ADVANCES IN CYTOLOGY AND GENETICS OF BEES[1]

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Seven previous papers have reviewed the genetics, cytology, and evolution of bees (33, 37, 41, 59, 60, 63, 64). As is the case in every field of science good progress in bee genetics is being made, not only by those whose contributions deal directly with bees but also by those whose interest is in other Hymenoptera (15, 26, 69) and whose studies have an indirect but important impact on our understanding of the genetics of bees.

EVOLUTION OF HAPLODIPLOIDY

Haplodiploidy or male haploidy is the mode of sex determination in which females are diploid whereas the males are haploid and develop either from an unfertilized egg or from a fertilized egg in which there has been an elimination of the paternal set of chromosomes.

Four main hypotheses have been put forward in explanation of the origin of haplodiploidy: (*a*) hypothesis of parahaploidy (65); (*b*) hypothesis of racial hybridization (79); (*c*) genetical hypothesis (4); and (*d*) ecogenetical hypothesis (26).

Brown (4) opened a completely new field for the understanding of the evolution of haplodiploidy by showing that it could evolve from a diplodiploid population by a process similar to the normal substitution of one allele for another. Subsequently, his conclusions were considerably enlarged by Hartl & Brown (26) who devised an ecogenetical hypothesis that has all but replaced the former ones. Hartl and Brown examined two situations: (*a*) in which a change in the environment stimulates the development of unfertilized eggs, and (*b*) in which a newly arising mutation causes the development of unfertilized eggs. Common to both models is the assumption that the population has a mode of sex determination that causes haploids to be male and a genetic system that allows haploids to be at least partially viable and fertile.

[1]The research on bees by the author and his colleagues of the Department of Genetics, herein reported, was financed by the Fundação de Amparo à Pesquisa do Estado de São Paulo, the Conselho Nacional de Pesquisas, and Coordenação do Aperfeiçoamento do Pessoal de Nivel Superior.

In their environmental model they assume that a fraction u of eggs remain unfertilized when a diploid female mates with a diploid male and that a fraction v of eggs remain unfertilized when the mating is with a haploid male. They then proceed to show that when all unfertilized eggs develop into haploid males, the haploids, in time, will completely replace the diploids if $2vs/(1-u) \geqslant 1$ where s is the probability of survival and reproduction of a haploid male relative to a diploid male. If $s = 1/2$ or less it is very difficult to establish a haplodiploid race or species.

The genic model supposes a mutation H that allows unfertilized eggs to develop in a given environment. Assuming that haploid and diploid males reproduce in the ratio s:1, Hartl and Brown demonstrate that the frequency of H will become 1 provided $s \neq 0$. Glaser (see 4) carried out simulations in the computer and found that, for $u = v = 1/10$, $s = 1/2$, fixation of H occurs in 400 to 500 generations. For values of u greater than 1/10 and s greater than 1/2, fixation may occur in 100–200 generations (30 to 100 years). Mutations that increase the frequency of development of unfertilized eggs will accumulate in the population until virtually all unfertilized eggs develop. Then, provided the ecological and genetic conditions are met in the population, haplodiploidy (arrhenotoky) will evolve wherever $2vs/(1-u) \geqslant 1$.

These models suggest that the evolution of haplodiploidy is rather easy and that many groups and species should be male haploid. However, arrhenotoky originated independently only six times among insects, once in arachnids, and once in rotifers. Furthermore, thelytokous parthenogenesis and such variations in chromosome number or structure as polyploidy, fusions, inversions, and translocations have a scattered occurrence in many families, tribes, and genera, while haplodiploidy occurs in blocks involving large taxonomic groups. Whiting (79), Hughes-Schrader (65), Brown (4), and especially Hartl & Brown (26), provide a plausible solution for these puzzles. They consider the following preconditions (or, in evolutionary language, preadaptations) necessary to make the evolution towards haplodiploidy possible.

1. Structure of Ancestral Populations

These should be constituted so that the genetic load is very low. This conclusion agrees with the high frequency of endogamous Hymenoptera and with the low values of lethal equivalent found by Kerr (31, 32; unpublished observations) in *Apis mellifera* (0.28) and in seven species of meliponids (0.13).

2. Breakdown of Barriers that Prevent Development of Unfertilized Eggs

This precondition is not difficult to achieve since many mechanisms, environmental or genetic, may induce unfertilized eggs to develop (acids, temperature, water, level of enzymes, viruses, etc).

