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Laranja: A New Honey Bee Mutation

Gene dosage and maleness of diploid drones

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A DESCRIPTIVE list of twenty-nine mutations in the honey bee has recently been published by Woyke[15]. Some of these mutations have helped to solve practical questions concerning natural mating[11,16], the physiology of insemination[13], and sex determination[19]. New mutations are therefore of interest. A genetic marker of the sex chromosome would be especially valuable in the study of gene dosage.

Woyke[12] determined that fertilized eggs (zygotes) homozygous for alleles at the sex locus are male, and by special techniques[14] can be made to survive and produce diploid drones. Diploid drones provide the means to more readily investigate genetic problems such as the effect of gene dosage[5,9], including that of the sex genes[1]. Diploid males as well as five visible mutations were used in the studies reported here.

Source and Description of the Mutation

The mutation, laranja, was discovered by the author in 1957. One drone with light orange eyes was found among several hundred drones with normal black eyes that were produced by an *Apis mellifera adansonii* Latr. queen in the apiary of the Department of Genetics, Faculty of Medicine, University of São Paulo, Ribeirao Preto, S.P., Brazil. This is the first mutation known to have occurred in this race of honey bees. It was propagated by artifically inseminating a normal virgin queen with semen of the mutant drone and rearing heterozygous daughters.

The eyes of newly emerged mutant drones are light orange. The proposed mutant name is laranja, which means "orange" in Portuguese; the suggested symbol is *la*. According to the Ostwald's color charts[8], the eyes of newly emerged laranja drones vary between 4 *ea* and 4 *gc*. On aging the eyes darken to reddish brown. At 10 days of age, the eyes range between 5 *pg* and 5*pe* and reach 6 *ng* to 6 *pe* in 2-month-old drones.

The eyes of newly emerged workers are orange-red brown (6 *ie* to 6 *le*) and darken to reddish brown (6 *pi*, 6*pl*, 7 *pi*) in 10-day-old workers. Thus, the eyes of workers are much darker than those of drones. This great difference between mutant eye color of drones and workers can be useful in investigations of gene dosage and maleness of diploid drones.

Methods

The virgin queens were reared by the usual methods. They were emerged, marked, and kept in queen cages until introduced into colonies for natural mating or were artifically inseminated. The artificially inseminated queens were clipped and introduced into colonies provided with queen excluder over the entrances. Drone comb was given to colonies that provided drones for insemination or classification.

The combs with brood of drones that were to be used for artificial insemination were caged a few days before emergence in comb cages having queen excluder sides. Most of the emerged drones were matured in a queenless rearing colony. Diploid drones were reared by the method of Woyke[14]. Combs of brood yielding progeny to be classified were placed in a comb cage having screened sides and were kept in an incubator. The emerged bees were classified once a day, and the eye colors described with the aid of an Ostwald color chart[8].

Test for Dominance

Queens heterozygous for the *la* gene were allowed to fly and mate naturally. The only drones in the mating area had the normal wild-type black eye phenotype or were brick (*bk*) mutants.

Queens homozygous for the *la* mutation were mated artificially to wild-type black eyed drones, and queens that were homozygous black eyed were inseminated with semen from mutant *la* drones.

All F_1 workers from these matings were black eyed, indicating that *la* is recessive to wild type (Table I).

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Segregation Test

Segregation of the *la* gene from its black allele was demonstrated by the phenotypes of the drone progenies of heterozygous queens (Table II) and the worker progenies of heterozygous queens mated to *la* drones (Table III). In all cases, the phenotypic ratios were close to 1:1 indicating that the laranja phenotype is due to a single Mendelian gene.

Nevertheless, there were quite consistently fewer *la* drones and workers than wild-type bees. Chi-square calculated on totals of drone segregants was 0.196 ($P = 0.75$–0.50) showing no significant deviation from 1:1. However, the chi-square calculated on totals of workers was 6.41 ($P =$ close to 0.01) showing a significant departure from the expected 1:1 ratio. Probably a very low semilethal action of the *la* mutation is visible here, resulting in 2.3 percent fewer *la* workers than wild type. This might prove to be the case in drones also when larger numbers of segregants are available for study.

Test for Allelism

Virgin queens were reared from a heterozygous laranja (*la*/+) queen that was mated to a laranja drone. Thus all of the virgins that were phenotypically laranja were homozygous for that gene, and all black eyed virgins were heterozygous. Virgins that were either homozygous laranja or were heterozygous were mated by artificial insemination to brick (*bk*), chartreuse (*ch*), garnet (*g*), or umber (i^u) drones. In Table IV it is shown that all worker progenies were wild type. Thus it can be assumed that *la* is not allelic to *bk, ch, g,* or i^u.

