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LINKAGE TEST BETWEEN A SEX-LIMITED COLOR GENE AND SEX ALLELES IN THE HONEY BEE

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ABSTRACT

Honey bee queens heterozygous for the sex limited body color gene y^{ac}, were mated to related drones carrying the same gene or the black body color genes y^{bl}. Also queens heterozygous for two body color genes Y/y^{bl} were mated to related y^{ac} drones. Diploid drones were reared from eggs, homozygous at the sex locus (X/X).

All crosses resulted in diploid drones of two body color classes: yellow and dark. The data indicate that no linkage exists between the sex limited body color gene y^{ac} and consequently, not between the other body color alleles (Y and y^{bl}), and the sex locus X.

INTRODUCTION

A series of homozygous lethal X-alleles was described by Mackensen (1951), in the honey bee. Mackensen (1955) found 11, and Laidlaw *et al.* (1956) 12 alleles, to exist in the honey bee population. Woyke (1976) showed only 6 of them to be present on the isolated Kangaroo Island and Adams *et al.* (1977) as many as 19 in a larger population of honey bees in Brazil. Woyke (1953) showed that homozygosis at the X locus results in diploid drone larvae. The larvae are eaten by worker bees within 6 hours of hatching from eggs (Woyke, 1963a,b), and therefore the brood looks scattered. However, adult diploid drones can be reared from these larvae by a special method (Woyke, 1969). When a queen is mated to a drone whose allele differs from

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those of the queen, offspring survival is about 100%, but when the drone has the lethal allele in common with one of the two of the queen, all homozygous larvae are eaten and survival is only 50% ($X_1/X_2 \times X_1 \rightarrow \frac{1}{2} X_1/X_1 + \frac{1}{2} X_1X_2$). With multiple mating the survival rate of the offspring may vary between these extremes depending on the rate of drones with alleles identical to those of the queen.

Production of diploid drone larvae, and thus losses of honey bee offspring, could be avoided if the sex alleles could be identified. Therefore, linkage investigations between sex alleles and visible mutants have been conducted. Mackensen (1958) did not find linkage between sex alleles and eight visible mutants, when the surviving worker progeny was tested. Woyke (1973) checked for linkage between sex alleles and the *laranja* mutation (s^{la}) within worker as well as diploid drone progeny. There was no evidence of linkage, nor was linkage found between size of diploid drone testes and the sex allele locus (Woyke, 1974). Kerr (1969) described an *ac* gene (abdome castanho = brown abdomen) in African bees which is limited to drones only and inhibits the 7 polygenes responsible for the yellow abdomen color. As a result, African queen and worker bees are yellow but the drones are dark. Later, Woyke (1977) showed it to be a major body color gene which is affected by the yellow polygenes and, therefore, the symbol was changed to y^{ac}.

Because of the sex limited action of this gene, the present study was undertaken to test linkage to the sex locus. Since the y^{ac} gene expression is limited to drones, linkage between it and the sex locus can only be detected in diploid drones. The effect of the y^{ac} color gene in homozygous diploid drones (y^{ac}/y^{ac}) results in dark body color (Woyke, 1971) and in drones heterozygous for it and the yellow (y^{ac}/Y) in yellow color, and in diploid drones heterozygous for it and the black gene (y^{ac}/y^{bl}) in dark color (Woyke, 1977).

METHODS

In order to produce diploid drones, queens must be mated to a related drone having its sex allele in common with one of the two that the queen has. Results concerning 528 diploid drones reared from 15 queens are presented here. African bees used in this experiment originated from Pretoria, South Africa, and were maintained pure in Ribeirão Preto through instrumental insemination. Yellow Italian queen No. 7 originated from the USA, where it was mated naturally. The race of drones mating with this queen was determined by the color of drones produced by its daughters. All crosses were single drone matings done by instrumental insemination. Four types of crosses were made (Table I).

Queens heterozygous for y^{ac} were mated to their y^{ac} brothers. Thus, African queen No. 618, producing only dark African drones, was mated to one yellow Italian drone ($y^{ac}/y^{ac} \times Y$). Each hybrid daughter was mated to its African brother

Table I - Linkage relationship between the sex locus X and sex limited body color gene y^{ac}, as well as two other color alleles (yellow - Y and black - y^{bl}) tested in diploid drones homozygous at the sex locus XX.

