# NEW APPROACHES IN THE GENETICS AND CYTOGENETICS OF BEES (1)

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1. INTRODUCTION The development of genetics in *Apis mellifera* began with the memorable works of Watson (1928), Laidlaw (1944), Mackensen (1947), Mackensen & Roberts (1948), and recently Camargo (1972a), which put the control of the reproductive process on a sound basis. Only two genes had been described before 1948 (and these without the precautions taken today) and none before Watson's publication. Bumble bees (Sladen 1912, Frison 1927, Pouvreau 1963) and, recently *Melipona quadrifasciata* (Camargo 1972b), may also be crossed at the will of the experimenter, since one male and one female of proper age mate if put together in a small box.

The aim of this article is not to review the genetics of bees but it is to indicate to the participants of this Congress, some new lines and new approaches in research in genetics and cytogenetics of bees.

2. SEX AND CASTE DETERMINATION Garófalo (1973), in our laboratory, was able to mate a queen of *Bombus atratus* with her own son; this inbreeding resulted in $\frac{3}{4}$ diploid males to $\frac{1}{4}$ workers, suggesting a cross of the type female $x_1^a/x_2^a;x_1^b/x_2^b$ with any of her potential haploid sons (genotypes $x_1^a;x_1^b, x_1^a;x_2^b, x_2^a;x_1^b, x_2^a;x_2^b$). For instance her mating with $x_1^a;x_2^b$ would produce $\frac{3}{4}$ of the bees with one or two homozygous alleles (that are diploid males) and $\frac{1}{4}$ double heterozygous (the females). Further, Garófalo (1973) obtained triploid females by crossing these diploid drones with normal females. Virgin triploid females laid eggs that developed into 10 haploid males, 4 diploid males, 2 intersex, 15 females; chromosome numbers in these offspring varied between 20 to 59, demonstrating undisputably that sex in Hymenoptera is a balance between maleness and femaleness genes (Kerr 1973a,d).

Possibly caste determination in *Melipona* evolved from a system like *Bombus* in which the homozygous diploids were sterile females. Kerr (unpubl.) estimated the Malahanobis generalized distances between workers, queens and drones, in *M. quadrifasciata* and found that workers are closer to males than to females as far as external morphological characters are concerned.

Chaud (1973), also using the Malahanobis generalized distance, found that adult diploid males are metamales (as far as the generalized result of 22 randomly taken morphological characters are concerned) and triploid females are somehow masculinized. This indicates that the maleness genes are slightly additive as proposed by Kerr (1973a).

Tarelho (1973) found in *Trigona (Tetragona) quadrangula*, within

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a natural hive, $\frac{3}{4}$ workers and $\frac{1}{4}$ diploid males, indicating that workers can be heterozygous for two genes or for one and that diploid males are homozygous for two alleles.

The species of *Melipona* in which experiments have been carried out have queens doubly heterozygous while homozygosity for one or two alleles produces sterile females (workers) (literature in Kerr 1969). Camargo (1972 a, b) developed the technique of raising Trigonini queens in artificial wax queen cups, provided with food and maintained at a constant temperature of 28° to 34°C, according to the species. This technique applied to *Melipona* by Kerr & Camargo (1973) made possible experimentation with groups of cells using different amounts of food. Kerr, Montenegro and Stort (1966) found that young pupae of *M. quadrifasciata* weighing 25 to 76 mg or less were all workers while the ones weighing 76 to 140 mg segregated into $\frac{3}{4}$ workers to $\frac{1}{4}$ queens. Kerr & Camargo (1973) raising the bees in controlled conditions found that the ones receiving 93.0 mg of food produced only workers, and the ones that received 155, 186 and 218 mg of food segregated into $\frac{1}{4}$ queens to $\frac{3}{4}$ workers.

These results strengthened the assumption that sex in bees is determined by a small number of genes, probably two or three female determining and two or three male determining genes. The hypothesis of genic balance (literature in Kerr 1969, 1973 a, b, c) is the one that fits the existing data for sex determination in bees. It says that sex is determined by a balance between completely additive or almost completely additive female determining genes with slightly additive male determining genes.

Caste determination in *Apis mellifera* (literature in Rembold 1917), in *Trigona (Scaptotrigona) postica* (Camargo 1972) and in *Melipona* species (Kerr 1969) fits quite well in the "battery of genes" hypothesis of Britten & Davidson (1969). However more experiments are needed in order to establish the steps of genic control.

