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POPULATION GENETIC STUDIES ON SEX ALLELES IN THE HONEYBEE USING THE EXAMPLE OF THE KANGAROO ISLAND BEE SANCTUARY *

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Summary

The common practice of crossing different races of honeybees has created a demand for the establishment of isolated reserves for pure ecotypes of the main races. On Kangaroo Island, S. Australia, a bee sanctuary 100 years old, the average survival rate of brood was found to be 75·6%. Mortality of 6·5% was caused by factors other than sex alleles, so the survival rate resulting from sex alleles was 82·1%.

Equations are presented for calculation of survival rate of brood (S) in populations in equilibrium, $S = (N\text{-}1)/N$, and in unstable populations, $S = 1 - pfpm - qfqm - rfrm \dots$. Equations are developed for calculating the frequencies of sex alleles in subsequent generations, and after the introduction of new alleles (and new queens), and also differences between the frequencies of sex alleles in the two sexes.

In natural selection, sex alleles in honeybees represent an example of overdominance, with total elimination of all homozygotes in populations with multiple alleles and different frequencies of those alleles in the sexes.

Calculations show that 36% of the drones that mated with the queens in the sanctuary carried the same sex alleles as the queens.

Only 6 of 12 sex alleles known to occur in the honeybee were present among the bees in the sanctuary. An increase in the number of alleles above 8-10 in a population gives very little improvement in the percentage of surviving larvae. Theoretical calculations shows that the frequencies of different sex alleles must be equal throughout the bee population on Kangaroo Island. When a new allele is introduced into a population with few alleles, even in a very low proportion, its frequency increases rapidly, and thus the survival of brood increases. The maximum survival rate of brood in a population is reached when the sex alleles are in equilibrium. Before reaching equilibrium for different sex alleles, any generation of queens produces virgins and drones with different frequencies of these alleles. When there are few alleles, there is a diminishing oscillation in the frequency of the same sex allele between the two sexes, in subsequent generations. The best way to increase the number of sex alleles in a population and to increase the survival of brood, with a minimal change of other characters, is to introduce one or very few queens inseminated by several (not many) different drones.

Although the survival rate of brood on Kangaroo Island was rather low, after 100 years of isolation, the colonies were still good honey producers.

Introduction

The phenomenon of heterosis in the honeybee (*Apis mellifera*) is well known, and the crossing of different lines or races of honeybees is therefore becoming a common practice in more and more countries. But many bee breeders now fear that no pure races will be available in the future, and appeals have been launched at recent bee congresses to conserve the local honeybee ecotypes. Some countries have already started to create reserves for local strains. But when the population is small and well isolated, some inbreeding may occur, which leads to the destruction of a proportion of the brood. Therefore population genetic studies are necessary, to establish the expected viability of brood in such populations, the frequencies of alleles responsible for viability, and the expected changes under natural selection and when new alleles are artificially introduced in order to improve brood viability in those populations.

The principles of population genetics and gene frequencies in populations may be found in manuals such as Li (1955), Falconer (1960) and Wright (1968). Of the original

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papers, the earliest (Fisher 1922, Haldane 1927) discussed the effect of a dominant factor in a population. The frequency of two alleles at a single locus was studied by Wright (1937), and the equilibrium at a sex-linked locus by Mandel (1959*a*). The multiple allele situation has been investigated by Kimura (1956), Mandel (1959*b*) and Kingman (1961). These authors showed that under natural selection the mean viability of the population increases from one generation to the next, and the gene frequencies tend to a limit, which is the stable equilibrium corresponding to a local maximum of the mean viability. Parson and Bodmer (1961) argued that overdominance is commonly the evolutionary phenomenon. (Overdominance means that the heterozygote for two alleles is superior in mean fitness to either homozygote.) Kerr and Wright (1954) found in *Drosophilia* very strong selection against homozygotes in both viability and productivity. Talis (1966) proposed an algorithm for finding all the equilibria of a multiple-allele system.

