LONGEVITY OF AFRICANIZED WORKER HONEY BEES (*Apis mellifera*) CARRYING EYE COLOR MUTANT ALLELES

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ABSTRACT: The dark coloration of insects eyes is attributed to the accumulation of the brown pigment insectorubin, a mixture of ommochromes, xanthommatin and several ommins, biosynthesized from tryptophan. When any of the events in the synthesis chain is interrupted, formation and accumulation of pigments other than insectorubin occurs, and a new eye color will appear. The aim of the present work is to evaluate the longevity of worker honey bees *Apis mellifera,* homozygous and heterozygous for the mutant alleles cream (cr), snow-laranja (s^{la}) and brick (bk). Eye pigmentation and average longetivity of bees are very closely related. Mutant bees carrying lighter eye pigmentation are unable to return to the hive; there is, therefore, a close association between the eye pigmentation and honey bees lifespan. Experiments ran in confinement cages confirm the orientation problems of mutant honey bees, which kept in a limited space, were able to return to the hive and had an extended lifespan in comparison to that observed in the nature, and did not present statistical difference (P>0.05) relative to the control group. Confinement to restricted areas improves honey bees orientation abilities and facilitates return to the hive.
Key words: eyes, mutants, cream, snow-laranja, brick

LONGEVIDADE DE OPERÁRIAS *Apis mellifera* AFRICANIZADAS PORTADORAS DE MUTAÇÕES PARA A COR DOS OLHOS

RESUMO: Os olhos das abelhas selvagens adultas apresentam coloração marrom-escura, devido à presença de pigmentos denominados omocromos-xantominas e diferentes tipos de ominas. Os principais passos da cadeia metabólica que determinam a biossíntese desses pigmentos iniciam-se a partir do triptofano e qualquer interrupção em um dos seus passos fará com que a cor marrom-escura seja substituída por uma nova cor. Estudou-se a longevidade de operárias de *Apis mellifera* portadoras dos alelos mutantes para a cor dos olhos cream (cr), snow-laranja (s^{la}) e brick (bk). Existe nítida relação entre o grau de pigmentação dos olhos e a longevidade média das abelhas. As abelhas mutantes que apresentam a cor dos olhos mais clara perdem-se no campo, quando realizam os primeiros vôos, não retornando ao ninho, o que mostra um acentuado problema de orientação capaz de interferir na longevidade. Também experimentos realizados em situação de confinamento confirmam estes resultados, uma vez que, estando retidas em um espaço limitado, as abelhas, em especial aquelas portadoras de qualquer mutação caracterizada pelo escurecimento progressivo dos olhos, apresentaram uma longevidade que não se diferenciou do grupo controle (P>0,05), sugerindo que o confinamento a áreas restritas contribui de forma positiva para a orientação dessas abelhas, facilitando o seu retorno à colméia.
Palavras-chave: olhos, mutantes, creme, snow-laranja, brick

INTRODUCTION

Mutations for eye color in honey bees, *Apis mellifera* L., described thus far are as follows: cream (cr) (Rothenbühler et al., 1952); brick (bk), ivory (i) (Laidlaw et al., 1953); pink (p) (Cale et al., 1963); snow (s), snow-tan (s^t), pearl (pe), garnet (g) (Laidlaw et al., 1964), umber (i^u) (Laidlaw & Tucker, 1965), snow-laranja (s^{la}) (Woyke, 1973; Soares & Chaud-Netto, 1982), Bayer (by), spade (sp) (Laidlaw, personal communication; quoted by Chaud-Netto et al., 1983) and chartreuse (ch) (Laidlaw et al., 1953; Lee, 1969; Soares, 1981). Eye color in mutant bees differs from wild bees for the complete absence or a decrease in the amount of ommochromes in the first; however, mutants differ from one another not only in the amount of ommochromes, but also with regard to the amount of other chemical compounds present (Dustmann, 1987).