3. CYTOGENETICS Many papers have been published recently on the cytology of bees and other Hymenoptera, most of them using the normal cytological techniques. However some new trends are appearing. Kerr (1972) described some aberrations that were observed when very young male pupae of *Melipona marginata* were kept at 18° to 20°C (the normal colony controlled temperature is 32°C); interruption of the 2nd division and diploid 2nd spermatocytes were observed. Silveira (1972) published a preliminary report of her extensive experiments treating male prepupae and white eyed male pupae of *Apis mellifera* with different temperatures from 5°C to 50°C at several different times. Between 15° and 25°C the same aberrations were found plus double headed spermatozoa; many other types of aberrations were also found. These two experiments show a possible way through which diplo-tetraploid species could have evolved in bees, besides the production of diploid males by homozygosity of sex alleles.

M. L. S. Mello *et al.* are using polarization and interference microscopy and special histological techniques for the study of several tissues of bees. In her remarkable paper (Mello 1972a) on the linear dichroism on the peritrophic membrane of Melipona quadrifasciata which showed that protein and acid mucopolysaccharide molecules are differently oriented as the larva develops, probably adapting the larva for modifications in food types to be digested. Many of the properties of the acid mucopolysaccharide molecules of the peritrophic membrane are similar to those of non-mature collagen fibers. Mello (1972b) measured, with an interference microscope, the dry mass of nuclei of silk glands and Malpighian tubes of bees. She found that the amount of histones extracted from silk gland nuclei is 4 times greater than in Malpighian tubes. Mello thinks that this is due to a greater need of suppressor histones in silk glands since polyploidization is very high but the type of protein to be produced (silk for the bee cocoon) is of only one type, and probably will involve one or few genes, while in Malpighian tubes several proteins are produced. Also, by measuring cytophotometrically the nuclear basophilia of the silk gland cells, stained with Toluidine Blue at different pH, Mello (1972c) demonstrated that with increase in ploidy there is a sharp increase in the blocking of the DNA $-O-PO_3$ groups (perhaps due to this increase of histones). These two papers agree with the remarkable findings of Susuki, Gage & Brown (1972) which showed that, in the silk worm (Bombyx mori) there is no selective amplification of the DNA homologous to fibroin mRNA, in posterior silk gland cells, that is, the fibroin genes are replicated in proportion to the rest of the genome in such polyploid cells. By finding that only 0.0022% of B. mori DNA hybridizes with fibroin mRNA, and having both the haploid genome size and the molecular weight of this mRNA, they concluded that there are less than 3 fibroin genes and there may be only one for each haploid complement of DNA. According to Mello's studies reported above, the other genes would be repressed by proteins, very likely by histones.

4. POLYMORPHISM Kerr (1951) produced the first very simple mathematical treatments in bee populations showing the possibility of the existence of genetic polymorphism due to overdominance (for definition of genetic polymorphism see Ford 1965, Morton, Krieger and Mi 1966, and Dobzhansky 1970). After his trip to Central Brazil, Kerr (1971, 1973d) was convinced that the greatest maintainer of genetic polymorphism is the enormous variation in environment plus details of behaviour, including methods of communication and of defense, characteristics of the nesting site, range of flight, species of flowers preferred, etc. The polymorphism can be detected both through the chromosomal variations (in structure or in numbers) and the frequencies of alleles.

Seven independent cases of polyploidy have been found (Kerr and Silveira 1972). Immediately after polyploidy is established, deletions, mutations, monosomy begin to be established and consequently diminish the DNA content. This causes an increase in the variation of the chromosome length of diplo-tetraploid species when compared with the set of haplodiploid species. Over 65% of the present species of social bees have had a polyploid event in their evolutionary history. Tarelho (1973) found in 29 species of Apidae, 16 cases of independent fusions, 6 of fissions and 4 of pericentric inversions.