The homozygous lethal X alleles in the honeybee were discovered by Mackensen (1951, 1955) and confirmed by Laidlaw, Gomes and Kerr (1956) and by Hachinoe and Jimbu (1958). Mackensen estimated their number as 11 and Laidlaw et al. as 12. Woyke (1962) showed that eggs homozygous at locus X are viable, but that larvae developing from them are diploid drones (Woyke 1963*a*) and are eaten by the workers within 6 hours after hatching (Woyke 1963*b*) : they are thus eliminated from the colony. The destruction of brood homozygous at the sex locus X may resemble the self-sterility or self-incompatibility known to occur in plants (e.g. Mayo, 1966). But the phenomena in plants are quite different : any S_1 or S_2 pollen grain tube fails to grow in an S_1S_2 style, whereas in the honeybee any X_1 or X_2 drone mating with an X_1X_2 queen produces viable X_1X_2 offspring, and only the homozygous X_1X_1 or X_2X_2 larvae are eliminated. Destruction of these larvae (diploid drones) is rather similar to elimination of homozygotes in the selection. Thus in the honeybee complete overdominance occurs in natural selection. The phenomenon is complicated, however. The distribution of genotypes in subsequent generations of diploid animals resulting from an $AA \times aa$ cross is $1AA : 2Aa : 1aa$ in either sex, starting from the F_2 generation, and does not change in the next generations. But in the honeybee, the drones develop from unfertilized eggs, so the cross $AA \times a$ results in different genotypes in each generation and approaches an upper limit in F_∞ of $4AA : 4Aa : 1aa$ for females and $2A : 1a$ for males, as shown by Demianowicz (1957). This study was not concerned with multiple sex alleles, but their distribution is also different in males and females produced by the same queen. Woyke (1963*c*, 1972) examined the combination of sex alleles, and the survival of brood, after different methods of controlled mating were applied. When a queen is mated with drones from one colony, she may produce brood of which 50%, 75% or 100% survives on the average. But when she is mated with drones from several different colonies, the average survival of brood is less than 100% : from unrelated matings the average was 91·7% (12 alleles) and from related matings 87·5%. To avoid the inconvenience of low brood survival in breeding work, Maul (1972) started a programme to identify the alleles in his breeding lines. In theoretical studies, Shaskol'skii (1968) showed that both the number of sex alleles (A) and the number of drones (D) mating with a queen determined the distribution of queens producing different proportions of homozygotes : $\left(\frac{A-2}{A}+\frac{2}{A}\right)^D$. But the number of matings (D) does not change the whole proportion of homozygotes in the population. Thus, apart from ignoring the number of matings, studies on the average survival of brood in a population are possible.

Neither the survival rate of brood in isolated honeybee populations, nor the frequencies of sex alleles in subsequent generations, has previously been investigated. The honeybee sanctuary on Kangaroo Island, Australia (Eckert, 1958) proved an ideal place for investigating this problem, and preliminary results on brood survival have been already presented (Woyke, 1975). A few colonies of Italian bees were introduced to the Island in 1884; in the next year a bee sanctuary was created, and importation of new honeybees was prohibited. It is the oldest bee sanctuary in the world, and has remained relatively uncontaminated (Ruttner, 1976).

Materials and Methods

There are 288 registered honeybee colonies on Kangaroo Island. They belong to 13 beekeepers, only 7 of whom have more than 10 colonies. The survival rate of brood was investigated in 34 colonies (12% of the total population) located in six apiaries distributed all over the Island (Fig. 1).

FIG. 1. Sketch map of Kangaroo Island, scale 1 : 8 500 000. The survival rate of brood was investigated at : 1. Flinders Chase, 2. Karatta, 3. American River, 4. Cygnet River, 5. Birchmore, 6. Airport.

In order to investigate the survival of newly hatched larvae, the queen was placed under a queen-excluder cage on one side of a comb; an area containing 200-250 eggs in the centre of another comb was selected. It was marked with a plastic strip 1 cm wide fastened to the sides of bottom and top bars of the frame with drawing pins, so that it marked the edge of the selected comb surface. Row-numbers of cells were written on the strip; starting from it, individual cells containing eggs were recorded. The comb (with the strip) was returned to the colony, in the centre of the brood nest and next to the reverse side of the comb with the caged queen. The marked comb was removed from the colony 3 days later, and a record made of the number of surviving larvae from eggs in the selected cells; the larvae were then 0-3 days old.

In a few colonies the queen could not be found. Several brood combs were then placed in the hive box above the queen excluder, with the selected comb in the centre.

