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Selection and Breeding of Honey Bees

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1. Introduction

The question is, why after crossing queens from colonies producing 20 kg of honey with drones from colonies producing 20 kg of honey, many times the new generation of bees do not produce 20 kg, but less. What to do to breed new queens whose colonies will not produce oniy 20 kg of honey, but more.

Bee breeders mostly rear queens and drones from colonies producing much honey, and then cross them naturally or instrumentally. This way, some progress can be achieved. However, much better breeding results are achieved when the breeding background is known.

Honey production depends upon two main factors: 1. heritable factors and 2. environmental characters. The final result is the interaction of heritable and environmental characters.

Below we will get acquainted with those characters. Heritable characters depend upon genes responsible for those characters. The genes are located in the chromosomes.

2. Introductory Cytogenetics

The honey bee develops from an egg. The egg is composed of an outside chorion and the inside cytoplasm and nucleus. Inside the nucleus is the chromatin, which during division of the cell is transformed into several chromosomes. Inside the chromosomes are located the genes which are responsible for heritable characters of animals and plants. In an unfertilised egg of honeybee there are 16 chromosomes. During insemination of an egg, the head of a spermatozoon penetrates inside the egg. The head inside the egg cytoplasm transforms into a nucleus. Inside that nucleus there are also 16 chromosomes. Next the two nuclei unite, and the new nucleus contains 32 chromosomes. The process of uniting of the egg nucleus with the sperm nucleus is called fertilization.

In honey bee both, unfertilised as well as fertilized eggs can develop. From unfertilised eggs develop drones and from fertilized ones, workers and queens.

The sperms develop from reproductive cells in testes of drones, and the eggs from cells in the ovaries of queens. Before they become functional spermatozoa or eggs they undergo a maturation process. During that process in egg, a division called MEIOSIS occurs. Two similar chromosomes come close together. They lay side by side. Thus 16 pairs of chromosomes appear. Next the two chromosomes of each pair separate and go to opposite direction. Membrane is created around them, and thus two new cells appear. This way a reduction of the number of chromosomes occurred. Each new nucleus has now only half of the original number of 32chromosomes, it is 16.

It is a matter of chance, which chromosome of each pair go to one of the new nucleus. This way different combinations of chromosomes occur. This process is called segregation. With 2 pairs of chromosomes 4 combinations occurred it is 22. With more pairs the number of combinations is 2n, where n is the number of pairs of chromosomes. With 16 pairs of chromosomes the number of combinations in unfertilised eggs is 216 it is 65536.

After the egg is fertilized, again 32 chromosomes are present in the egg.

3. Sex determination

In honey bees, workers and queens develop from fertilized eggs and drones from not fertilized. However, Woyke (1963) showed that drones develop also from fertilized eggs. They are not seen in the hive, because workers eat them within 6 hours after hatching from eggs (Woyke 1967). As a result empty cells occur and the brood is scattered. However, Woyke (1969) worked out a method to rear these drones to the adult stage.

In the honey bee there are not 2 sex genes X and Y like in many animals and plants. Instead there are about 12 sex genes called sex alleles: Xa, Xb, Xc, Xd, Xe,·.

A queen inseminated by one drone XaXb x Xc produces haploid males Xa and Xb from unfertilized eggs and heterozygote females XaXc and XbXc from fertilized eggs (see figure)

No diploid males are produced from this mating. If the virgin queen XaXc is inseminated by her brother Xa, she produces two types of fertilized eggs: heterozygotes XaXc, from which females develop, and homozygotes XaXa from which diploid males develop. Half of the diploid progeny from this mating is female and the other half is male. Because the diploid drones will be eaten, the survival of brood will be 50%.