The chemotype of a mutation for eye color relates to its position in the metabolic pathway that determines the biosynthesis of ommochromes from tryptophan. This metabolic pathway was established out of studies performed with several types of organisms (Kikkawa, 1941; Green, 1949; Dustmann, 1968, 1974, 1981).

Many mutations for eye color in *Apis mellifera* are normally used as genetic markers, but little is known about the behavior of bees carrying this type of mutation. The objective of this work was to make observations on the longevity of worker bees carrying the mutations cream (cr), snow-laranja (s^{la}) and brick (bk), determining to what extent the degree of eye pigmentation can influence the behavior of those individuals.

MATERIAL AND METHODS

The study on the longevity of worker bees was carried out in Ribeirão Preto-SP, Brazil, (altitude 621 m, 21°12'S, 47°52'W) both in hives maintained in the open field and in confined hives. Bees came from colonies that had heterozygous queens instrumentally inseminated with semen from mutant drones, which gave rise to mutant and heterozygous (wild phenotype) worker bee descendants in a 1:1 segregation. A brood comb with ready-to-emerge bees was removed from the colony and maintained in an incubator at 34°C and 80% relative humidity, for approximately 24 hours. After that period, 100 recently-emerged bees from each group were individually marked with small colored and numbered plastic disks (Opalithplättchen): mutant workers (cr/cr, s^{la}/s^{la}, bk/bk), heterozygous (cr/+, s^{la}/+, bk/+) and wild (+/+) workers, the latter coming from wild nests and deemed as a control in the experiment. Then, all bees were placed inside a nucleus-type, Langstroth model hive, with capacity for four frames and a mean population of 8,000 bees. The experiment was repeated twice for each experimental setting (field and confinement).

A confinement cage was constructed for the worker bee longevity study, based on the model by Waddington (1982) and Cartar & Dill (1990). It consisted of a wooden enclosure measuring 2 m x 2 m x 2 m, covered with white nylon screen (fine mesh = 1 mm). An experimental nucleus supplied with frames containing honey and pollen was placed inside the cage. Marked workers were introduced by following the same procedure previously described. Experiments were conducted in the fall and in winter.

The variance means were analyzed according to the Kruskall-Wallis, non-parametric test. The Dunn multiple comparison test was applied to determine which samples differed from aech other.

RESULTS AND DISCUSSION

The longevity of worker bees was estimated from life tables constructed according to Sakagami & Fukuda (1968), from which it is possible to obtain the mean expectation of life (MEL) of individuals at different age intervals.

Longevity of worker bees maintained in free hives

The MEL found for worker bees observed in each group was 2.70 and 4.05 days for cr/cr bees; 16.80 and 22.60 days for cr/+ bees; 2.95 and 4.40 days for s^{la}/s^{la} bees; 14.60 and 19.05 days for s^{la}/+ bees; 5.70 and 8.75 days for bk/bk bees; 23.60 and 25.70 days for bk/+ bees; 16.95 and 24.85 days for wild bees. Longevities of heterozygous and wild bees did not differ among themselves, but both show highly significant differences (P<0.001) when compared with the mutant bees, for both replicates conducted in open field (Table 1).

Several studies on the longevity of *Apis mellifera* have been carried out involving wild bees, both africanized and European. Some data from these studies were summarized in Table 2 for improved visualization and comparison.

Adult, European worker bees can have a much longer lifespan than africanized bees (Winston et al., 1983). This can be explained in view of the hibernating behavior exhibited by European bees toward the process of thermoregulation, which is a behavior adopted by the entire colony and provides excellent temperature control in temperate climate regions.

For temperate climate bees, there are two different longevity stages that occur over annual cycles (Sakagami & Fukuda, 1968). These stages correspond to a period when the colony is active and another when it undergoes a resting or hibernating phase. Bee longevity in temperate regions is very distinctive. In the winter, workers have a long lifespan and in the summer, which is the active stage, they are short-lived. In tropical and subtropical regions bees are generally active year-round, and the seasonal difference cannot be observed.