In order to determine the proportion of larvae that failed to survive for other reasons than homozygosis at the sex locus, 16 queens originating from Kangaroo Island were each instrumentally inseminated with semen of a single drone. Only two degrees of survival rate could then occur : 100% when sex alleles of queen and drone were different e.g. $X_1X_2.X_3 \rightarrow X_1X_3$, X_2X_3, or 50% when the sex allele of the drone was the same as one allele of the queen e.g. $X_1X_2.X_1 \rightarrow X_1X_1$, X_1X_2. Other factors besides sex alleles could cause a deviation from these percentages. Each of 9 queens was mated instrumentally to a drone that had been flying free on Kangaroo Island, and each of 7 queens to a

Carniolan drone from the mainland. The survival rate of brood produced by the instrumentally inseminated queens was investigated in Adelaide, by the same method as on the Island.

Altogether 10 000 eggs were recorded, and the surviving larvae were re-examined. The experimental data formed the basis for theoretical calculations.

Results*

Survival rate of brood in colonies on Kangaroo Island

In many colonies on Kangaroo Island the brood was scattered (Fig. 2). The survival rate of brood in the 34 colonies studied ranged from 53% to 98%. Fig. 3 shows only 1 colony with an extremely low survival rate (below 60%) and 3 colonies with a high survival rate (above 90%). In almost all colonies the survival rate was between 60% and 90%, commonly between 70% and 80%, and the average survival rate of brood in all the colonies investigated on Kangaroo Island was 75·6%.