3. Adjustment of Gene Dosage to Haplodiploidy

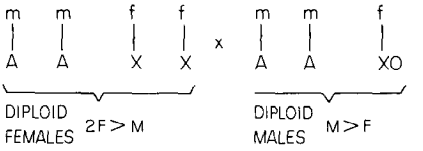
The first requisite established by Hartl & Brown (26) requires that the haploid male should not be lethal or sterile. Some mechanisms have been suggested in order to compensate for the haploidy: (*a*) Dosage in males is amplified by somatic polyploidy

(26) as found in *Bracon hebetor* (79), *Apis mellifera* (50, 52), and *Melipona quinquefasciata* (51). Mello (50) showed that polyploidy in various tissues has been selected for optimization in function and is independent of the original number of chromosomes; (*b*) genes limited to the diploid state (32–35); a great number of female sex-limited genes have been found in various species of Hymenoptera. In *Apis mellifera* they are about 20 to 40% of the total number of genes (22, 32). Using the recent techniques developed by Woyke (production of diploid drones), Chaud (13) has demonstrated that some sex-limited genes are actually diploid-limited.

4. Sex Determination

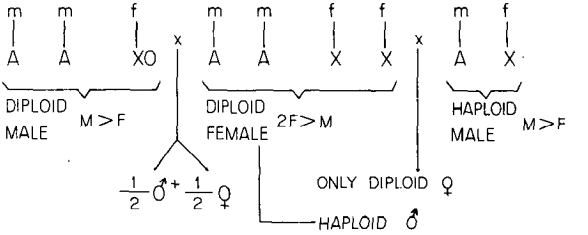
The importance of this point was emphasized by Hartl & Brown (26) but their discussion of the details of sex determination in the Hymenoptera was more brief than might be desired. The balanced hypothesis for sex determination in the Hymenoptera was first proposed by Cunha & Kerr (16) and later slightly modified (31, 35, 37, 39, 43). The hypothesis (39) proposes that sex in Hymenoptera is produced by an interaction between male-determining genes that, in double doses, are not additive or slightly additive, and female-determining genes that are totally or almost totally additive. The insect orders related to Hymenoptera (Coleoptera, Strepsiptera, Neuroptera) are characterized by sex determination in which males are either XY or XO. The ancestral diplodiploid species, being XO or XY, must have its maleness genes much less additive than femaleness ones, otherwise the haploids would be females, and not males. Therefore, the first steps in the hymenopterous evolution may have followed this scheme (in which *m* represents one or more male-determining genes and *f* one or more female-determining genes; *A* stands for the autosomes):

A– ANCESTRAL POPULATION



B– PRODUCTION OF HAPLOID MALES IS POSSIBLE

C– INTERCROSS OF DIPLOID FEMALES WITH BOTH TYPES OF MALES



No difficulties would be encountered if the males of the original stock are XY. However if the females are XY, then half the males would be lethal and it would be almost impossible to evolve a haplodiploid race. This probably explains why there are no haplodiploid Lepidoptera. In spite of having all known genetic and ecological conditions, there is no case of arrhenotoky known in Diptera. However, parahaploidy is present in Sciaridae and Cecidomyiidae (77) and many species have enormous X chromosomes, with frequent translocations of autosomes in the X, which results in a large number of genes becoming effectively haplodiploid. The X chromosome represents 20% of the genome in *Drosophila melanogaster* and 40% in *D. saltans* and *D. pseudoobscura,* causing them to be intermediate systems between haplodiploidy and diplodiploidy.

5. Gametogenesis

Since the newly appeared haploid males must be fertile or partially fertile, any mechanism that avoids random segregation of chromosomes in meiosis should be advantageous. Three of these mechanisms are: (*a*) a small number of chromosomes with random segregation (26); (*b*) an increase of the pairing protein (70) that results in all chromosomes sticking together; and (*c*) a permanent nuclear spindle that would not allow separation of the chromosomes in the first division (38, 45).

BEE CYTOLOGY

Cytological studies of bees received a considerable spur with the publication of the papers by Kumbkarni (46), Hoage & Kessel (28), Kerr (36, 37), Kerr & Silveira (45), Silveira (66–68), and Garófalo (21). All together, chromosome numbers of 41 new species were determined and a general appraisal was made by Kerr & Silveira (45) suggesting: (*a*) That polyploidy is an important mechanism in the evolution of bees, especially the recently evolved group of social bees; they suggest at least seven polyploidy events in these 41 species studied. (*b*) After polyploidy, cytological mutation such as Robertsonian translocation (that diminishes the number of chromosomes), simple translocations, inversions, and deletions (that diminish total DNA content) can take place; chromosomal variation is much greater in polyploid species than in the ones with $n = 8$ or $n = 9$ chromosomes. Among 19 species of bees with $n = 18$ and $n = 17$, Kerr & Silveira (45) found 10 having $n = 17$; among these 10 there are at least 4 independent fusions. In all species studied, Kerr and Silveira (38, 45) confirmed the presence of an intranuclear spindle and persistent nuclear membrane in the first meiotic division. Another discovery by Kerr (38) is the secondary (somatic type) pairing of chromosomes in species with $n = 18$ chromosomes in such a way that 9 pairs of chromosomes are easily seen in the meiotic prophase of *Pebleia* species and of *Meliponula bocandei* (38, 42). Kerr & Silveira (45) interpret this as natural selection that had not reduced the effects of the pairing protein (as seen in females), resulting in species with, and others without, secondary pairing.