Sex determination in honeybee

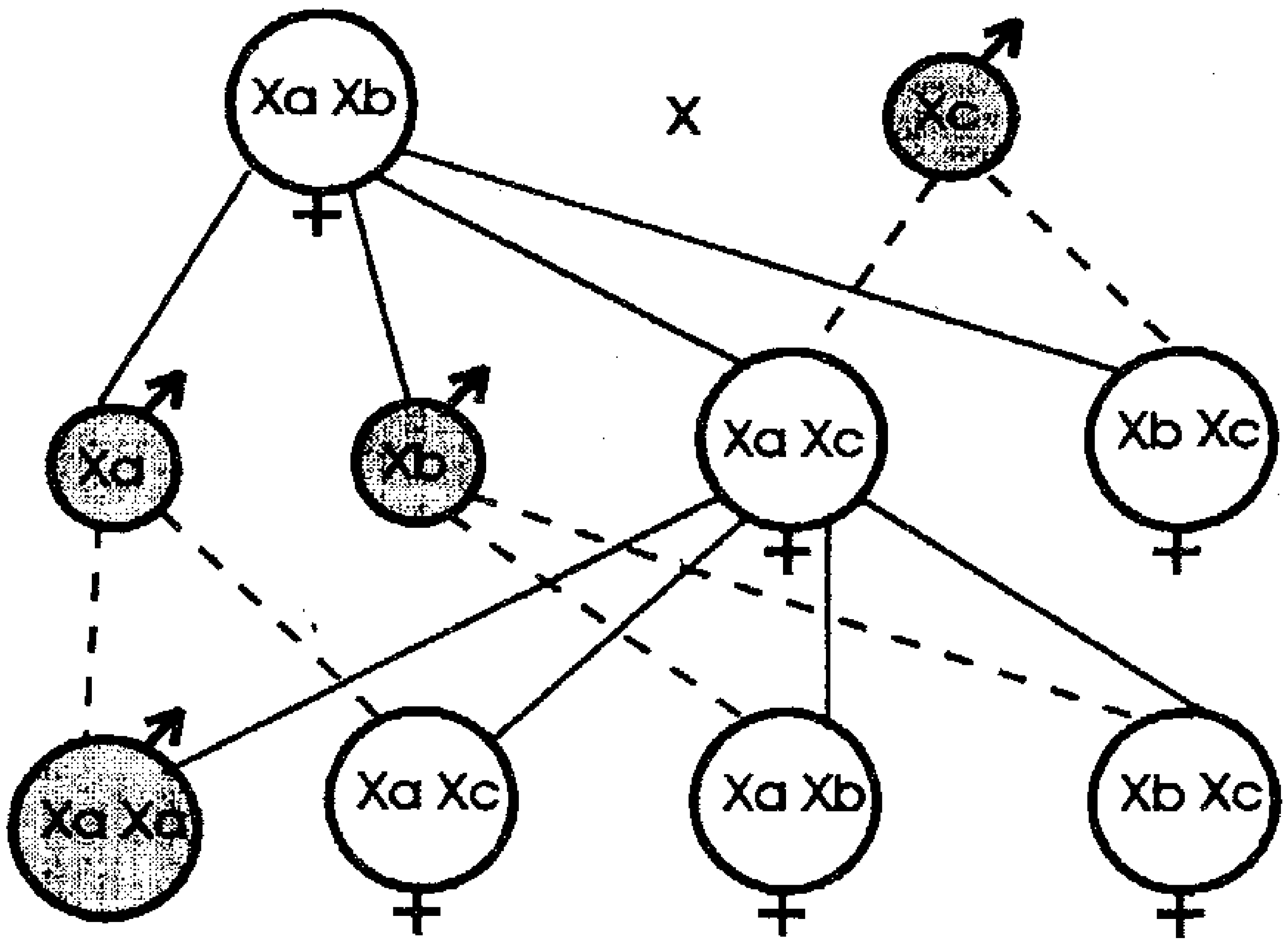
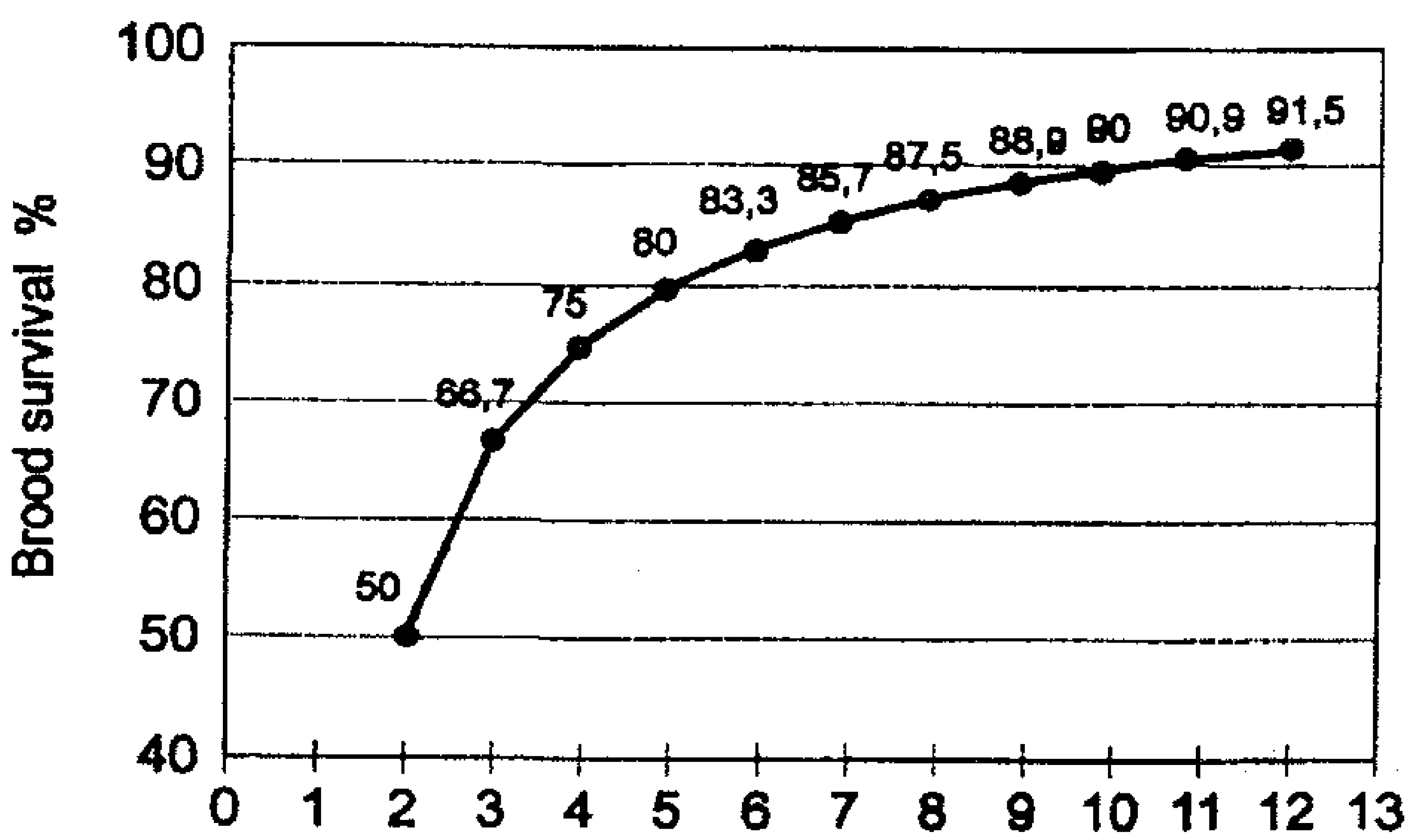