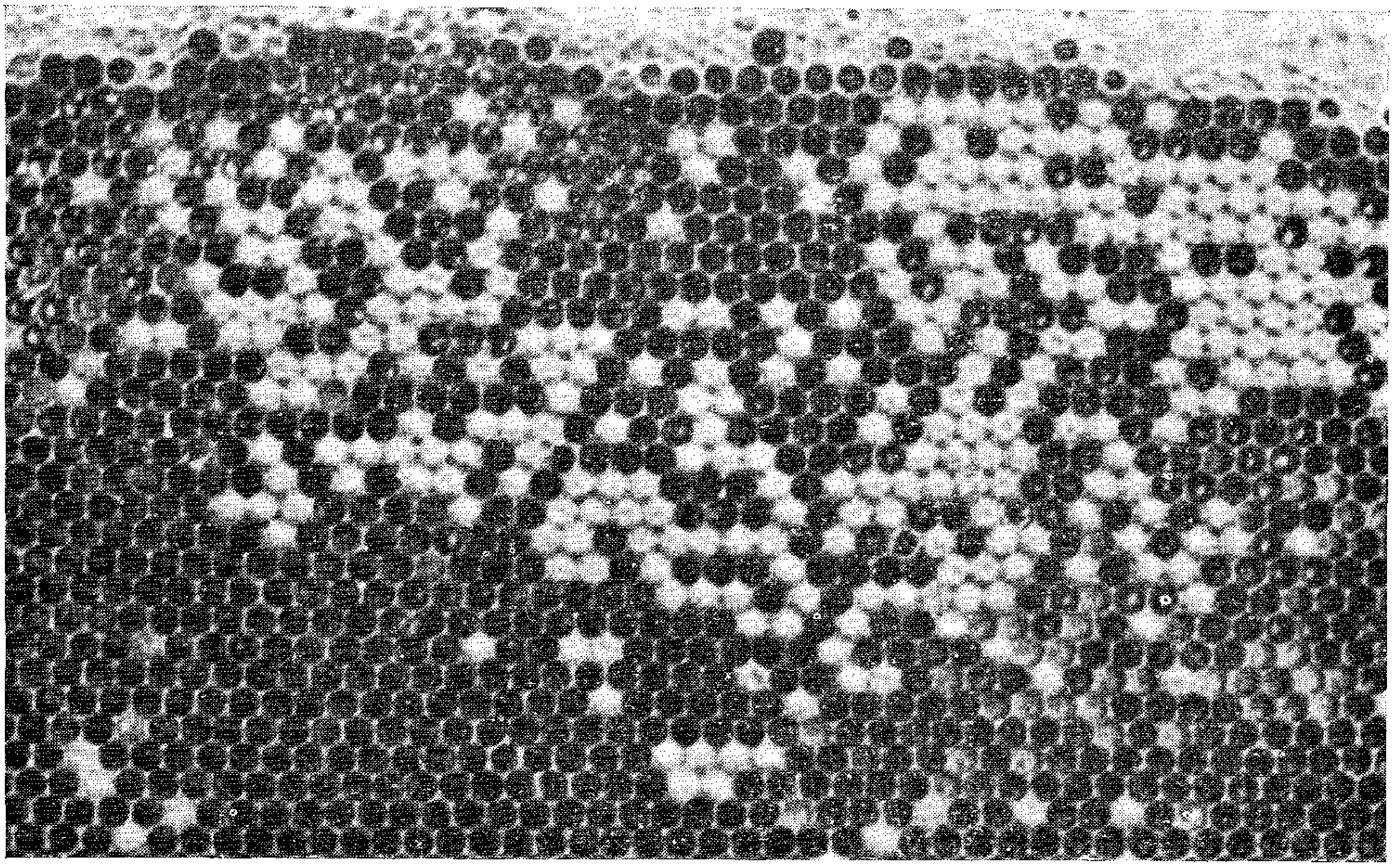

FIG. 2. Scattered brood in a colony on Kangaroo Island.

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* Fig. 4—Fig. 10 summarize the different situations. Full Tables have been prepared giving allele frequencies and brood survival rates in the various circumstances described, and Tables 1-3 are extracts from them. The full Tables are deposited in the Library of the International Bee Research Association, and are designated here as " IBRA Table . . . " They are also available in the 1976 annual PL 480 report for USDA, and will be included in the 1978 final report for USDA.

