

CHAPTER 18

THE STORY OF DIPLOID DRONES IN THE HONEYBEE

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After Dzierzon's paper in 1845², it was generally accepted that female honeybees develop only from fertilized eggs, and drone honeybees only from unfertilized eggs.

In the 1950s some queen honeybees were found to produce scattered brood following sibling mating^{3,6}. Daily counts of brood from these queens showed that the greatest loss of brood occurred at hatching, i.e. between the third and fourth day after egg laying. It was concluded that eggs that were homozygous at the *X*-locus did not hatch and were removed by the workers. Eleven⁷ and twelve⁴ lethal alleles responsible for this phenomenon were found.

Mosaic drones, in which some patches of eye tissue were diploid have been found^{1,8}, and it was suggested that this diploid tissue was able to survive only by virtue of its association with normally viable haploid tissue in the drones. Throughout the 1960s the author conducted a series of researches which showed that eggs that were homozygous at the *X*-locus were in fact viable, and that the larvae were removed by the bees within 6 hours of hatching¹⁰. Histological studies showed that these larvae were drone¹¹, and cytological studies showed that they were diploid and had developed from inseminated and fertilized eggs^{30,31}. Thus in addition to normal haploid drones in the honeybee, diploid forms could develop from fertilized eggs.

Diploid drone larvae do not survive in nature, and investigations on the behaviour of the worker bees towards them, and on the viability of these larvae, revealed the fact that the workers eat the diploid drone larvae within a few hours of their hatching^{12,13,16}. It was thought that diploid drone larvae might be less viable than the haploid ones, or than normal worker larvae, but this was not so, and in fact their viability may be even higher than that of normal haploid drones.

Another possibility was that these larvae were destroyed because they were in worker cells. However, when larvae from sibling-mated queens—which may produce up to 50% diploid drones—were transferred to drone cells, the diploid drones were still eaten, while female larvae were reared normally¹⁷.

In other studies diploid drone larvae were grafted into cells already containing normal worker larvae¹⁹. Both larvae were eaten, irrespective of whether the drone larvae were alive or dead when they were added to the cells. However, if the diploid drone larvae had been extracted in a suitable lipid solvent before being grafted into cells containing normal worker larvae, the latter were not destroyed, but were reared through to maturity. This indicated that diploid drone larvae might be producing a pheromone which induced worker bees to eat them. This pheromone—which Woyke called “cannibalism substance”—was produced mainly during the first day of larval life and to a lesser extent during the second day¹⁹.

A technique for rearing diploid drones in the colony was then developed^{20,21}. Low-survival brood was hatched in the incubator and the larvae grafted on royal jelly and held in the incubator for 2–3 days after hatching, and then transferred to the colony where they were reared normally. Several thousand diploid drones were reared in this way.

The bi-parental origin of these drones was demonstrated^{15,29}. Mutant queens were mated to drones of the wild genotype, or to drones carrying a mutant gene of a different locus from that carried by the queen. Normal haploid drones produced from eggs laid in drone cells by these queens differed phenotypically from those reared from eggs laid in worker cells. The participation of the father in the origin of these drones was shown quite clearly. Androgenesis was also excluded, and the bi-parental origin of the diploid drones was proved.

Diploid drones are heavier than the haploids, and many body parts are larger. In some morphological characters, such as the number of hairs on the wings, development is less in haploids than in the females, and still less in diploids. It is considered that diploid males show “supermale” characters^{22,23}.

Spermatogenesis in diploid drones is very similar to that in haploid drones. No pairing or reduction of chromosomes occurred in diploid drones during spermatogenesis. In haploid drones 16 chromosomes were found in all stages of spermatogenesis, and in diploid drones 32 chromosomes occurred in all stages, including II anaphase^{32,33}. The DNA content of the heads of spermatozoa of the diploids was twice as high as that in the haploids^{25,28}. Thus it must be concluded that diploid drones produce diploid spermatozoa, and it might be possible to produce triploid bees if a queen were inseminated with the semen of diploid drones.

Unfortunately diploid drones ejaculate amounts of semen that are too small to be collected in the tip of the syringe for instrumental insemination of queens. Possible causes for this were shown by dissection of adult diploid and haploid drones: although all other parts of the reproductive organs of the diploids were similar to those of the haploids, the testes of diploids commonly had only one-tenth the volume of the testes of haploids, and contained less than half as many testicular tubules. These tubules in turn were much shorter than those of the haploids²⁴.

The inheritance of size of testes was then examined in an attempt to rear diploid drones with larger testes, which would produce more semen. Homozygosity of X-alleles resulted in a reduction in the size of the testes in diploid drones. However there was no detectable influence of the different additive ability of the sex alleles upon the size of the testes, nor did higher homozygosity increase their size, as has been suggested. The inheritance of size of testes was in accord with the additive action of special polygenes, and this must be the basis for rearing diploid drones with larger testes²⁷.

In a study on the size of testes in different races of honeybee, the largest were found in diploid drones of the African bee *A. mellifera adansonii* (these were reared in Brazil). It was found that very few of the spermatozoa of diploid drones transferred from the testes to other parts of the reproductive organs; nevertheless the sperms in the testes were alive, their viability being highest in drones 7 days old. Therefore a study was made in which queens were inseminated with sperms taken directly from the testes of diploid drones; the results of this experiment are not yet available.

This discovery of diploid drones in the honeybee has provided a useful method of testing certain hypotheses that could not otherwise have been tested. The results of early experiments demonstrated that the so-called "lethal" alleles⁷ are in fact sex alleles¹¹. Heterozygous combination of the sex alleles— $X_a X_b$ —results in females, azygotes— X_a or X_b —in haploid males, and homozygotes— $X_a X_a$ —in diploid males. The haploid drones are uni-parental, and the females and diploid drones are bi-parental. Adult diploid drones are not seen in nature because the larvae are eaten by the workers a few hours after hatching from the egg.

A second question answered by the use of diploid drones is that of gene dosage compensation. It has been supposed^{5,9} that differences between males and females might be caused by different gene dosages in the two sexes—i.e. haploid in males and diploid in females. However, with the aid of the mutation *laranja*, it was possible to show that the characters of diploid drones are similar to those of males and not to those of females²⁶. So gene dosage is not responsible for all the differences between diploid females and haploid males.

The use of diploid drones has also made a contribution to the solution of the inheritance of body colour in honeybees. Haploid drones of (Brazilian) African bees (*A. m. adansonii*) are black, even in colonies in which the queen and workers are yellow. This is caused by an *ac* gene, which expresses its action not only in hemizygotes but also in zygotes²³.

The diploid drones have also helped to solve the question of body colour inheritance in the various patterns of yellow and black coloration in the yellow Italian and black European races of bees. The results of this investigation are however not yet published.

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