Fig. Small circles are haploids, large - diploids.
White circles are females; gray are drones, haploid and diploid.

If the queen is inseminated by 2 drones of which 1 has the same sex allele as the queen XaXb x Xa, Xc she will produce fertilized eggs with the following alleles XaXa, XaXc, XbXa and XbXc. 25% of eggs will be homozygous (XaXa) and the survival rate of brood will be 75%.

With multiple mating the survival of brood depends upon the number (N) of sex alleles in the population according to the formula $S\% = 100\ [(N - 1)/N]$ (presented in the figure).

Effect of sex alleles on brood survival



No of sex alleles

4. Dihybrid cross - Resistance to bee diseases

The inheritance of different pairs of genes must be known. A cross in which two pairs are involved is called a dihybrid cross. A good example for such cross is the phenomenon of hygienic behaviour of *A. mellifera* bees. Dr Rothenbuhler (1964) found that hygienic behaviour depends upon two recessive genes: 1. *u* - (uncapping) responsible for detecting brood cells with dead larvae and uncapping them, and 2. gene *r* - (remove) responsible for removing dead brood from open cell. When gene *u* is absent, worker bees do not open cells with dead brood, and absence of gene *r* cause that worker bee do not remove dead larvae from comb cells. Worker bees showing the hygienic behaviour are resistant to American foul brood and also to *Varroa destructor*

The genes are located in two different chromosomes. Consider two lines of bees. One homozygous for the *u/u* genes uncaps cells with dead brood but does not remove it. The second line homozygous for *r/r* genes, remove dead brood only after the cells are open naturally or artificially.

After queens from one line are crossed with drones from the other line, the new F1 generation is produced. All the offspring (workers and queens) are heterozygous for both pairs of genes *u/u, +/+ x +/+, r/r = u/+, r/+* (+ = wild dominant gene). Because both hygienic genes are recessive to the wild dominant non hygienic genes, the offspring will not show any hygienic behaviour. Thus a surprising result is obtained. After bees uncapping cells are crossed with those removing dead larvae, the offspring neither opens the cells not removes dead larvae. No any variation in hygienic behaviour occurs in the F1 generation.

Each double heterozygous queen *u/+, r/+* produces 4 types of gametes (unfertilised eggs, or drones producing sperms): *u/ r/; u/ +/; +/ r/* and *+/ +/*.