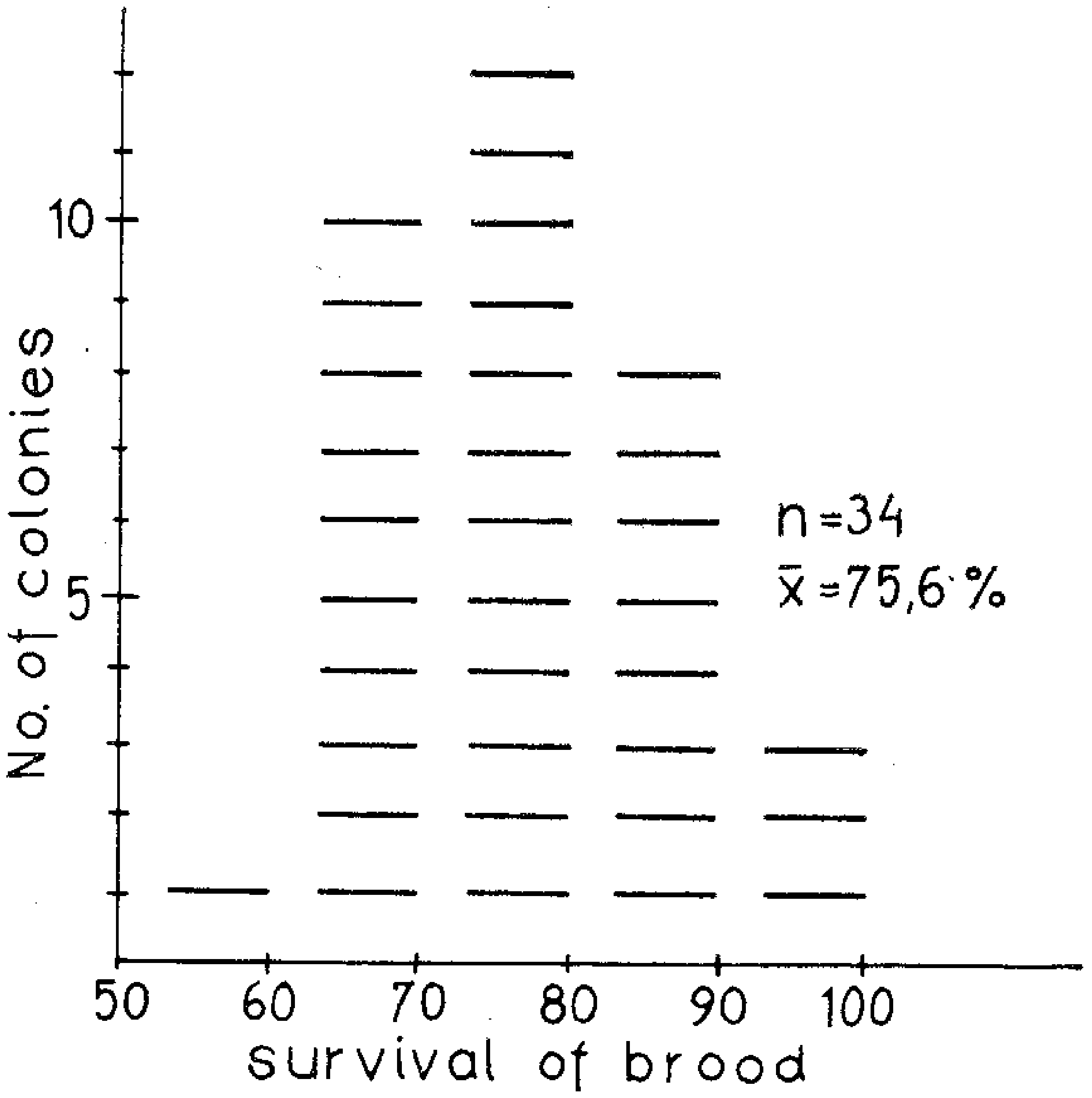

FIG. 3. Survival rate of brood in 34 colonies on Kangaroo Island.

Survival rate of brood in colonies headed by queens mated instrumentally to one drone

For the 7 Kangaroo Island queens instrumentally mated to a Carniolan drone, this ranged from 90·4% to 96·1%; the average was 93·5% (Fukuda & Sakagami, 1968) reported 94% for naturally mated queens). Of the 9 queens mated to a Kangaroo Island drone, 7 produced brood with a survival rate ranging from 87·8% to 99·2% (average 93·5%, like the Carniolan group). The other 2 queens produced brood with survival rates of 51·7% and 58·2% respectively. Queens producing brood of which about 50% or rather less survived were expected ; the slightly higher results could have been accidental, or due to the low number of eggs counted. Unfortunately the queen producing brood of which 58·1% survived was lost, and re-examination was impossible.

It seems reasonable to assume that 6·5% of brood is lost in the first 3 days of larval life by factors other than sex alleles.

Percentage of drones with the same allele as the queen they were mated to

When the queen mates with many drones carrying the same alleles as her own, the survival rate of her brood is low, and from it one can calculate the percentage of the mating drones that had the same alleles as the queen.

A queen X_1X_2, mated to one drone with the X_3 allele, produces eggs that are all heterozygous; 100% of the brood survives. A queen X_1X_2, mated to one drone with allele X_1 (identical to one of the queen's alleles), produces 50% homozygous eggs, and only the other 50% heterozygous brood survives. When a queen X_1X_2 is mated to two drones, one with allele X_3, and the other with X_1, then all eggs fertilized with X_3 spermatozoa are heterozygous ($X_1X_2.X_3 \rightarrow X_1X_3$, X_2X_3), and half the eggs fertilized with X_1 spermatozoa ($X_1X_2.X_1 \rightarrow X_1X_1$, X_1X_2). Only 25% of eggs are homozygous at the sex locus, and the survival of brood is 75%.

Only half the eggs are homozygous after being fertilized by spermatozoa with an allele identical to one of the queen's, and the other half, as well as all eggs fertilized by other drones with different alleles, are heterozygous. Thus, for instance, if 10% of drones carry alleles identical to queen's, only 5% of the eggs will be homozygous. On this basis Table 1 was set up. The percentage (D) of drones with the same allele as the queen with which they mate is twice the percentage of homozygous eggs. Thus :

$$D = 2b = 2(1 - S) = 2(100 - S\%), \tag{1}$$

where S = survival rate of the brood ($S\%$ as a percentage). The average survival rate of brood found on Kangaroo Island was 75·6%; 6·5% must be accounted lost through other factors, so the survival due to sex alleles alone is 75·6 + 6·5%, i.e. 82·1%. Thus $D = 2(100\text{-}82{\cdot}1) = 2\times 17{\cdot}9 = 35{\cdot}8$. Free-flying queens on Kangaroo Island thus mated with drones of which on average 36% carried the same alleles as their queens.

TABLE 1. Survival rate of brood in relation to the percentage of drones that had the same sex allele as the queen they mated with.