The number of surviving mutant bees decreased markedly from their first days of existence and there seems to be a clear relationship between the degree of eye pigmentation and longevity (Table 1). From age 6 to 10 days, bees carrying the cream mutation (white eyes) showed survivorship indices of 4% and 31%; snow-laranja worker bees (orange eyes with progressive darkening) showed indices of 9% and 38%; while the group of bees carrying the brick mutation, which show reddish-brown eye pigmentation, therefore closer to the normal coloration, had 42% and 81% of the surviving individuals in the same age class, and also a survivorship index of 17% and 31% in the age interval 11-15 days for the 1st and 2nd experiments, respectively. Data corroborate those obtained by Almeida & Soares (1992), while studying the chartreuse-limão mutation, described by Soares (1981), as they found MEL values below 5 days of age.

Longevity of worker bees maintained in a confinement hive

The longevity of worker bees maintained under confinement was 3.05 and 4.00 days for cr/cr bees; 10.80 and 16.35 days for cr/+ bees; 11.30 and 10.50 days for s^{la}/s^{la} bees; 7.40 and 10.10 days for s^{la}/+ bees; 18.35 and 24.40 days for bk/bk bees; 17.15 and 23.45 days for bk/+ bees; 15.30 and 19.55 days for wild bees (Table 3).

Bees carrying the cream mutation, even in a restricted area, have very reduced longevity, relative to the other mutants. This occurs since these bees have no eye pigmentation at all, their eyes remaining permanently white and not undergoing any alteration with age, which could cause a degree of photosensitization capable of interfering with their orientation, preventing them from returning to their nest, even if it is confined and is the only option available for the bees. Therefore, the experiments performed under confinement confirm that the absence of eye pigmentation influences bee orientation in any condition, since even when they had a reduced space for flight activity, there was a difference between the longevities of heterozygous and wild bees when compared to bees carrying the cream mutation. The *Pr>F* values were 0.012 for the 1st experiment and 0.020 for the 2nd.

Table 1 - Number of surviving worker bees in two experiments carried out under natural conditions (open field) and respective mean expectations of life (MEL) for each observed group.

Age (days)	*cr/cr*		*cr/+*		s^{la}/s^{la}		$s^{la}/+$		*bk/bk*		*bk/+*		*wild*	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
01-05	100	100	100	100	100	100	100	100	100	100	100	100	100	100
06-10	04	31	75	75	09	38	64	77	42	81	76	89	85	80
11-15	0	0	66	64	0	0	48	65	17	31	70	79	60	73
16-20			52	63			41	57	03	07	68	71	36	67
21-25			44	58			36	45	01	05	58	71	30	60
26-30			31	46			27	34	01	01	48	61	25	50
31-35			17	37			17	22	0	0	42	41	21	40
36-40			10	28			08	16			21	28	18	24
41-45			0	14			01	13			17	13	08	22
46-50				11			0	02			08	10	02	19
51-55				03				01			07	01	02	08
56-60				02				0			06	0	01	04
61-65				01							01		01	0
66-70				0							0		0	
MEL	2.70 b	4.05 B	16.80 a	22.60 A	2.95 b	4.40 B	14.60 a	19.05 A	5.70 b	8.75 B	23.60 a	25.70 A	16.95 a	24.85 A

Kruskall-Wallis; significance level: *P<0.001*
Dunn Test: groups not followed by the same letters are statistically different (lower and upper case letters refer to the 1st and 2nd experiments, respectively).

Table 2 - Longevity of *Apis mellifera* workers.

Longevity (days)	Season	References
150.0 - 200.0 **	Winter	Free & Spencer-Booth (1959)
30.0 - 50.0 **	Summer	Sakagami & Fukuda (1968)
24.3 * / 18.7 *; 26.3 *	Winter / Summer	Terada et al. (1975)
21.8 (NI)	NI	Winston (1979)
28.5 (NI)	Fall	Winston (1980)
32.0 *; 35.0 *; 12.0 - 18.0 **	NI	Winston et al. (1981)
21.5 - 28.9 (NI)	NI	Woyke (1981)
27.6 *	NI	De Jong & De Jong (1983)
28.1 **	NI	Winston et al. (1983)
12.27 *; 20.15 **	Summer	Morini (1990)
19.88 *; 20.72 **	Fall	Morini (1990)
21.5 *; 23.92 **	Winter	Morini (1990)
30.19 *; 27.92 **	Spring	Morini (1990)
29.0 * / 32.5 *	Fall/Winter	Modesto-Zampieron (1991)
19.9 *	Spring	Souza & Simokomaki (1997)

