical guidelines comparing workers of the AHB with the EHB so that apiary inspectors and other apiculture specialists will be able to have a clear reference to differentiate the immature worker brood. We are currently working with drone brood from these lines to compare developmental rates and visual differences among these EHB lines and the AHB. We are also measuring volatile chemical compounds that are unique to the different bee lines.

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