Two conditions may drive a bee species toward polyploidy. One is low temperature shock. Kerr (36) showed that males of *Melipona marginata* left at 18–21°C (the temperature in a hive is practically constant at 32°C) produced two types of aberrations: (*a*) spermatocytes that had not entered second division and had a haploid number of chromosomes, some of which were outside the nuclear membrane; and (*b*) polyploid spermatids. Silveira (66, 67) experimented with *Apis mellifera,* by submitting prepupae and white-eyed pupae to temperatures varying from 5°C for periods of 5 min to 85 hr and 20 min. Many cytological aberrations were found; polyploid spermatozoa were found more intensely between 15 and 25°C, thus confirming the observations made in *M. marginata.*

The second method by which polyploid species could arise is by production of fertile diploid males. Some bees may have developed the same system of sex determination as is found in *Bracon* and *Apis,* in which diploid males may be produced by endogamy. In such cases, triploid queens can be produced and segregation can stabilize tetraploid races.

ADVANCES IN SEX DETERMINATION

Four hypotheses have been formulated for the explanation of sex-determination in Hymenoptera: cytoplasmatic, attributed to Goldschmidt; multiple alleles (48, 78); genic balance (16, 23, 34, 37, 39, 43); and multiple heterozygous loci (15). In the genic balance hypothesis (34, 37), sex determination is seen as the result of a balance between nonadditive or slightly additive male-determining genes and totally additive, or almost totally additive, female-determining genes. These genes may exist in small numbers or in great numbers and are scattered in the chromosomes. The cases of *Bracon* and *Apis,* in which a series of eight or more *x*-alleles in a single locus produce males in homo- or hemizygosity and females when in heterozygosity, is interpreted by Cunha & Kerr (16) as a major female-determining gene that has lost the property of being additive unless heterozygous. Whiting, Greb, and Speicher cited by Martin, 49) describe the gene *gy* (gynoid) and von Borstel & Smith (2) describe the gene *i* (intersex) that I interpret as mutations in two male-determining genes.

According to Crozier (15) sex in Hymenoptera is determined by a number of sex loci. Females are heterozygous at one or more loci while males are homozygous or hemizygous at all sex loci. The main difficulty with Crozier's hypothesis is the existence of many endogamous species of Hymenoptera in which diploid males have not been detected, notwithstanding their extensive investigation by cytologists, entomologists, and agronomists.

Recent discoveries on sex determination have shed new light on this problem. Investigations on the following three species have been particularly illuminating: the primitive Symphyta *Neodiprion nigroscutum* (69), the neotropical bumble bee *Bombus atratus* (20, 21), and the stingless bee *Trigona (Tetragona) quadrangula* (74).

Smith & Wallace (69) interpret *Neodiprion nigroscutum* segregations as being produced by three (a, b, c) complementary sex alleles in one single locus. Their

interpretation of the segregation of 1104 females to 466 diploid males is that "not all diploid males are recovered equally" and they tentatively explain their results as due to selective fertilization. Looking at the data in their Figure 1, the following reinterpretation is proposed: out of six families three families segregate 1 female:1 diploid male (56♀ ♀:47 ♂ ♂ $2n$) and 3 families segregate 3 females:1 diploid male (60♀ ♀:26♂♂ $2n$). Also, the global data presented in their Table 1 are: mating type one—388 diploid females and no diploid males; mating type two—1288 diploid females and 1282 diploid males (which agrees with a 1:1 segregation); and mating type three—1104 females to 466 diploid males, which suggests a 3:1 segregation. The distortions in the segregation (a lack of females) can be explained by mortality (there was 29% mortality in this sample) or possibly by an error in sorting haploid from diploid males. A two-loci system would offer a likely explanation for these results, with at least two alleles per gene, in which males would be homozygous for both genes while heterozygosity for one or two genes would produce females. In this way the observed 3:1 segregation would be produced by x_1^a/x_2^a; x_1^b/x_2^b female with any male, for instance $x_1^a;x_1^b$. In this cross the wasps x_1^a/x_1^a; x_1^b/x_1^b would be diploid males. The 1:1 segregation would occur in the case in which the females are homozygous in one loci, like x_1^a/x_1^a; x_1^b/x_2^b, that crossed to any male, for instance, x_1^a,x_1^b that would produce 1/2 diploid males (x_1^a/x_1^a; x_1^b/x_1^b) and 1/2 females ($x_1^a x_1^a$; x_1^b/x_2^b).

