Androgenesis with Zygogenesis in Gynandromorphic Honeybees (*Apis mellifera* L.)[1]

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DISCOVERY OF A STRAIN OF HONEYBEES in which sex mosaics occur with high frequency in the progeny of some queens presented the opportunity for experimental study of the cytogenetic origin of the component male and female tissues (*1*). The question of this origin has been discussed from time to time since 1864 (*2*), but until now no genetical or cytological data have been secured from living specimens. No absolutely controlled matings were possible until the development of artificial insemination of honeybees by Watson (*3, 4*) in 1927, and such control was not completely practical until the more recent improvements of the insemination technique by Laidlaw (*5*) and by Mackensen and Roberts (*6*).

Several hypotheses, nevertheless, have been advanced to explain the occurrence of gynandromorphs in bees. After studying the body colors of a few museum specimens of the now-famous Eugster gynandromorphic bees, Boveri (*7*) concluded that the male parts possessed only traits inherited from the mother, whereas the female parts possessed traits inherited from both parents. He suggested that the egg pronucleus divided once, twice, or more prior to fertilization. One of these cleavage nuclei then united with a sperm pronucleus, and this zygote gave rise by zygogenesis (origin from a zygote) to the female parts of the resulting mosaic. The other cleavage nucleus (or nuclei) did not unite with any sperm but did develop into male tissue by gynogenesis (origin from a female).

Morgan (*8*), on the other hand, reasoned that the mosaic sex types could be explained if an accessory sperm undergoes cleavage along with the zygote resulting from normal syngamy. It is known that polyspermy normally occurs in fertilization of the bee's egg (*9*). Male parts, under Morgan's explanation, would possess the genetic characters of the father only (androgenesis—origin from a male). The morphological descriptions of von Englehardt (*10*) provided possible support for this mechanism (*11*), but later developments in *Drosophila* work prompted the following explanation.

After sex-chromosome elimination was found to be important in the production of Drosophila mosaics, Morgan and Bridges (*11*) favored it as the explanation of the honeybee gynandromorphs. A normal diploid individual might lose one sex chromosome from an early cleavage nucleus. Tissue derived from such a deficient nucleus was expected to be male, whereas nondeficient tissue would be female. Whether male parts of a gynandromorph showed paternal, maternal, or biparental characters would depend in this case on sex-linkage or nonsex-linkage of the hereditary traits being followed, and on whether a paternal or maternal chromosome were eliminated. This explanation, possibly, possessed the advantage of reconciling the opposing observations of Boveri and von Englehardt.

Work of P. W. Whiting and Anna R. Whiting in *Habrobracon* sex determination (*12,* and earlier papers) gave rise to another explanation by Altenburg and Muller (*13*), which involved dispermic fertilization of a binucleate egg. If one of the zygotes of such an egg contained heterozygous and the other contained homozygous sex factors, female and male tissue, respectively, would be produced in one individual. Both kinds of tissue would be biparental as to the derivation of their genetic constitutions.

Use of our recently discovered stock has permitted tests of the above and other hypotheses as to the origin of honeybee sex mosaics. Experimental matings of three different types were used in these analyses. Resulting data and conclusions were presented in part at the 1951 meeting of the Genetics Society of America (*14*).

Type I evidence: Use of different marker genes in parents of the mosaic. This involved queens homozygous for the recessive ivory-eye gene (*i*) artificially inseminated with semen from drones hemizygous for the recessive gene for chartreuse eyes (*ch*). Data from several matings established that ivory and chartreuse are nonallelic and nonlinked. Female progeny from these matings were black-eyed (wild type), and male progeny were ivory-eyed. Four samples of progeny from three queens contained 347 living gynandromorphs. Their female eye facets were black, but their male facets were chartreuse. Since neither the ivory nor chartreuse gene appears to be located in any possible sex chromosome, this result provides evidence for the androgenetic origin of the male parts.

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Type II evidence: Progeny tests of the genetic constitution of the supposed androgenetic tissue. This type of experiment was carried out by using as sperm sources for inseminations gynandromorphs having male reproductive organs. The gynandromorphs were obtained from a mating of a queen homozygous for the recessive ivory-eye gene by drones hemizygous for the recessive cream-eye gene (*cr*). Cream is nonallelic with either ivory or chartreuse (for females from such matings have black eyes) and, on the basis of limited data, is not linked with ivory. There are no data on linkage of cream and chartreuse. Gynandromorphs from the ivory female by cream males mating had mutant male facets, but it was impossible to be sure whether they were cream or ivory because of color similarity of the phenotypes of the two genes. On the basis of results from the Type I experiment, however, the male parts of these gynandromorphs were expected to be cream. To test this, semen produced by seven such gynandromorphs was used to inseminate three cream queens. Samples of brood from the three queens yielded 311 worker progeny. Every worker bee had cream eyes. This establishes that the male parts of the gynandromorphic fathers were cream, and adds further evidence for androgenesis in combination with zygogenesis as the origin of the sex mosaics. Production of worker progeny in numbers establishes that the male parts of these gynandromorphs did produce functional sperms.

Type III evidence: Use of two genetically different kinds of sperms in an insemination. Only one of two such mixed-sperm inseminations will be described here. Both, however, were consistent with the hypothesis of androgenesis. An ivory queen was inseminated with both chartreuse-bearing and ivory-bearing sperms. She produced in two samples 3363 black workers (ivory egg × chartreuse sperm) to 381 ivory workers (ivory egg × ivory sperm). This count shows that 89.8 per cent of the functioning sperms are chartreuse and 10.2 per cent are ivory. If an accessory sperm is giving rise to the male parts of gynandromorphs, there ought to be both ivory and chartreuse male parts in various individuals, and each of these should occur in combination with both ivory and black female parts in predictable numbers. Fifty-one gynandromorphs collected from this mating had both male and female eye parts, and were classified as shown in Table 1. The class showing chartreuse male parts in combination with ivory female parts constitutes critical evidence. Two genetically different sperms must be involved for this result to be realized in its expected frequency. The results of the mixed-sperm insemination are in accord with results of the first two experiments.

TABLE 1

Combination	Chartreuse ♂ Black ♀	Chartreuse ♂ Ivory ♀	Ivory ♂ Black ♀	Ivory ♂ Ivory ♀
Expected	41.1	4.7	4.7	0.5
Observed	41.0	4.0	6.0	0.0

Androgenesis in combination with zygogenesis has been advanced as the preferred explanation for only a few cases of mosaicism prior to this time. Crew and Lamy (*15*) reported on 49 mosaics in *Drosophila pseudoobscura*. Forty-five of these appeared to have only paternal chromosomes in part of their tissue. One of these mosaics provided evidence resembling our Type III data, one was somewhat like our Type II, and the remainder bore some resemblance to our Type I. In the latter group some individuals were ambiguous (as pointed out by the authors), but others were clear. Polyspermy is normal in Drosophila, but Crew and Lamy did not attribute the exceptional tissue, in most cases, to cleavage of an accessory sperm.

Whiting (*16*) described 13 mosaics in *Habrobracon juglandis*, which were interpreted as having tissue of androgenetic origin. Twelve of these mosaics were equivalent to our Type I individuals, but the other one gave evidence somewhat resembling our Types I and II. Hollander (*17*) concluded that androgenesis was the probable origin of the exceptional parts in seven mosaics in pigeons (*Columba livia*). To varying degrees six of these individuals resembled our Type III, and one was somewhat like our Types II and III.

That both a sperm nucleus and a fusion nucleus may undergo cleavage in the same egg and subsequently give rise to a mosaic individual appears to be established as a biological phenomenon.

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