When F1 virgin heterozygous queens are mated to drones from other heterozygous F1 queens, then 16 different combinations of genes occur, which are presented in the figure. Although the number of genetic combinations is 16, the number of different phenotypes is lower. This occurs because phenotypic expression of some genetic combinations is the same. E. g. 3 genetic combinations: *u/+, +/u, or +/+* result in the same phenotype of the not uncapping character. Thus, 4 genetic combinations of one pair of the *u* gene: *u/u, u/+, +/u, +/+*, result in 2 phenotypes: the uncapping phenotype and the not uncapping phenotype.

In our check board of 16 genotypes there are 4 genotypes of the *u* gene resulting in a phenotype of cell uncapping, and 12 genotypes resulting in another phenotype of not uncapping the cells. Hence, the distribution of uncapping to not uncapping is 1 : 3 (see fig. on overhead). The same distribution 1 : 3 concerns the other character (*r*) removing and not removing. When both characters are taking into account, the distribution is (1 : 3)2 = 1 : 3 : 3 : 9. Thus, the distribution of phenotypes is 1 colony uncapping and removing dead larvae, 3 uncapping brood cells, 3 removing dead larvae and 9 neither uncapping nor removing.

Hence, due to independent segregation of two pairs of genes (second Mendel law), new combinations of genotypes occurred, which were not present in the original populations. These are the bees showing hygienic behaviour which uncap the cells and remove dead larvae, and the non hygienic bees which neither open cells nor remove dead larvae. The phenomenon of appearance of new genotypes in the F2 generation is the genetic background of breeding better bees with characters, like hygienic behaviour, which were not present in the original population.

Summarizing, there is not sufficient to cross bees uncapping cells with those removing dead larvae to breed hygienic offspring. The new offspring will neither uncap brood cells nor remove dead larvae. The F1 non hygienic generation must be crossed to produce the F2 generation. Due to the segregation, new genotypes will occur in the F2 generation. Now the breeder beekeeper should select bees with desired characters.

There are two methods to detect hygienic bee colonies. 1. A piece of 5 x 6 cm of sealed brood is cut, and frozen for 24 h and then returned to the hive. 2. Sealed brood is killed by inserting a pin through the cappings. Colonies in which killed brood is removed within 48 hours are considered hygienic.