The case described by Tarelho (74) is very similar. She found in one colony of *Trigona (Tetragona) quadrangula* a comb with females, diploid males, and haploid males. The proportion between females and diploid males was 3:1, which can be explained by the method described above. However, the results of Garófalo (20, 21) are slightly different from those of Tarelho. He was able to cross a normal queen of *Bombus atratus* with her own son. This resulted in the queen producing 14 workers and 27 diploid males ($2n$ = 40 chromosomes). Garófalo suggests two different loci with at least two alleles each (they may have more). In this case, a queen (or any female) is x_1^a/x_2^a; x_1^b/x_2^b and diploid males are any bees with one or two genes in homozygosity. Therefore, his case would be: x_1^a/x_2^a; x_1^b/x_2^b crossed to a haploid male x_2^a, x_1^b and producing; females 1/4 x_1^a/x_2^a; x_1^b/x_2^b, diploid males: 1/4 x_1^a/x_2^a; x_1^b/x_1^b, 1/4 x_2^a/x_2^a; x_1^b/x_1^b, 1/4 x_2^a/x_2^a; x_1^b/x_2^b. Under these circumstances the evolution of sex determination could have resulted from a scheme similar to that presented in Figure 1.

Hung, Imai & Kubota (30) reported for the ant *Pseudolasius* sp (near *P. emeryi* Forel) n = 14 from testes of 20 male pupae and found 28 chromosomes in testes of 7 males (diploid). This is the seventh case in which diploid drones or sterile workers result from homozygosity of female sex alleles. An eighth case was just discovered in *Melipona quadrifasciata* (C. Camargo, personal communication) and is not considered in Figure 1.

NEW MENDELIAN CHARACTERS

The review paper of 1968 by Rothenbuhler, Kulinčevič & Kerr (63) listed the known genes of *Apis mellifera* in their Table 1. Since then, only three new genes have been described in *Apis mellifera* rendering unnecessary the publication of a new general table. Bambi (3) showed that the yellow color of the Egyptian bee (*Apis mellifera*