Queen no.	Frequencies of body color		χ^2
	Yellow y^{ac}/Y or y^{bl}/Y	Dark y^{ac}/y^{ac} or y^{ac}/y^{bl}	
1. Sibling mated cross Y/y^{ac} x y^{ac}			
623	20	20	0.0
626	31	22	1.53
665	25	47	6.72*
666	13	12	0.004
Total	89	101	0.76
2. Blackcross Y/y^{bl} x y^{ac}			
44	11	4	3.26
125	17	8	3.24
131	4	21	11.56*
167	19	7	5.54*
Total	51	40	0.26
3. Next sibling mated generation Y/y^{ac} x y^{bl}			
46	15	9	1.50
78	11	11	0.0
79	5	18	7.35*
97	19	5	8.17*
122	3	20	12.57*
Total	53	63	0.86
Overall	193	204	0.30
4. Test cross Y/y^{bl} x y^{bl}			
A. Diploid drones			
2302	28	45	3.96*
2317	6	52	36.48*
B. Worker bees			
2302	67	71	0.12
2317	54	70	2.06

*$P < 0.05$

(y^{ac}/Y x y^{ac}). The low survival of brood produced by some queens indicated that these queens had one sex allele in common with their brothers, (X_1/X_2 x X_1), and thus diploid drone larvae were produced.

Queens from a second group consisting of hybrids for yellow and black body color genes were mated to related drones carrying the sex limited y^{ac} gene. For this experiment, daughters were reared from the naturally mated yellow Italian queen No. 7. A check of haploid drones produced by five of her daughters showed that half of the drones were black and half yellow, indicating that the original queen No. 7 was mated to black drones (Y/Y x y^{bl}). Hybrid daughter No. 120 (Y/y^{bl}) was mated to a dark y^{ac} drone originating from pure yellow African queen No. 63 (y^{ac}/y^{ac}), and produced exclusively dark African drones. From that cross (Y/y^{bl} x y^{ac}), several of the grand-daughters were reared. These could be Y/y^{ac} or y^{bl}/y^{ac} and therefore of yellow phenotype, which made it difficult to distinguish them. However, the first group of queens produced both, yellow (Y) and dark (y^{ac}) haploid drones and the second group dark (y^{bl} and y^{ac}) drones only. Dark drones (y^{ac}) from queens producing both color classes were used to backcross daughters of the original queen No. 7. Five daughters producing scattered brood ($X_1/X_3, Y/y^{bl}$ x X_3, y^{ac}) were used to produce diploid drones.

Queens hybrid for the sex limited y^{ac} gene were also mated to related black (y^{bl}) drones. For this experiment, the next generation of the backcrossed queen No. 125 (Y/y^{bl} x y^{ac}) was reared and sibling mated. Two groups of queens could occur, *i.e.* – y^{ac}/Y and y^{ac}/y^{bl}. Phenotypically they would be yellow and thus difficult to distinguish. But after the queens were mated to y^{bl} brothers, those from the first group would produce yellow (Y/y^{bl}) and dark (y^{ac}/y^{bl}) diploid drones, and also both color classes of haploids, while those from the second group – only dark diploid drones (y^{ac}/y^{bl} and y^{bl}/y^{bl} both dark), and also only dark haploid drones. Results concerning diploid drones from the first group of queens (Y/y^{ac} x y^{bl}) are presented here. A detailed diagram of the last two crosses was presented by Woyke (1974).

Worker bees of the Y/y^{ac} and Y/y^{bl} genotypes, heterozygous for the sex locus X_1/X_2, are all yellow and cannot be used to determine the distribution of the two color types. Therefore a Y/y^{bl} x y^{bl} test cross was conducted which resulted in yellow Y/y^{bl} and black y^{bl}/y^{bl} phenotypes in both X_1/X_1 diploid drones and in X_1/X_2 worker bees.

Diploid drones were reared by the method of Woyke (1969). Their diploid character was verified, after the drones were dissected, by the small size of the testes.