5. Quantitative genetics - Honey production

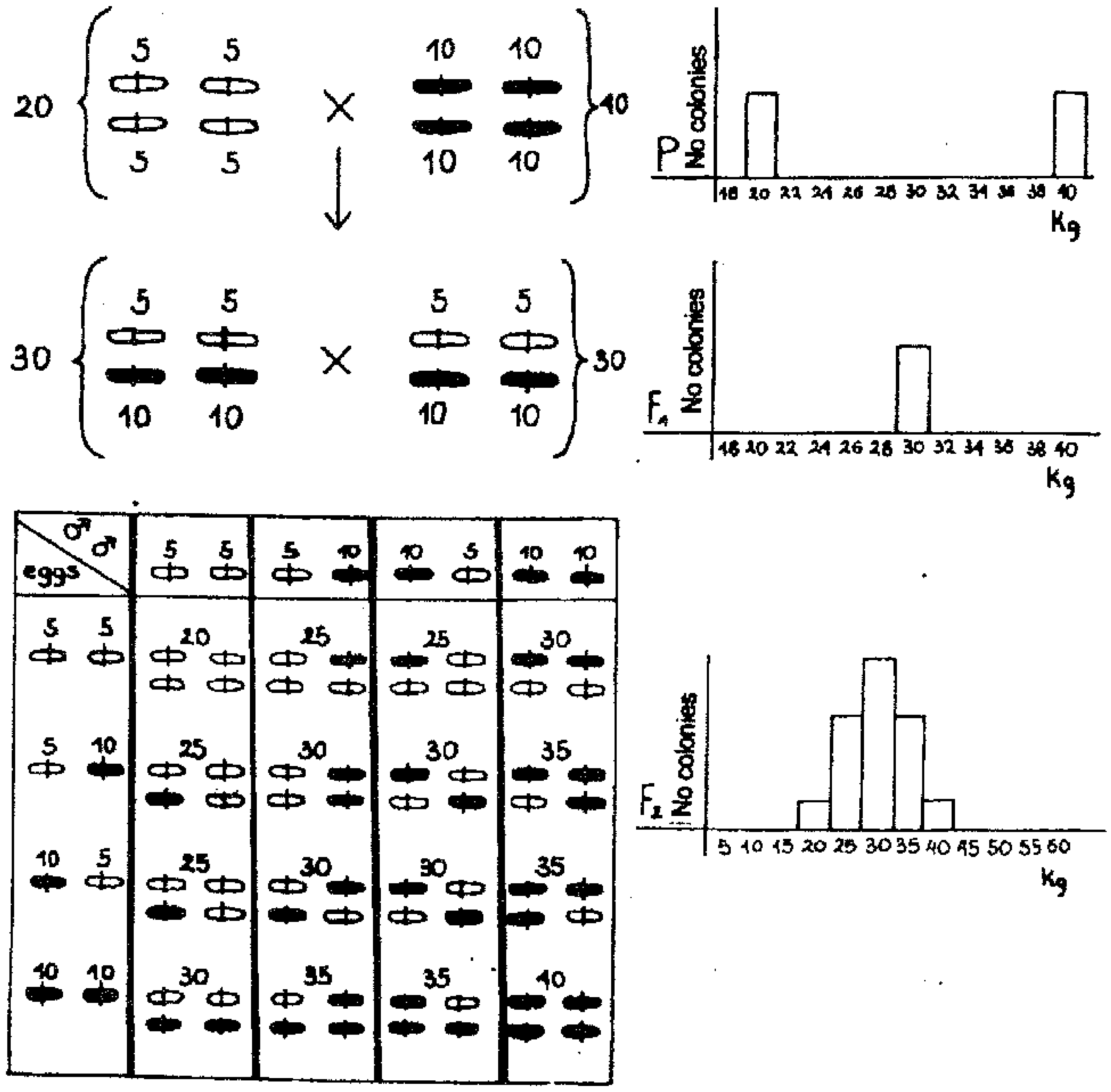
A model of this inheritance is presented below. To simplify the explanation, assume that the production of honey depends upon two pairs of genes on two chromosomes segregating independently. Assume two lines of honey bees, one producing little honey and the other much. The low producing line has the following genotype: *m1/m1, m2/m2*. Each of those alleles results in the production of 5 kg of honey, in the given circumstances. The effect of these alleles is cumulative. As a result, bees with that genotype would produce 4 x 5 kg = 20 kg of honey. The high productive line is of the following genotype *M1/M1, M2/M2*. Each of those alleles results in the production of 10 kg of honey. As a result bees with the last genotype would produce 4 x 10 kg = 40 kg of honey. After the two lines are crossed, an F1 generation is produced with the genotype of *M1/m1, M2/m2*. Thus each colony would produce 30 kg of honey (see the figure below). This amount corresponds to the average of the production of the two original lines.

After the F1 queens are crossed to drones of their sisters, combinations presented for F2 in the figure are obtained. There appeared 16 genotypic combinations. However, after the combinations yielding the same production are gathered together into classes, then only the following 2 x 2 + 1 = 5 classes producing 20, 25, 30, 35 and 40 kg of honey are present (see figure). Combinations yielding the lowest production of 20 kg of honey as well as that of the highest production yielding 40 kg of honey are present. The most numerous is the middle class yielding the average production of 30 kg. After production of all the combinations is added and then divided by the number of individual combinations, the average production of 30 kg is obtained. This average is equal to the average of the F1 generation. But the main difference between the F1 and F2 is that in F1, each cross yielded the same average production and in F2 very great variation occurred.

Now, the responsibility of the bee breeder is to select colonies yielding the highest honey production for breeding the next generations of bees.

6. Selection

Selection is a breeding procedure by which the best bee colonies are chosen to become the source for the next generation of queens and drones for the reproduction. Thus, the best colonies are chosen, whose average value of the character desired is above the average value of the whole population.



Quantitative inheritance

(n = No of pairs of genes = 2)

Zygotic combinations $2^{2n} = 2^{2x2} = 2^4 = 2 x 2 x 2 x 2 = 16$

Frequency $(a + b)^{2n} = (a + b)^4 = a^4 + 4ab^3 + 6a^2b^2 + 4ab^3 + 4b^4$

No classes $2n + 1 = 4 + 1 = 5$

Selection differential

As an example, honey production will be discussed (see figure below). Quite high variation in honey production occurs in any apiary. Let say that the average production of all bee colonies is 30 kg. A group of bee colonies is chosen for queen and drone rearing, and the average production of that new selected group is 50 kg. The difference between the average production of the selected group and the average of the whole population is called <u>selection differential</u>. DS = 50kg 30kg = 20 kg. When the selection differential is low, slow progress occurs, when it is higher quicker progress is made. But too high selection differential eliminates to many valuable parents from the breeding group.