There are six cases of genic polymorphism well described in bees, not counting the $x^a$ and $x^b$ femaleness alleles envolved in sex determination, namely: 1) the alleles *Am* and *am*, which determine presence or absence of yellow color in the first metasomatic segment of *Melipona marginata* (Kerr 1969). In the populations around São Paulo the allele *am* was found in a frequency of 81%. 2) The allele *N* and *n* which determines, respectively, 5 and 4 ganglia in the abdominal nerve cord of workers (and the opposite, that is, 4 and 5 in queens) in *Melipona quadrifasciata* and *Melipona marginata* (Kerr and Nielsen 1966). In *quadrifasciata* this effect is only clearly observed in young pupae, however in *M. marginata* this is also observed in the adult. In *M. quadrifasciata* n has a frequency of 34.8% (confidence interval 0.20 to 0.55). *3 and 4*) Mestriner & Contel (1972) using gel electrophoresis found in *Apis mellifera* two genetically independent genes with two alleles each, P-$3^F$ and P-$3^S$, (this last one with frequency of 99.5% in *A. m. adansonii* and 53.1% in *A. m. ligustica*) that control migration of the protein p-3, and the alleles Est$^F$ and Est$^S$ (the last one with frequency of 97.2% in both subspecies) that control migration of one esterase. *5 and 6*) Contel (1972) found in *Melipona subnitida* two independent polymorphic genes, one with three alleles: Est-$3^F$, Est-$3^M$, Est-$3^S$ with respective frequencies of 13.0%, 62.6%, and 24.4%, controlling migration of esterase-3, and another locus with two alleles (Est-$2^F$ and Est-$2^S$ with respective frequencies of 1% and 99%) controlling migration of esterase-2.

Out of 21 loci tested in *Drosophila pseudoobscura* Hubby & Lewontin (1966) found 9 (that is 43%) with electrophoretic variants. Mestriner (1970) found 2 polymorphic genes out of a total of 6 analysed (33%); Contel (1972), in *Melipona subnitida*, out of 7 possible loci found 2 polymorphic (28%) and in *Melipona quadrifasciata* out of 6 found none (0%) polymorphic. If all these four species are compared by a $X^2$ test, only *M. quadrifasciata* is different, having a smaller number of polymorphic loci than the others. It is interesting to mention that Kerr (1973c) considers *D. pseudoobscura* as possessing an intermediate system haplo-diploid and diplo-diploid, since the X chromosome of this species is 40% of the total genome.

5. *DIPLOID DRONES* Woyke became famous for studies in diploid drones (literature in Woyke and Adamska, 1972). Step by step, he was able to raise diploid drones, to show their paternal origin. He explained why they are eliminated, counted the average sperm content, produced them in various subspecies and measured the size of their testes in different lines.

Chaud (1972, 1973), who worked with Woyke on the production of diploid drones, taking advantage of the climate of Ribeirão Preto where queens and diploid drones can be produced throughout the year, studied the biology of diploid drones showing that they have difficulties in orientation, but go eagerly toward a balloon with queen-pheromone. Chaud (1972), using the technique developed by Camargo (1970) of diluting sperm in coconut water (pH 7), was also able to cross diploid drones with normal queens and is now in the process of analysing the triploid workers and queens.

6. *QUANTITATIVE GENETICS* The group of studies in which there are the greatest advances, in which more modern and sophisticated methods are being used in bee genetics, is on quantitative characters. Four quite

different lines have been started and are yielding good results.

a) *Sex limitation*. Kerr found, in 1951, mathematically, that the equilibrium among two alleles, A and a, for normal genes in a haplo-diploid population is $\frac{p}{q}\frac{2s + s' - t' - 2ss'}{2t + t' - s' - 2tt'}$ where $s$ is the selection against *aa*, $s'$ against *a*, $t$ against *AA*, $t'$ against *A*, $p$ is the frequency of *A* and $q$ of *a*. When $s = s'$, $t = t'$ the formula becomes: $\frac{p}{q} = \frac{3s - t - 2s^2}{3t - s - 2t^2}$. The application of this formula for hymenopteran populations is further limited by the greater value that $t$ can assume, that is $t < \frac{3 - \sqrt{9-8s}}{4}$. However when the gene is limited to the female sex, the equilibrium between *A* and *a* becomes $\frac{p}{q} = \frac{s}{t}$, that is, exactly equal to the equilibrium in diploid populations; that is the reason why Kerr (1951, 1973d) says that with genes of this type the bee population can exploit the genetic advantages of diplo-diploid populations. In fact the following percentages of female sex limitations have been found: a) In *Nasonia vitripennis* 35.9% of the genes cause sterility in females but not in males (estimated from the data of Saul et al., 1965); b) In *Bracon hebetor* 21.2% of the genes are limited to the females in some of their effects (estimated from the list and description of Martin, 1947); c) 20.1% of the lethal equivalent estimated by Kerr (1969) for *Apis mellifera* is limited to females; d) studying 11 quantitative characters of *Apis mellifera* Gonçalves (1970) found that 5 of them had genes limited to such an extent to females that the total genetic variance of each of these characters was sex limited.

b) *Disruptive selection*. Rothenbuhler in 1964 presented the first case of disruptive selection in bees giving the results of tests of the Squires Resistant and Susceptible lines (for American Foul Brood). These two lines were derived from the progenies of one naturally mated queen. Each line was subjected to a few generations of selection in opposite directions.