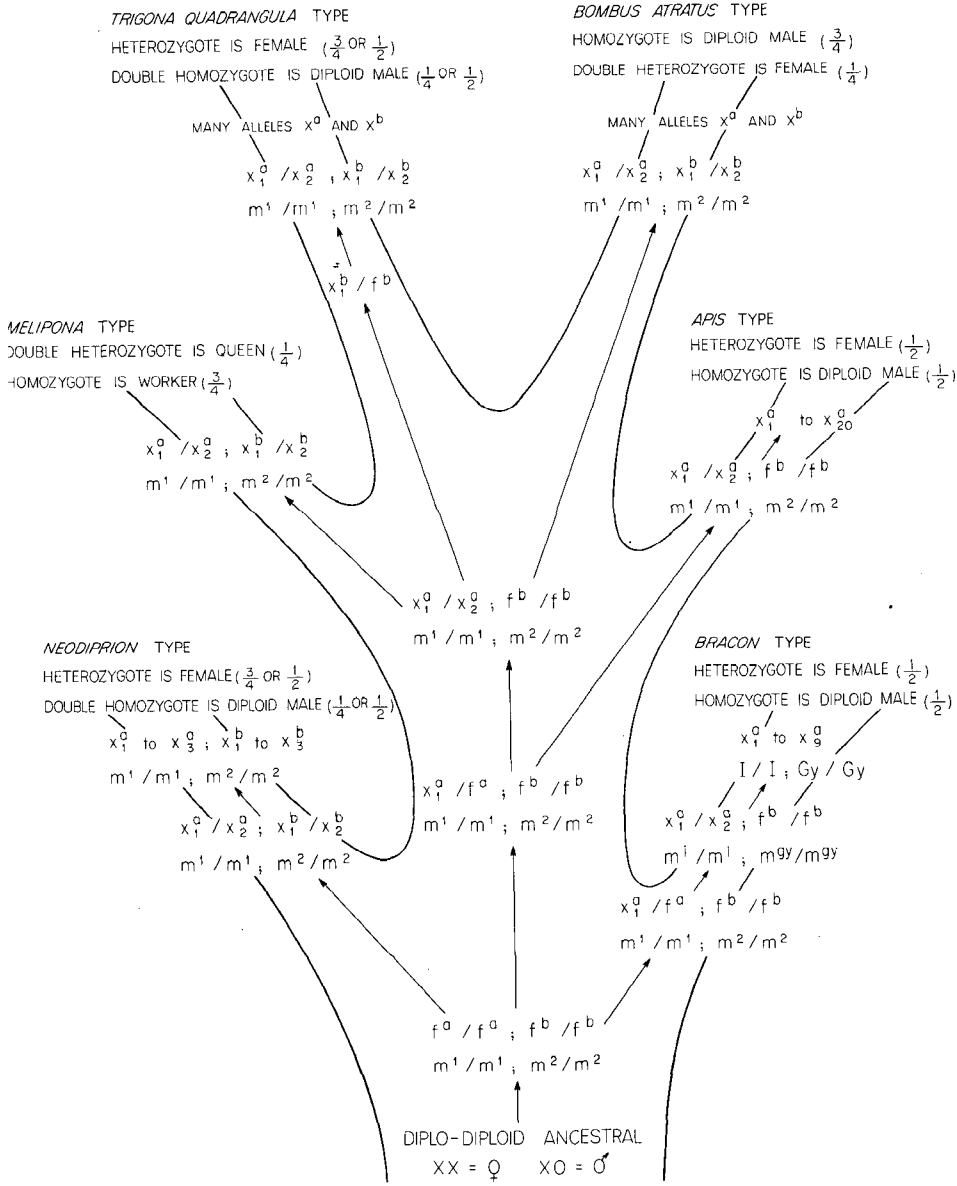


Figure 1 Sex determination in the Hymenoptera. The scheme shows the female genetic constitution in seven types of sex determination found in different species of Hymenoptera as being a product of independent mutations occurring in two femaleness genes (f^a and f^b) of the basic type $f^a/f^a; f^b/f^b; m^1/m^1; m^2/m^2$. In the line of *Bracon* type it is suggested that the maleness genes are the normal alleles of *i* and *gy*, therefore m^1 and m^2 are substituted by *I* and *Gy*. The fractions in parentheses are the proportions of female to diploid males (or sterile workers in the case of *Melipona*) expected under inbreeding.

lamarckii) is dominant to the grey color of Carniolans and due to an only pair of alleles. These alleles will not be considered among the three new genes because they may be alleles of the locus *bl* (black), already described in the literature.

Woyke (82) describes a new gene *la* (laranja) that affects the eye color; bees emerge with orange eyes which later darken. This gene confers good viability and is being used as a marker by Chaud (12) in the production of triploid workers.

The other two genes have been studied by Mestriner (53) and Mestriner & Contel (54). They used the technique of protein separation in starch gel electrophoresis and analyzed homogenates of individual blackeyed worker and drone pupae and detected two polymorphic systems: 1. the gene *P-3* with two intermediate alleles, $P\text{-}3^F$ and $P\text{-}3^S$, that control the proteins p-3 fast and p-3 slow. The allele $P\text{-}3^F$ has a frequency of 0.005 in *Apis mellifera adansonii* and 0.469 in the subspecies *A. m. ligustica;* 2. the gene *Est* with two codominant alleles Est^F and Est^S that control the different mobility of an esterase in the same individuals tested for p-3. The population frequency for the allele Est^S is equal in both subspecies *ligustica* and *adansonii,* and is 0.972. Mestriner (53) found, furthermore, that these two genes are not linked and that the molecular weight of this esterase is 45,000. Also, the heterozygote workers $P\text{-}3^S/P\text{-}3^F$ produce only an intermediate band, instead of the normal two.

A total of 27 genes are now known in *Apis mellifera* of which three, namely *P-3, Est,* and the *x*-alleles, are polymorphic. However, if only the bands of enzymes and proteins are considered, Mestriner & Contel (54) found 2 polymorphic genes out of a total of 6, that is 33%; this value does not differ from that of 9 out of 21, found for *Drosophila pseudoobscura* by Hubby & Lewontin (29). Kerr (35) considers *Drosophila pseudoobscura* an intermediate between a haploid system (like bees) and a diplodiploid one, since the X of this species is 40% of the total genome. Furthermore, *Apis mellifera* has about 20% of her genes sex limited (32, 34). Sex-limited genes in Hymenoptera act under the same principles as regular genes in diplodiploid populations (32).

In animals it is usual for each individual to look for environmental conditions to which it is most suited. Queens of stingless bees (Meliponinae) fly once while swarming and a second time during the nuptial flight; after that they never fly again. Therefore, except for the choice made by the workers in selecting a nest site, they cannot select any special conditions under which to live. In this respect, these social bees are similar to plants, that is, they have very limited means of movement, and therefore, their living sites are of enormous selective importance. The adaptation of populations to different ecological niches within the same reproductive area appears to be the main factor for maintaining polymorphism in bees (40).

QUANTITATIVE GENETICS

Comparisons between African and Italian bees and their hybrids are being extensively investigated by several Brazilian scientists. One of these experiments (44) consisted of observing 20 hives, 10 with Italian and 10 with African bees, each of which was headed by one artificially inseminated queen. When the Italian queens

died, they were replaced by Italian X African hybrids that superseded them (by natural methods) and continued to be observed. The hives were given the necessary space, but were neither fed nor provided any aid in defending themselves against moths, ants, diseases, or any enemy. Some of the results obtained are as follows: (*a*) no difference in swarming (under these conditions); (*b*) no Italian absconded, 1 African and 2 (out of 6) hybrids did; (*c*) after 14 months no Italian colonies were alive and only one of the hybrid colonies was alive, after 14 months 4 African colonies still survived; (*d*) During the year, the 10 colonies which started with Italian queens, plus the hybrids that superseded some of them, gave a sample of 205,077 Italian workers (23%) and 190,357 (22%) hybrid workers, while the African produced 481,397 bees (55%); (*e*) artifically inseminated queens, both of Italian and African breeds, lived equally long (4.8 ± 2.8 months), the average age for the naturally bred and naturally inseminated queen was 7.9 ± 4.9 months; (*f*) queens that weighed 220–230 mg lived longer than those that weighed 200–220 or 230–250 mg (stabilizing selection around the mean); (*g*) out of 20 hives, treated as if they were natural swarms, only 4 survived (as colonies) after 14 months, which indicates that survival success of a swarm is slightly smaller than 25%. This value is greater than the figure of 12.6% obtained by Autuori (1) in the ant *Atta sexdens.*