Test for Linkage and Mutant Interaction

Queens homozygous for wild-type body color and also for *la* were artificially inseminated by drones that were either *bk, ch, g,* i^u, or *cd* (cordovan) so that daughters reared from these matings were doubly heterozygous. The daughter queens were inseminated artificially or were mated naturally. Two of the daughters, numbers 787 and 788, were not inseminated and they laid unfertilized eggs in

Table I. Test for dominance

Queen no.	Parents: Genotype of queen		Parents: Genotype of drone	Phenotypes of worker progeny
130	+/+	×	*la*	all black
494	+/+	×	*la*	all black
497	+/+	×	*la*	all black
675	+/+	×	*la*	all black
676	+/+	×	*la*	all black
2	*la*/+	×	+	all black
4	*la*/+	×	+	all black
5	*la*/+	×	+	all black
13	*la*/+	×	+	all black
31	*la*/+	×	+	all black
70	*la*/+	×	+	all black
99	*la*/+	×	+	all black
611	*la*/*la*	×	+	all black
612	*la*/*la*	×	+	all black

Table II. Segregation of drone progeny from heterozygous queens

Queen no.	Genotype of queen	Phenotypes and frequencies of drone progenies: Laranja	Black
444	*la*/+	205	197
582	*la*/+	230	247
2008	*la*/+	16	19
2009	*la*/+	41	43
	Total	492	506

Table III. Segregation of worker progeny from heterozygous queens testcrossed to the mutant

Queen no.	Parents: Genotype of queen		Parents: Genotype of drone	Phenotypes and frequencies of worker progenies: Laranja	Black
503	*la*/+	×	*la*	945	1046
582	*la*/+	×	*la*	1037	1069
824	*la*/+	×	*la*	219	227
834	*la*/+	×	*la*	194	241
2008	*la*/+	×	*la*	823	853
2009	*la*/+	×	*la*	863	861
2011	*la*/+	×	*la*	1391	1447
			Total	5472	5740

Table IV. Test for allelism: F_1 worker phenotypes from cross of different mutants

Queen no.	Parents: Genotype of queen		Parents: Genotype of drone	Phenotypes of worker progenies
639	*la*/*la*	×	*bk*	all black
643	*la*/*la*	×	*bk*	all black
641	*la*/*la*	×	*ch*	all black
642	*la*/+	×	*ch*	all black
2080	*la*/*la*	×	*g*	all black
2087	*la*/*la*	×	*g*	all black
2063	*la*/*la*	×	i^u	all black
2064	*la*/*la*	×	i^u	all black
2078	*la*/*la*	×	i^u	all black
2079	*la*/*la*	×	i^u	all black

worker cells. The drones emerging from these cells were included in the calculations. Drones from the other daughter queens were reared in drone cells.

Three phenotypes were found among the drones from *bk*/+, *la*/+ queens. Some one-day-old laranja drones could have been confused with newly emerged brick drones but nevertheless nearly twice as many drones with the laranja phenotype were found as those that were brick or wild type, showing that laranja is epistatic to brick (Table V). The unusual ratio of the progeny of queen number 787 was probably caused by the semilethal action of the *bk* gene[7] on drones reared under highly unfavorable conditions, in this case in worker cells.

Queens doubly heterozygous for *la* with either *ch*, *g*, or i^u produced drones of four phenotypes, with the combination class in each case lighter in eye color than that of the interacting mutations. The eye color combination of the class *ch, la* was light buff, and corresponded to 1 *ea* of the Ostwald table; *g, la* drones emerged with white eyes having pink ventral tips, the eye color being between 3 *ea* and 3 *gc*; the combination i^u, *la* produced a tannish-cream phenotype that corresponded to Ostwald color 1 *ca*.

Laranja–Sex Locus Linkage Relationship

Heterozygous queen number 503 (*la*/+) was mated to one *la* mutant drone. All black eyed daughters were therefore heterozygous at the *la* locus. Black eyed daughters were each artificially inseminated with semen from one *la* brother drone. Then the survival rate of the brood in worker comb was tested. A survival rate of 50 percent indicates that one of these two sex alleles of the queen is identical to the one sex allele of her mate. The 50 percent of the brood that did not survive was homozygous for the sex alleles and the homozygous larvae were genetically diploid males that were destroyed by the bees.