RESULTS AND DISCUSSION

Diploid drones are reared only from eggs homozygous for the sex locus X/X. Table I.1 shows that queens heterozygous for the sex-limited gene, when sibling mated to y^{ac} drones (y^{ac}/Y x y^{ac}) produced diploid drones of yellow (y^{ac}/Y) and dark (y^{ac}/y^{ac}) color. Thus the cross resulting in diploid drones homozygous for the sex locus was: X_1/X_2, y^{ac}/Y x X_1, $y^{ac} \rightarrow \frac{1}{2}$ X_1/X_1, $y^{ac}/y^{ac} + \frac{1}{2}$ X_1/X_1, y^{ac}/Y males. The progeny with heterozygous sex alleles X_1/X_2 consisted of workers only, and all of them were yellow. The variation occurred in frequencies of both color classes of diploid drones produced by individual queens, is explained by different viability.

Queens heterozygous for yellow and black body color genes and backcrossed to drones carrying the sex-limited gene (Y/y^{bl} x y^{ac}) produced diploid drones of yellow (y^{ac}/Y) and dark (y^{ac}/y^{bl}) body color (Table I.2). Thus the cross resulting in diploid drones was X_1/X_3, Y/y^{bl} x X_1, $y^{ac} \rightarrow \frac{1}{2}$ X_1/X_1, $y^{ac}/Y + \frac{1}{2}$ X_1X_1, y^{ac}/y^{bl}. Significantly more dark than yellow diploid drones were reared from queen No. 131, but more yellow than dark drones were reared from queen No. 167.

Queens heterozygous for the sex-limited color gene, but sibling mated to black drones (y^{ac}/Y x y^{bl}) also produced yellow (Y/y^{bl}) and dark (y^{ac}/y^{bl}) diploid drones (Table I.3). Thus the cross resulting in diploid drones was X_1/X_3, y^{ac}/Y x X_3, $y^{bl} \rightarrow \frac{1}{2}$ X_3/X_3, $y^{ac}/y^{bl} + \frac{1}{2}$ X_3/X_3, Y/y^{bl}. Significantly more dark than yellow diploid drones were reared from two queens (No. 79 and 122), but more yellow than dark drones were reared from queen No. 97.

The significant X^2 for the distribution of color types in diploid drones originating from some queens led us to conduct a test cross with the two other alleles of the y^{ac} gene (y and y^{bl}). This resulted in both color types in diploid drones (X_1/X_1), as well as in worker bees (X_1/X_2). Table 1.4 shows that significant deviation from the 1:1 color distribution occurred in diploid drones from both queens, but not in the worker progeny. Thus the deviation from the 1:1 distribution must have been caused by some other phenomenon such as unequal cannibalism of diploid drones. It is already known, that many diploid drones are eaten by worker bees during the rearing period.

The above results, taken as a whole, show that diploid drones homozygous at the sex locus and of both body color types were reared. Color distribution of the total number of diploid drones reared did not differ from the 1:1 ratio. This evidence indicates that no linkage exists between the sex-limited body color gene y^{ac} and the sex locus X, and consequently, nor between the other body color alleles (yellow - Y, and black - y^{bl}) and the sex locus of the honey bee.

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RESUMO

Rainhas de *Apis mellifera* heterozigotas para o gene y^{ac} (que é o antigo ac, que confere abdome castanho escuro apenas aos machos, isto é, é limitado ao sexo masculino) foram cruzadas com machos irmãos que levaram o mesmo gene y^{ac} ou o alelo y^{bl}, que determina abdome preto. Também, rainhas heterozigotas para dois alelos da mesma série (Y/y^{bl}) foram cruzadas com machos y^{ac} seus aparentados (e que levavam um alelo X em comum). Criou-se, pelo método Woyke, a partir de ovos, machos diplóides, ou seja, homozigotos para dois alelos do locus sexual X/X.

Todos os cruzamentos resultaram em zangões diplóides de duas classes de coloração de corpo: amarelo e escuro.

Os dados indicam que não há ligação entre o locus sexual X e o gene limitado ao sexo masculino y^{ac} e, consequentemente, nem entre os seus alelos Y e y^{bl}. Os testes significativos foram devidos à maior mortalidade dos machos diplóides.

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