A global project on the genetics of the differences between *Apis mellifera ligustica, A. m. caucasica,* and *A. m. adansonii* (the African bee) has been carried out and the results of these investigations are currently being published. Gonçalves (22) obtained, from a series of crosses and back crosses between *adansonii* and *ligustica,* the genetic variance, the heritability, and the correlation of father to daughters, for 11 morphological characters. Five of these characters (width of the head, diameter of the median ocelli, clypeo-ocellar distance, width of the posterior wing, and width of the clypeus) had smaller genetic variance in the males than in the females, very low correlation of father to daughters, and low heritability. All these factors led Gonçalves to consider these five characters as determined by a global genic action limited to the female sex. Three of these characters (length of the anterior wing, number of hamuli, length of radial cell) had smaller genetic variance in males than in females, presented high heritability, and the correlations of father to daughters were high. These properties indicated that these characters are being determined by additive quantitative genes. Li (47) and Eickwort (19) demonstrated the possibility of a certain gene being selected for, in the males, and against, in the females, or vice-versa: these genes determine characters with differential selection. Two of the characters studied by Gonçalves, namely length of the flagellum and width of the anterior wing, have properties (genotypic variance in males smaller than the genotypic variance of females, father:daughter correlation very small or negative, low heritability) that suggest they are under this type of selection. One character, length of posterior wing, has the genotypic variance of the male smaller than the variance of the female, medium to high father:daughter correlation, and medium heritability, all of which suggest that the character is overdominant or heterotic.

The same crosses studied morphologically by Gonçalves were studied in relation to aggressivity (attack and stinging behavior) by Stort, who employed an ingenious methodology (71). Stort divided the aggressive behavior into five subcharacters:

(*a*) time in seconds to first sting in a leather ball, (*b*) time in seconds to become infuriated, (*c*) number of stings in a leather ball in one minute, (*d*) number of stings in the gloves in one minute, (*e*) distance (in meters) that bees follow the operator. Since the crosses were made under the Rothenbuhler method (61) each backcrossed colony has bees with the same genetic constitution, and this condition permits the observation of genetic segregation of the characters that influence behavior. In this way Stort (72) was able to estimate that African and Italian bees would have the following genetic constitution for each gamete indicating nine dominant or co-dominant and two recessive genes in the African bees:

Subspecies	First sting	Number of stings in the glove	Number of stings in the leather ball	Distance the bees follow observer
African:	$Ag_1\ Ag_2\ ag_3\ ag_4$	$F_1\ F_2$	$A^m\ B^{br}$	*Pr1, Pr2, Pr3*
Italian:	$ag_1\ ag_2\ Ag_3\ Ag_4$	$f_1\ f_2$	$A^{br}\ B^m$	*pr1, pr2, pr3*

The most important genes are the two which control the sting insertion in the leather ball and the three that control the behavior of attack at long distance. There is no linkage of these genes and *Ac* (*Ac* is a male-limited gene that confers dark brown color in the African drones; the worker bees are yellow). Stort (72) found a positive and significant correlation between "number of stings in the glove of the observer" and "number of stings in the leather ball," and "distance of persecution," which clearly indicates that some of the seven genes, (F_1, F_2), (A^m, B^{br}), and (*Prl, Pr2, Pr3*), are the same. This reduces the number of 11 genes to a maximum of 8 genes.

Certain interesting correlations between morphological characters and aggressive behavior were found by Stort (73). In Italian bees an increase in the abdominal size diminishes aggressive behavior. In African bees a very high negative correlation (–0.929) was found between the length of the mesoscutum and the time taken to first sting a leather ball.

Adaptative values can be estimated through viability tests and mating competition. Several mutants, in spite of being equally viable, have difficulties in orientation and the rate between inbound and outbound flights can be very small or zero. For drones, the number of flights occurring at a suitable time is very important because mating takes place in the air, outside the hive. For normal (wild type) and mutant drones, Witherell (80) determined several parameters for drone activity including the two cited above. Using Witherell's figures, the following relative adaptative values were estimated for the males: normal 1.00; chartreuse-1 eye (ch^1) 0.284; chartreuse-1 eye with modifier gene (ch^1, *m*) 0.390; chartreuse-2 eye (ch^2) 0.038; Benson green eye (ch^B) 0.142; chartreuse-red eye (ch^r) 0.591; Walker red eye 0.728; Rothenbuhler yellow-green eye 0.157; tan eye (s^t) 0.015; umber eye (i^u) 0.043; brick eye (*bk*) 0.168; diminutive wing (*sh* ?) 0.376; wrinkled wing (*wr*) 0.101; golden body color 0.846; eyeless (*e*) 0.0; hairless body (*h*) 0.297.