Queen number 582, a daughter of queen number 503, produced brood of which 50 percent survived, and she was chosen for the test. Haploid drones were reared in drone cells and diploid workers were reared in worker cells. These were classified. Diploid drones were reared from larvae in worker cells by the method of Woyke[14] and were also classified.

Table VI shows that the segregation of mutant and normal was 1:1 in all cases. Thus, no linkage between *la* and the sex locus was indicated.

Gene Dosage Effect and Maleness of Diploid Drones

Mutant *la* haploid drones, as well as diploid mutant homozygous drones and workers, were reared from heterozygous queen number 582 (Table VI). Mutant haploid drones were also reared in worker cells for comparison with those reared

Table V. Test for linkage and gene interaction

Queen no.	Genotype of queen	Phenotypes and frequencies of drone progenies: *bk*	*ch*	*g*	i^u	*cd*	*ch,la* light buff	*g,la* umber	i^u*,la* white	*cd,la*	*la*	+
787	*bk*/+,*la*/+	15									101	80
791	*bk*/+,*la*/+	50									88	49
792	*bk*/+,*la*/+	110									286	135
788	*ch*/+,*la*/+		122				161				160	158
793	*ch*/+,*la*/+		10				19				20	26
817	*ch*/+,*la*/+		107				117				128	127
2166	*g*/+,*la*/+			210				174			323	246
2167	*g*/+,*la*/+			548				533			744	681
2168	*g*/+,*la*/+			552				599			688	724
2093	i^u/+,*la*/+				315				303		299	296
2164	i^u/+,*la*/+				210				315		251	265
2263	i^u/+,*la*/+				298				505		201	273
671	*cd*/+,*la*/+					266				260	309	275

Table VI. Linkage relationships of *la* mutation to the sex alleles (sibling mated queen producing brood of low survival rate)

Queen no.	Parents: Genotype of queen		Parents: Genotype of drone	Type of progeny	Frequencies of individuals: Laranja	Frequencies of individuals: Black
582	*la*/+,x_1/x_2	×	—	haploid drones	230	247
582	*la*/+,x_1/x_2	×	*la*,x_1	workers	1037	1069
582	*la*/+,x_1/x_2	×	*la*,x_1	diploid drones	64	60

in drone cells. They were smaller with smaller eyes than drones reared in drone cells. The haploids have only one *la* gene, which came from the mother, while the diploids of both sexes have two *la* genes, one from each parent.

All newly emerged individuals in this test were classified with the aid of the Ostwald color chart. Workers had the darkest eyes, from 6 *ie* to 6 *le*. Haploid drones reared in worker cells had lighter eyes, 4 *ie*, but the lightest eyes were those of drones reared from drone cells, and their color ranged from 4 *ea* to 4 *gc*. No visible difference could be detected in eye color between mutant haploid and diploid drones reared in similar cells.

Although there is a great difference between the mutant eye color of workers and drones, it is difficult to say what should be recognized as more fully expressed, the lighter or the darker eye color.

The identical eye color of the haploid and diploid mutant drones showed that there was no gene dosage effect of the *la* gene in drones. Investigations of polyploidization in the haploid and diploid drones now underway will cast more light on this question. The differences between the eye color of drones and workers are probably caused by other factors, such as caste determination and/or developmental conditions. This might be confirmed by the darker *la* eye color of small drones reared in worker cells. Perhaps in a smaller eye the pigment is more concentrated.

The fact that the eye color of diploid mutant drones is identical to that of the haploid ones casts some light also on the maleness question of diploid drones. As far as the *la* gene is concerned no inclination toward the female character was detected in the diploid drones. So in respect to this factor, the diploid drone shows a true male character.

Summary

The recessive laranja (*la*) eye mutation is lighter in drones than in workers; it is not allelic to, or linked with, brick (*bk*), chartreuse (*ch*), garnet (*g*), or umber (i^u), and it is not linked to cordovan (*cd*) or the sex alleles. It is epistatic to brick and interacts with chartreuse to produce light buff eyes, with garnet to produce umber eyes, and with umber, white eyes. All of the interaction phenotypes with *la* were lighter than those of the interacting mutations. The eye color of homozygous mutant diploid drones was the same as that of haploid drones. Thus, no gene compensation was detected, and the diploid drones showed the male character with respect to laranja.

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