DIPLOID TISSUE DERIVED FROM ACCESSORY SPERM IN THE HONEY BEE[1]

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THE entry of more than one sperm into the egg of the honey bee at fertilization is normal. A zygote is formed by the fusion of the pronucleus of one of the sperm with that of the egg; the pronuclei from the accessory sperm undergo abortive division and then disintegrate after the second cleavage of the zygote (NACHTSHEIM 1913). Fertilized eggs, with certain exceptions (MACKENSEN 1951; ROTHENBUHLER 1956, 1957; WOYKE 1962, 1963a, b) develop into females; unfertilized eggs, again with certain exceptions (ONIONS 1912; MACKENSEN 1943; TUCKER 1958), develop into males.

Bees with both male and female body parts appear occasionally among the normal bees of a colony. These sex-mosaics or gynandromorphs were studied intensively (ROTHENBUHLER, POLHEMUS, GOWEN and PARK 1949; ROTHENBUHLER, GOWEN and PARK 1952; ROTHENBUHLER 1956, 1957) and it was demonstrated that they originate through the cleavage of accessory sperm nuclei along with zygotic cleavage in the cytoplasm of fertilized eggs. Cleavage cells derived from sperm are haploid and give rise to male structures; those derived from the zygote are diploid and are progenitors of female structures.

Of the several occurrences of gynandromorphs which must have originated in some other fashion (MACKENSEN 1951; ROTHENBUHLER 1956, 1957; TUCKER 1958; WOYKE 1962) there were some reported by ROTHENBUHLER which could have been due to the union of two accessory sperm. One such case involved the mating of a homozygous ivory-eyed queen to hemizygous cream-eyed drones. Ivory and cream are indistinguishable white eye mutants, recessive to normal black eye and neither allelic nor linked to each other. Male eye facets are distinguishable from female eye facets in the upper three fourths of the compound eyes by their larger size, and can be recognized in mixtures of male and female facets. Male compound eyes are much larger and more bulbous than female eyes, and the two eyes meet at the middorsal line, whereas female eyes do not. In general, patches of male eye facets can be distinguished from female eye facets by gross observation. As was expected, the female eye facets of both the normal workers and the gynandromorphs of this mating were black and both the normal and gyandromorphic male eye facets were white. In some gyandromorphic individuals, however, the compound eyes consisted at least in part of white female eye facets, instead of black. ROTHENBUHLER suspected that these facets were cream

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and thus patroclinous. In a later paper, ROTHENBUHLER (1957) speculated that two gynandromorphs possessing small amounts of female eye tissue resulted from the fertilization of binucleate eggs or the union of two sperms to form a second zygote. Thus, while ROTHENBUHLER (1957) proposed the hypothesis of the origin of female tissue from the union of accessory sperm nuclei, his evidence could not discriminate between this and alternative hypotheses.

The possibility of union of accessory sperm, made plausible by ROTHENBUHLER's data, received additional support in the present study from the result of mating a homozygous normal-eyed queen of gyandromorphic stock to hemizygous drones possessing the recessive eye color, red (ch^r). The female facets of the gyandromorphic progeny of this mating were black and the male eye facets red as expected. One gynandromorph, however, had eyes entirely female and red, indicating patroclinous origin.

If somatic pairing of chromosomes occurs in honey bees, then somatic crossing over in the four strand stage between (ch^r) and the centromere followed by normal mitotic segregation of chromatids could conceivably account for this exceptional female and for at least some of those of ROTHENBUHLER (see STERN 1936). The fact, however, that these exceptional individuals appeared in stocks in which the sperm are known to undergo cleavage and the cleavage cells take part in embryo formation lent credence to the possibility of sperm fusion.

The material to test this hypothesis was acquired through the fortunate discovery of a new eye color mutant, tan (s^t), which proved to be allelic to snow (s), and which in combination with snow produces red female eyes (LAIDLAW, EL BANBY and TUCKER 1964). The critical observation in support of the hypothesis would be the occurrence of bees with female red eye tissue, when s^t and s are both of paternal origin.

If fusion of sperm pronuclei occurs it should cause distinctive gynandromorphs to appear among the progeny of a gynandromorphic stock queen inseminated with a mixture of tan and snow sperm. Accordingly, a gynandromorphic stock queen, No. 74-633, homozygous for the dominant normal black eye color was inseminated with mixed semen. She produced drones of normal size with normal eyes from unfertilized eggs, and normal females and gynandromorphs from fertilized eggs. Several worker combs of brood from this queen were caged in an incubator on the days immediately prior to eclosion, and a total of 2,401 bees emerged. Among these were two gynandromorphs with red female eye tissue: the eyes of one were both entirely female and red, and the eyes of the other were entirely female with the lower halves red and the upper halves black. As mentioned above, the red could occur only as a diploid heterozygote of tan and snow which were brought to the egg by the sperm.

It would be expected that if tan and snow sperm can combine to form diploid tissue, two snow or two tan sperm can also, and evidence of this might be found among the bees of the sample. Two gynandromorphs with white female eyes were observed.

In addition to the confirming bees above, 1,586 bees (66.1 percent) of the

sample appeared to be normal workers with black eyes, 653 (27.2 percent) were gynandromorphs with female eye tissue black and male eye tissue white, and 158 (6.6 percent) gynandromorphs had one or both eyes female, usually black but some with streaks of white which may have been female tissue. The eyes of the gynandromorphs were commonly mosaic with varying mixtures of male and female eye facets, and reddish pigmentation at the borders of wild-type and mutant tissues. Some of the reddish border areas could have been female tissue of s/s^t composition; or homozygous snow or tan with the reddish color due to diffusion of substances from adjacent wild-type tissues. No effort was made to determine the sex of these areas.

SUMMARY

Gynandromorphs are known to originate in certain lines of honey bees through the cleavage of accessory sperm nuclei along with the zygote in the cytoplasm of fertilized eggs. The sperm-derived cleavage cells are haploid and give rise to male structures; the zygote-derived cleavage cells are diploid and are progenitors of female structures. The eye color mutant alleles snow (s) and tan (s^t) together produce red female eyes. A queen from gynandromorphic stock homozygous for dominant normal black eyes was inseminated with a mixture of snow and tan sperm. She produced drones of normal size with normal eyes from unfertilized eggs, and normal females and gynandromorphs from fertilized eggs. Several gynandromorphs had some female eye facets red, and one had all facets of both compound eyes female and red verifying the origin of diploid tissue from accessory sperm.

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