NATURAL CROSSING BETWEEN *APIS MELLIFERA ADANSONII* AND *APIS MELLIFERA LIGUSTICA*[1]

WARWICK ESTEVAM KERR AND DAVID BUENO[2]
Department of Genetics, School of Medicine, University of São Paulo, Ribeirão Prêto, S. P., Brazil

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One of the reasons advanced for the extraordinary success of the African bee *Apis mellifera adansonii* in its new environment in South America is the supposed mating advantage of the African drones. On the other hand, another factor may be involved. Some researchers have proposed the hypothesis that a sort of sexual isolation might have evolved between *A. m. adansonii* Latr. and Italian bees, *A. m. ligustica* Fab. The experiments reported here were done in order to provide an evaluation of these hypotheses.

A total of 41 nuclei, with three combs and about 2 kg of bees in each, were utilized. Each of 20 nuclei had one African virgin queen; each of the other 21 had one Italian virgin queen. One very strong hive harbored 1000 drones, 500 of which were Italian and 500 African.

Queens were raised by the Doolittle method. Drones were raised from unfertilized eggs laid in a comb drawn from drone foundation. The rearing schedules were planned in such a way that the queens were 4 to 5 days old (nuptial flight age) when the drones were 11 to 14 days old (completely matured). The nuclei, complete with three combs, bees, and one virgin queen each, were put in an opening in a *Eucalyptus* forest (Rio Claro, Brazil), about 5–6 km from the next bee yard. They were distributed in a circle of 10-meters radius around the hive with the drones. Each nucleus entrance was provided with a queen excluder which was taken off when the queen was 4 days old and put back when she was 8 days old. This operation was repeated five times, and each time an equal number of Italian and African queens were used. Twenty-five to 30 days after the queen began laying eggs, about 200 emerging bees in each hive were counted to determine the proportion of the queen subspecies and hybrid workers produced by each queen. The results of the countings are in Table 1.

Analyses and Discussion

The first analyses of these data were done in order to determine if there is some degree of reproductive isolation between the two subspecies. The number of bees obtained from Table 1 can be added and since they represent the fusion of two sets of gametes, the number of spermatozoa and eggs of each subspecies can be estimated and analyzed by a χ^2 contigency test as in Table 2. The results indicate partial isolation within the population. Consequently, this sample of two subspecies can not be treated as a single population.

The second step was to determine the proportion of males of both subspecies that inseminated the African queens and the Italian queens. This information can be obtained directly from the data as follows: for African queens the frequency, p, of African drones is $p = 0.58$ (confidence interval 0.57–0.60) and the frequency, q, of Italian drones is $q = 0.42$. For Italian queens the frequency p' of African drones is $p' = 0.35$ (confidence interval 0.34–0.37).

Finally, with these data it is possible to estimate, on the average, how many

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[2] Present address: P.O. Box 178, Rio Claro, S.P., Brazil.

TABLE 1. *Proportion of African, Italian, and hybrid workers obtained from African and Italian queens that made their nuptial flights in a forest opening in which existed great, but equal, numbers of drones of both subspecies.*

Number of the queens	Race of the queen	Number of African workers	Number of Italian workers	Number of hybrid workers	Percentage hybrid workers
90-1-66	African	40	–	160	80
53-1-66	African	29	-	171	85.5
63-1-66	African	187	–	13	6.5
75-1-66	African	150	–	50	25
89-1-67	African	104	–	96	48
71-1-67	African	145	–	55	27.5
90-1-67	Afrcian	123	–	77	38.5
93-1-67	African	122	–	78	39
83-1-67	African	200	–	0	0
78-1-67	African	36	–	164	82
91-1-67	African	24	–	176	88
71-2-67	African	126	–	74	37
94-1-67	African	178	–	22	11
10-1-67	African	170	–	30	15
82-1-67	African	186	–	14	7
93-2-67	African	82	–	118	59
89-2-67	African	78	–	122	61
26-1-67	African	130	–	70	35
79-1-67	African	73	–	127	63.5
93-3-67	African	154	–	46	23
73-1-66	Italian	–	98	102	51
78-1-66	Italian	–	150	50	25
92-1-66	Italian	–	182	18	9
34-1-66	Italian	–	103	97	48.5
53-2-66	Italian	–	152	48	24
73-2-66	Italian	–	148	52	26
25-1-67	Italian	–	36	164	82
94-2-67	Italian	–	53	147	73.5
10-2-67	Italian	–	106	94	47
63-1-67	Italian	–	45	155	77.5
75-1-67	Italian	–	0	200	100
34-1-67	Italian	–	200	0	0
53-1-67	Italian	–	180	20	10
25-2-67	Italian	–	142	58	29
75-2-67	Italian	–	188	12	6
83-2-67	Italian	–	184	16	8
34-2-67	Italian	–	143	57	28.5
53-2-67	Italian	–	156	44	22
71-3-67	Italian	–	78	122	61
10-3-67	Italian	–	200	0	0
26-2-67	Italian	–	182	18	9
Total	Africans	2337	–	1663	
	Italians	–	2726	1474	