Quantitative genetics studies in disease resistance in bees have been carried out by Rothenbuhler and his group for more than ten years. Rothenbuhler (62) found that bees' behavior toward larvae killed by American foulbrood depends upon their genetic constitution. The Brown disease-resistant line uncaps and removes (ingests) foulbrood-killed larvae soon after their death, whereas the VanScoy susceptible line does not. They are designated hygienic and nonhygienic, respectively. Colonies with 50% of each kind of bee display hygienic behavior (76).

In some experiments (literature in Momot & Rothenbuhler, 55) hygienic behavior toward cyanide-killed larvae was studied. Momot & Rothenbuhler (55) measured the influence of bee age and nectar flow on these genetic lines and found that mixed colonies, having old nonhygienic and young hygienic bees, quickly remove dead brood under both conditions of food shortage and nectar flow. Mixed colonies with old hygienic and young nonhygienic bees, removed dead brood slowly in dearth, but clean out dead brood rapidly during a honey flow. Therefore, older hygienic bees engage in removal of dead brood during a nectar flow but not during a dearth.

Cosenza & Silva (14) inserted in each colony to be tested, a 10 X 10-cm piece of comb with brood, killed by leaving it 48 hr in a freezer (slight modification of the Rothenbuhler technique); the colonies were 4 African, 3 Caucasian, and 4 F_1 hybrid colonies. The uncapping and removing behavior obtained is shown in Table 1. The generalized distances of Mahalanobis (D^2) were estimated and showed that F_1 is more distant from the Italian and is closer to the African in every hour studied.

Table 1 Uncapping and removing behavior expressed in percentages, in Caucasian, F_1 hybrids and African bees (14)

Hours after insertion of comb containing dead brood	Caucasian		F_1 Hybrid		African	
	Uncapped	Removed	Uncapped	Removed	Uncapped	Removed
5	41.8	30.4	36.5	28.9	65.1	36.8
20	63.6	57.4	70.9	63.2	78.6	72.2
25	64.3	59.3	79.1	70.8	88.3	77.3
40	69.5	67.4	92.6	88.2	93.3	91.2
48	75.8	74.4	94.4	92.0	97.9	96.9
70	80.0	78.3	96.9	97.0	99.4	98.4
86	87.5	85.9	100.0	99.2	100.00	100.0

In the literature there are several established instances (11) of disruptive selection, both in plants (for instance, oil and protein; 81) and in animals (an excellent revision and 140 references are in Thoday, 75); not only morphological but also behavioral characters are being studied, for instance phototaxis and geotaxis in *Drosophila* (17, 27, 58). Since 1966, Drescher (18) has been selecting two lines of *Apis mellifera* for high and low numbers of hamuli and has produced interesting data for disruptive selections in bees. These lines showed the following results, after 10 generations, for workers (18): initial population, 21.41 ± 1.12 hamuli; Line High, 27.06 ± 1.51 hamuli; Line Low, 14.49 ± 1.07 hamuli.

Figure 2 shows the progress of 12 generations of selection (18, 23). The crossing of the lines High X Low produced workers with 20.15 ± 0.93 hamuli. These data, and those for the drones, suggest that the polygenes that control this character have an additive action. This result of Drescher's agrees with those of Gonçalves (22), which were obtained by employing a completely different approach. Additional data on this problem were published by Gonçalves (24) working in the staff of Drescher and with his same lines. Nine crosses were made between high and low lines; the hybrids produced wings with 19 to 21 hamuli indicating that the weight of the selected genes is quite intermediary. Moreover, a regression between the average number of hamuli of daughter workers (X) and the average number of hamuli of both parents (Y) of 46 crosses resulted in the equation $X = 1.99 + 0.95\ Y$. The correlation parent:workers is $r = 0.96$. The values obtained in this last generation are very close to the theoretical expected limit estimated by Gonçalves (24). A comparison of Drescher's disruptive selection experiments with the many experiments in diplodiploid populations shows that evolution is faster in bees. This confirms Hartl's (25) studies through which he demonstrated that haplodiploid populations evolve one third faster than diplodiploid ones.

ADVANCES IN TECHNIQUES

The technique for the artificial insemination of bees was considerably improved by the work by Camargo (7, 9); she discovered that coconut water is an almost perfect diluent for bee sperm. The pH of coconut water is raised to 7.0 to 9.0, after which this fluid is filtered through a Seitz filter, stored in sterilized vials in a refrigerator, and used when necessary. Camargo found that this diluent is outstanding for maintaining viable spermatozoa for prolonged periods of storage (up to 6 months at 10°C) and for ensuring good migration of spermatozoa to the spermatheca, under artificial insemination. When the semen from one drone was employed for insemination, dilution in the proportion 1 part of sperm to 4 parts of coconut water resulted in a 12-fold increase in the number of spermatozoa migrating to the spermatheca.