Mackensen & Nye (1969) after 6 generations of selection obtained a line of High-Alfafa-Pollen-Collectors in which 88% of the bees collect alfafa pollen.

Drescher (1971) published a paper on the work he had been carrying out since 1966 for 10 generations, and Gonçalves (1972) reported on two more generations in this same research, on the selection for high and low number of hamuli. In the 12th generation the selection is entering a plateau, which shows that, when compared to similar data in *Drosophila*, bees respond to selection more quickly than diplo-diploid organisms; this confirms Hartl's (1972) studies that demonstrated that haplo-diploid populations evolve 33% faster than diplo-diploid ones.

c) *Analyses of behavioural characteristics.* The first genetic intervention in bee behaviour was done by Rothenbuhler 1964 (literature in Rothenbuhler 1967) who showed that bees can be selected for hygienic or non-hygienic behaviour toward dead larvae. Parallel to this work Rothenbuhler also found that bees differ, genetically, as far as temperament is concerned.

In 1969 Isaias Pessotti received his Ph D degree for thesis work carried out in our Dept., in which he demonstrated a high degree of learning ability of bees: bees learned to discriminate between two levers, pulling correctly the one with light *on* if the towel around the apparatus was *yellow* and the lever with light *off* if the towel was *blue.* This made possible the study of heritability of learning in bees, along with great numbers of experiments in bee psychology now being carried out in São Paulo, Salvador and Ribeirão Preto with Pessotti's technique.

d) *Evolution of social life.* Hamilton, in 1964, filled a gap in the theory of evolution, providing the explanation for the origin of social life that the biologists were waiting for. William Hamilton pointed out that, if a bee mates with only one male, she will pass 75% of her genes on in raising a sister and 50% of her genes in producing a daughter of her own. Therefore, in such a system, any gene that would increase altruistic behaviour of a sister toward others would have a high selective advantage. Since then, many social insect specialists including the Brazilian group of bee biologists, have been collecting data that shows the validity of the Hamilton theory. The most important finding is that all the stingless bee queens mate just once. Also, since what matters is passing the genes to the following generation, 50% or more of the drones in many stingless bee species are sons of workers and not of the queen (Beig 1972, Tambasco pers. com., Contel 1972).

7. *GENETICS AND COMPARATIVE BIOLOGY OF APIS MELLIFERA ADANSONII.* An increasing program of research is being carried out in this subspecies. Indeed we are now collecting more genetic and biological data for this bee than for the other subspecies.

The first large scale research was published by Kerr, Gonçalves, Blotta & Maciel (1970) whose main conclusions, after 15 months of observations of 20 hives, 10 of *ligustica* and 10 of *adansonii*, in which 876831 bees were observed and computer analysed, are: 1) The Italians swarmed twice, hybrids once, and Africans once. 2) No Italians absconded; two of the Africans did. 3) Of the 10 Italian colonies, 7 were alive after 6 months, one after 12 months and only one (the first that changed queen, was superseded by her daughter inseminated by an African drone) was still alive after 15 months; of the 10 African initial colonies 6 were alive after six months, 5 were alive after 12 months, and 4 were alive and strong after 15 months (notice that all colonies were treated as swarms: they were not fed, not defended and only observed internally once a month in order to check queen substitution). 4) The unbiased sample of 876831 bees contained 55% Africans, 23% Italians and 22% Hybrids (that superseded the Italians). At the end of the experiment (14 months) the Africans had produced 10% more bees. 5) Nine strong colonies were checked for 13 months to establish the laying ability and no great difference existed between them: the Italians averaged 450.74 eggs per day, the Hybrids 249.50 (both together 350.19) and Africans 406.14 eggs per day; 6) The peak of activities of Italians is at 8h30 and the African and hybrids work decisively more than the Italians after 11 a.m. when the highest temperatures occur; 7) Some observations were made concerning swarming: in Cearé, out of 175 swarms

10 had two or more queens (all inseminated). None of the 175 swarms fragmented themselves into smaller ones. There are two peaks of swarming: August and December. Of these 10 multiple queen swarms, 5 happened in August to October and 5 in January and February, that is, in the second half of the curve, which suggests production of smaller swarms and subsequent fusion of them.

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