drones had inseminated each queen. Taber and Wendel (1958) reviewed this subject and corrected earlier estimates. According to these authors, the maximum likelihood estimate of the average number of drones (N) which inseminate each queen can be obtained on the basis of three observations: number of queens producing only progeny of her own subspecies (N_1), number of queens producing only hybrid progeny (N_2), and number of queens producing progeny of both kinds (N_3).

TABLE 2. χ^2 *test between the products of the union of spermatozoa and eggs (workers) of African and Italian bees. The expected values are in parentheses.*

	African spermatozoa			Italian spermatozoa			
African eggs	(1859)	2337	African workers	(2141)	1663	Hybrid workers	4000
Italian eggs	(1952)	1474	Hybrid workers	(2248)	2726	Italian workers	4200
		3811			4389		8200
$\chi^2 = 448.3$	d.f. = 1		significant below 0.1%				

These observed values are used to calculate N in the following formula: $N = \lambda + 1$, where

$$\lambda = 2 \log_e \frac{N_1 + N_2 + N_3}{N_1 + N_2}.$$

However, these estimates only apply for the case in which $p = q = \frac{1}{2}$. Since this does not apply to our case, we have to go back to the original formula:

$$\frac{N_1 q + N_2 p}{N_3 p q} = \frac{e^{-\lambda q} + e^{-\lambda p}}{1 - p e^{-\lambda q} - q e^{-\lambda p}}.$$

Using values of λ from 3.0 to 15.0 in a short program in a 1130 computer, the value of λ which fitted the data for African bees was 6.48, which indicates that on the average our African queens were inseminated by an average 7.5 males. The same thing was done with data for the Italian queens and the figure obtained indicates that they were inseminated by an average of 5.3 males.

These figures, especially for Italian bees, are slightly below the results obtained from four studies of *Apis mellifera* (revision, bibliography, and data, Taber and Wendel, 1958), which indicated that the average number of drones that inseminated a queen was between 7 and 10.

So far, the only apparent difference which has been found in the mating behavior is the speed of ejaculation, which is faster in *adansonii* males. This could somehow desynchronize the copulation process and prevent the Italian queen from receiving sperm of some of the fastest African drones. Data in Table 2 show that 58.5% of the inseminations received by African queens were contributed by African drones. Similarly 64.8% of the inseminations received by Italian queens were derived from Italian drones.

The preponderance of inseminations of queens by drones of their own subspecies indicates that one or more isolation mechanisms are beginning to form between these two subspecies. African and Italian *A. mellifera* have been geographically isolated less than 11,000 years. Prior to this time, during the last glacial advance, the Sahara region was a warm, moist area covered by vegetation, providing no geographical isolation between European and African populations of *Apis mellifera*. Since the European *Apis mellifera* were reconstituted from middle Asian and African stocks, it is likely that *adansonii* and *ligustica* are now some of the extreme forms of the species.

SUMMARY

Twenty *Apis mellifera adansonii* (African) queens and 21 *A. m. ligustica* (Italian) queens were allowed to take nuptial flights in an area which had been provided with 500 drones of each subspecies. An estimate indicates that an average of 7.5 males inseminated each African queen and 5.3 males inseminated each Italian queen. No advantage of

African males over Italian males was observed in the total numbers of drones involved in the inseminations. However, African queens were inseminated by African drones in 58.5% of inseminations, and Italian queens were inseminated by Italian drones in 64.8% of cases. This selective mating indicates that an isolation mechanism has been partially established in the period since the Sahara desert became a natural barrier (less than 11,000 years).

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