Regression

When next generation is reared from the selected group then its average production is mostly lower than that of the parental selected group. The obtained value is shifted in the direction of the mean value of the whole population. This phenomenon is called regression.

Heritability

Some phenotypic characters like eye color depend only upon the genotype, but other like honey production depends in great deal upon environmental conditions. The ratio of the genetic variance (Vg) to the total phenotypic variance (Vp) is called heritability (h2)

$$h2 = Vg/Vp$$

The coefficient of heritability ranges from 0 to 1. The coefficient of heritability of honey production is about 0.25 to 0.30. This means that the variation of honey production depends only in 25% to 30% upon genetic factors.

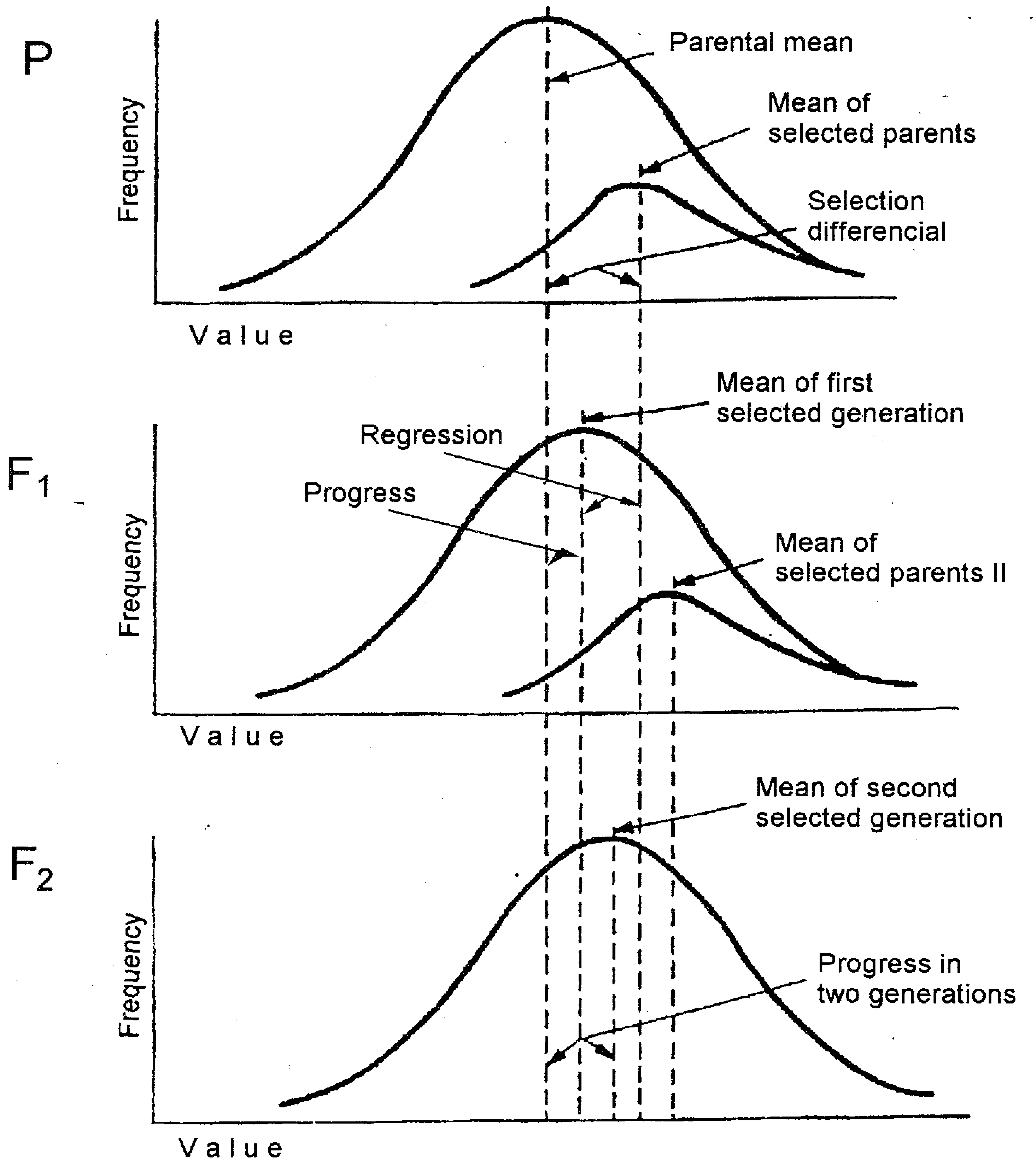
The coefficient of heritability enables to predicate the Progress of selection (Pr) in the next generation.

$$Pr = h2\ DS$$

Therefore this value is called also the response to selection. For our example of honey production the selection differential was DS = 50kg 30kg = 20kg and the progress expected is Pr = 0.25 x 20kg = 5kg. Thus even when the selected group produced 20 kg of honey over the population mean, only a gain of 5 kg can be expected in the next generation. Thus the average production of the next generation will be probably only 30 + 5 = 35 kg of honey.