In many experiments, the technique of counting spermatozoa with a hemacytometer is routinely employed. Kerr and Camargo found that counting is considerably improved if a solution of water plus 0.005% of water-radiator Bardahl is used.

Information on the artificial insemination procedures, with many drawings of the reproductive organs of *Apis mellifera,* were produced by Camargo & Gonçalves (5, 6).

Camargo (8) found that the social bee *Melipona quadrifasciata* can be crossed under controlled conditions by releasing one 8- to 15-day-old male and one 5- to 10-day-old virgin queen in a small box (4 X 5 X 10 cm) under light. This technique enables this species to be genetically analyzed.

An apparatus and methodology were developed by Pessotti (56, 57) that enables psychologists to test the various characteristics of learning ability under controlled conditions, and permits geneticists to estimate genetic parameters of such behavior. The bee is required to press one of two small levers to obtain a reward (50% sugar syrup) for a correct choice. This methodology was used by two of Pessotti's students

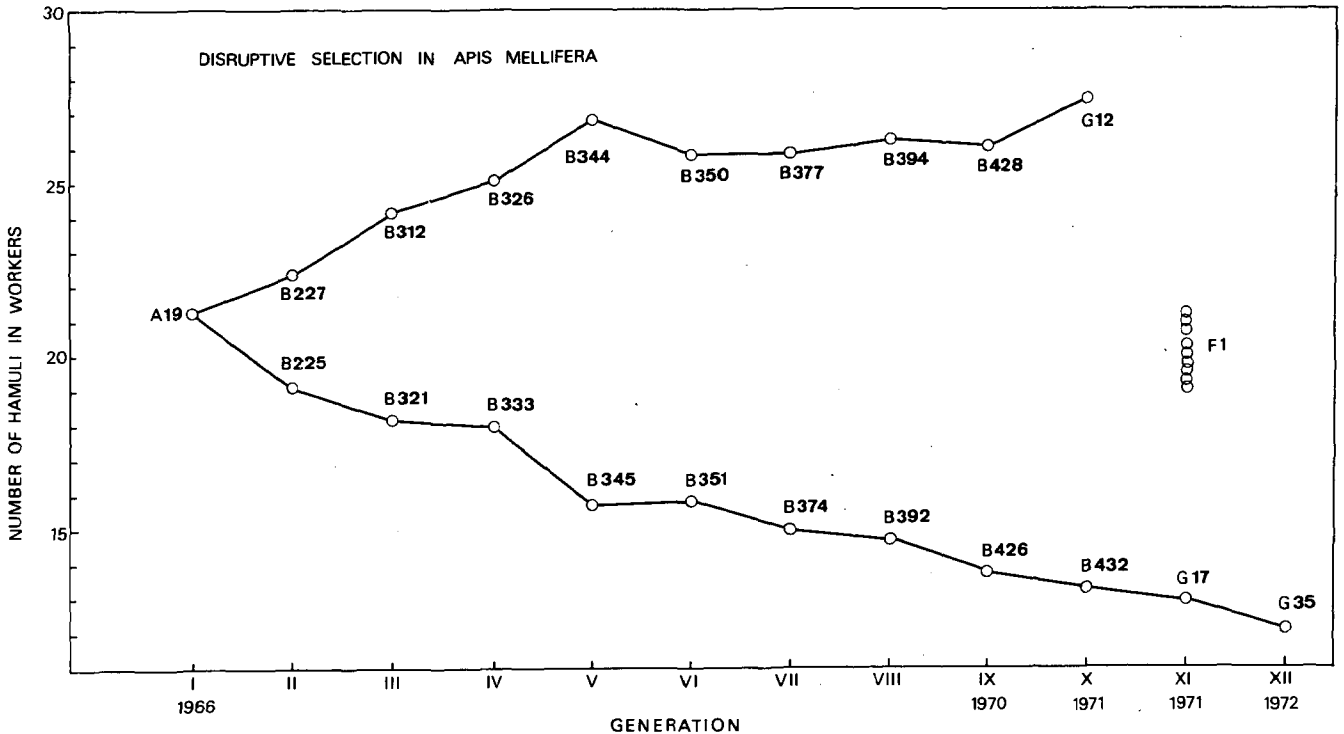


Figure 2 Number of hamuli in wings of workers in each of 12 generations under disruptive selection carried out in the University of Bonn (18, 23). Intermediate values of the eleventh generation show the results of the crosses between "High" and "Low" lines. Graph redrawn from Goncalves (23).

(10) and they were able to demonstrate: first, that overtrained bees have more difficulty in inverting the original discrimination, and second, that variation coefficient is greater at the beginning of the learning process than at the end. Experiments for detecting heritability (h^2) of learning are in progress.

ACKNOWLEDGMENTS

I wish to thank Dr. Daniel L. Hartl, Dr. Lionel Gonçalves, Dr. Walter Rothenbuhler, and Dr. Murray Blum for reading this manuscript and for their many suggestions. I would also like to thank Mr. J. M. Camargo who made the graphs and the drawing.

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