* = africanized bees; ** = european bees; NI = no information

Table 3 - Number of surviving worker bees, during the 1st and 2nd confinement experiments and respective mean expectations of life (MEL).

Age (days)	*cr/cr*		*cr/+*		s^{la}/s^{la}		$s^{la}/+$		*bk/bk*		*bk/+*		*wild*	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
01-05	100	100	100	100	100	100	100	100	100	100	100	100	100	100
06-10	10	18	49	63	56	44	56	48	96	88	88	98	50	68
11-15	01	08	37	52	28	28	18	20	76	84	68	84	40	64
16-20	0	03	27	39	21	20	10	18	52	68	40	64	32	64
21-25		01	22	30	20	20	06	08	28	64	36	56	30	48
26-30		0	12	19	17	20	06	04	28	60	28	56	30	48
31-35			07	16	14	16	02	0	16	40	16	36	26	24
36-40			07	15	11	08	02		08	12	12	16	22	12
41-45			05	10	06	04	02		08	12	04	04	18	08
46-50			0	07	02	0	0		04	04	01	04	06	04
51-55				06	01				01	04	0	01	02	01
56-60				06	0				0	02		0	0	0
61-65				03						0				
66-70				02										
71-75				02										
76-80				0										
MEL	3.05 b	4.00 B	10.80 a	16.35 A	11.30 a	10.50 A	10.10 a	7.40 A	18.35 a	24.40 A	17.15 a	23.45 A	15.30 a	19.55 A

Kruskall-Wallis; significance level: $P<0.05$
Dunn Test: groups not followed by the same letters are statistically different (lower and upper case letters refer to the 1st and 2nd experiments, respectively).

Studies on snow-laranja and brick mutats, which show progressive darkening in eye color with age, revealed that under confinement conditions the longevity estimates for bees carrying mutations for eye color and for heterozygous and wild bees were equal ($P>0.05$), showing that restricting these bees to a limited area still enables them to exercise flight activity and to return to the hive. However, bees maintained under confinement, generally exhibit a shorter mean longevity (Souza & Simokomaki, 1997). Probably, this decrease occurs because of the increased stress imposed to the bees by the confinement to small areas.

During the experiment, the bees in general showed a tendency to cluster around one of the cage's corners. Such agglomeration occurred throughout the day, from sunrise to sunset, always at the same location, where the presence of both mutant and wild bees could be observed. This behavior seems to be a common occurrence under confinement situations; however, it demands better explaination.

Experiments where wooden boxes were utilized for worker bees confinement in the laboratory have been performed by other researchers. Worker bees survivorship in small confinement boxes ranged from 4.3 to 13 days (Kulincevic & Rothenbuhler, 1973). Confinement, in this case, considerably affected longevity, since studies performed by several authors (Table 2) reveal higher longevity values for bees observed in the field.

Studying the longevity of chartreuse-limão mutant bees confined in the same type of boxes (Almeida & Soares, 1998), found MEL values of 14.8 and 11.9 days for mutant and heterozygous worker bees, respectively. The groups of mutant bees, when submitted to confinement, had greater longevities, confirming that the only factor responsible for the reduction in mean longevity values for bees in the field was, notably, the deficiency in eye pigmentation, which probably affects their orientation because of luminosity, and not because of a supposed lethal or semi-lethal action of the mutant gene.

CONCLUSIONS

Differences between the longevity of bees that are mutant for eye color and heterozygous and wild bees are associated exclusively with the degree of eye pigmentation, especially in field experiments. Light eye pigmentation impairs mutant bee orientation, decreasing their probability of returning to the hive.

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