Now the question is, how to achieve the production of 50 kg or more? To do this, the selection must be repeated for several generations. When we select the breeding group, which produces 20 kg of honey over the average of the apiary (Differential Selection = 20 kg), we can expect an increase of 0.25 x 20 = 5 kg in each next generation. With selection differential of 5 kg, we can expect an increase of only 0.25 x 5 = 1.25 kg per generation.

Selection


P
Frequency
Value
Parental mean
Mean of
selected parents
Selection
differencial
F1
Frequency
Value
Regression
Progress
Mean of first
selected generation
Mean of
selected parents II
F2
Frequency
Value
Mean of second
selected generation
Progress in
two generations

7. Breeding in moderate relationship

Some years ago, there was recommended to select the best colony in the apiary, which produced the highest yield of honey, From that colony, new queens and drones should be reared. Next, the drones and queens should be crossed.

However, that method resulted in two inconveniences:

1. Due to inbreeding, the same sex alleles occurred in particular eggs. This resulted in scattered brood and weak colonies.
2. Often, the queen in the best colony was a hybrid. As a result, gene segregation occurred in the next generation, and the production of particular colonies was very variable.

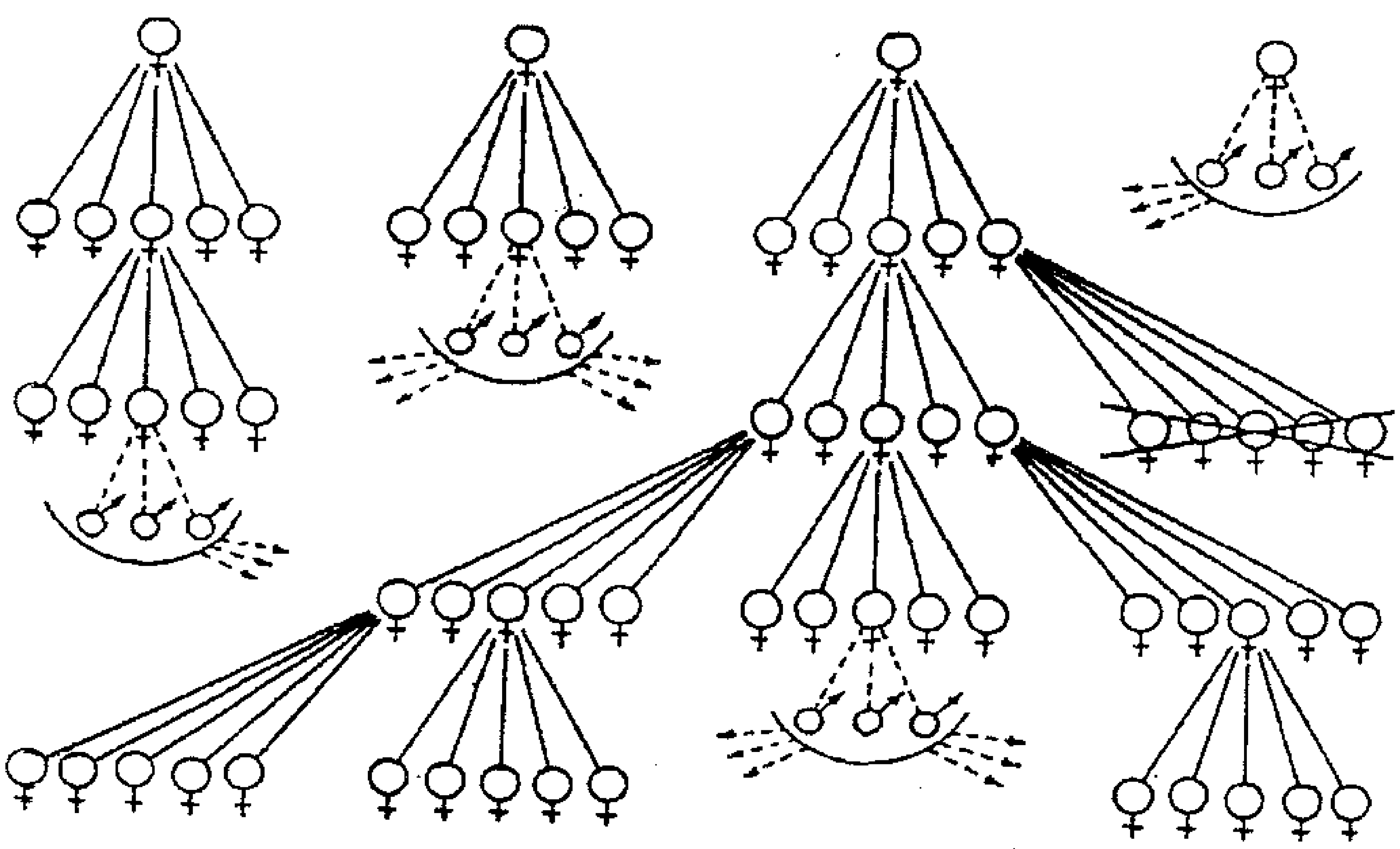
Therefore better method is to breed bees in a moderate relationship. This system is much easier than to breed at first pure lines and then to cross them to receive the hybrids. It is also easier than the so called breeding in closed population.

The recommendations are summarized below. To prevent disadvantages caused by homozygous sex alleles, and consequently by scattered brood, inbreeding should be avoided. The queen heading the colony, which produces drones, should be changed with each generation of virgin queens. Virgins and drones should not origin from the same colony, and even queens producing virgin and drones should not be sisters. The best is, when queens producing virgins belong to other group of sisters than queens producing drones.

An example of mating plan is described, and is presented in the figure below. At first, honey production of particular colonies in the apiary ·is evaluated. Next three best colonies are selected. New queens are reared from two colonies, and drones from the third one. After natural or instrumental insemination, honey production of colonies headed by those queens is evaluated. Next new virgin queens are reared from two best colonies of one group of sisters. Drones are reared from the best colony of the second group, and the whole procedure is repeated.

Better selection possibilities exist when the new virgin queens origin not from two, but from more queens. For example, four best colonies are selected. New virgin queens are reared from three colonies, and the drones from the forth one. Next year, drones are reared from a colony of one group of sister queens, and the virgins are reared from the three best colonies of the two remaining groups. Two breeder queens belong, to one group of sisters, and the third one to another group of sisters. It may happen that honey production of colonies headed by queens of one group of sisters is low. In such case, virgin queens should be not reared from that group of queens, but all three breeder queens should be selected from one sister queen group (see fig.).

It may happen that drones originating from the selected queen transmit the desirable characters in low proportion. Therefore a good idea is to rear drones not from one queen but from two. The virgins are not mated to drones originating from both queens, but one half of the number of queens belonging to each group of sisters is mated to drones from one queen and the other to drones from the second queen.



Breeding in moderate relationship

8. Closed population breeding

Introduction of new breeding material (new queens) into breeding population results after several generations in segregation and big variation. Therefore, breeding in closed population is conducted. A closed population is a breeding population, into which new uncontrolled genetic material is not introduced. These populations may be maintained closed by geographic isolation (e.g. on island, well isolated regions) or by instrumental insemination. All the breeding work improving honey production is conducted within that population. However, the problem of sex alleles and percentage of brood survival must be taken into account.

The number of sex alleles cannot be large in closed population. This is because some alleles are lost. Larger populations will have higher number of sex alleles and higher brood viability /Woyke 1976/, Yokoyama and Nei 1979, Page and Laidlaw 1982a, b/.

The number of sex alleles that can be maintained in a close population depends upon the number of parents taking part in reproduction. Figure in the overhead film presents the number of sex alleles, which can be maintained in population of different size. Page and Laidlaw /1982a/ computed also the number of expected alleles for 40 consecutive generations in closed populations of different numbers of colonies /Fig. overhead/.The queens were randomly mated, and the breeding colonies were selected by random, each generation. The results of this study show, that queens from 50 colonies must be selected as breeders, from each generation to maintain about 10 sex alleles in the population.

The next figure shows brood survival during 40 generations in closed population with different number of breeding queens. The number of 50 breeding queens assures over 85% survival of brood after 40 generations.

This is an important finding for its yields a first approximation of the necessary size of closed populations used for stock maintenance and stock improvement.

Later on, Page and Laidlaw /1982b/ showed, that the most effective method of maintaining high number of sex alleles and high survival rate is, when the breeder queens are not selected at random, but each queen mother is replaced by her own daughter, each generation. With this system a minimum of 35 colonies is needed for long term breeding that assure brood viability above 85% survival.