% drones with same alleles as queen (D)	*Alleles of queen*	*Alleles of drones*	*Proportion of homozygous eggs* (a)	*% homozygous eggs* (b)	*% surviving larvae* ($S\%$)
10	X_1X_2	$X_1, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}$	1/20	5	95
20	X_1X_2	$X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}$ or X_1, X_3, X_4, X_5, X_6	2/20 1/10	10	90
30	X_1X_2	$X_1, X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9$	3/20	15	85
40	X_1X_2	$X_1, X_1, X_2, X_2, X_3, X_4, X_5, X_6, X_7, X_8$ or X_1, X_2, X_3, X_4, X_5	4/20 2/10	20	80
50	X_1X_2	$X_1, X_1, X_1, X_2, X_2, X_3, X_4, X_5, X_6, X_7$ or X_1, X_2, X_3, X_4 or X_1, X_3	5/20 2/8, 1/4	25	75
60	X_1X_2	$X_1, X_1, X_1, X_2, X_2, X_2, X_3, X_4, X_5, X_6$ or X_1, X_1, X_2, X_3, X_4	6/20 3/10	30	70
70	X_1X_2	$X_1, X_1, X_1, X_1, X_2, X_2, X_2, X_3, X_4, X_5$	7/20	35	65
80	X_1X_2	$X_1, X_1, X_1, X_1, X_2, X_2, X_2, X_2, X_3, X_4$ or X_1, X_1, X_2, X_2, X_3	8/20 4/10	40	60
90	X_1X_2	$X_1, X_1, X_1, X_1, X_1, X_2, X_2, X_2, X_2, X_3$	9/20	45	55
100	X_1X_2	$X_1, X_1, X_1, X_1, X_1, X_2, X_2, X_2, X_2, X_2,$ or X_1X_2 or X_1	10/20 2/4, 1/2	50	50

The percentage of drones having the same allele as the queen with which they mated is the percentage of homozygous eggs × 2, i.e. $D = 2b$.

Relationship between the number of sex alleles in a population and the brood survival rate

In a population with many alleles, there is only a small chance that a queen will mate with a drone with the same allele as one of her own. Thus the average rate of brood survival in such populations is high. Conversely when the population contains few alleles, many queens are likely to mate with drones carrying the same alleles as their own. The average rate of brood survival will then be low. From the average rate of brood survival it is possible to calculate the number of alleles in the population. But first the difference between a three-allele cross and a population with three alleles must be clarified. A three-allele cross ($X_1X_2.X_3$) results in heterozygotes only (X_1X_3, X_2X_3), and the survival rate is 100%. But a population with three alleles (X_1, X_2, X_3) includes different diploid queens (X_1X_2, X_1X_3, X_2X_3), which may be mated by a three-allele cross (e.g. $X_1X_3.X_2$) as well as by a two-allele cross ($X_2X_3.X_2$).

Consider a population with several alleles (X_1, X_2, X_3) whose distribution in the population (frequency) is p, q, r, respectively, where $p + q + r = 1$. After a random cross is made $(p + q + r)^2$, the following frequencies of genotypes occur, whose sum is unity :

$$p^2X_1X_1 + q^2X_2X_2 + r^2X_3X_3 + 2pqX_1X_2 + 2prX_1X_3 + 2qrX_2X_3 = 1 \qquad (2)$$

All the homozygotes are eliminated by the worker bees, and thus the proportion of surviving heterozygotes is :

$$S = 1 - p^2 - q^2 - r^2, \qquad (3)$$

S being the survival rate of brood in the population. The percentage survival rate ($S\%$) is $100S$.

When there are N alleles, equally distributed throughout the population, the frequency of each is $1/N$. Hence equation 3 may be transferred into :

$$S = 1 - N\frac{1}{N^2} = 1 - \frac{1}{N} = \frac{N-1}{N}$$

and the survival rate :

$$S\% = 100\,\frac{N-1}{N}. \qquad (4)$$

This equation was used to calculate the data presented in Fig. 4. It can be concluded that an increase in the number of sex alleles to about 5 results in a rapid increase in the percentage of surviving brood in the population (17%—5% increase in survivals per added allele). A further increase from 5 to 8 alleles will improve the survival of brood much less—under 3·5% per added allele. Thereafter, the survival of brood is increased by less than 1·5% per added allele.

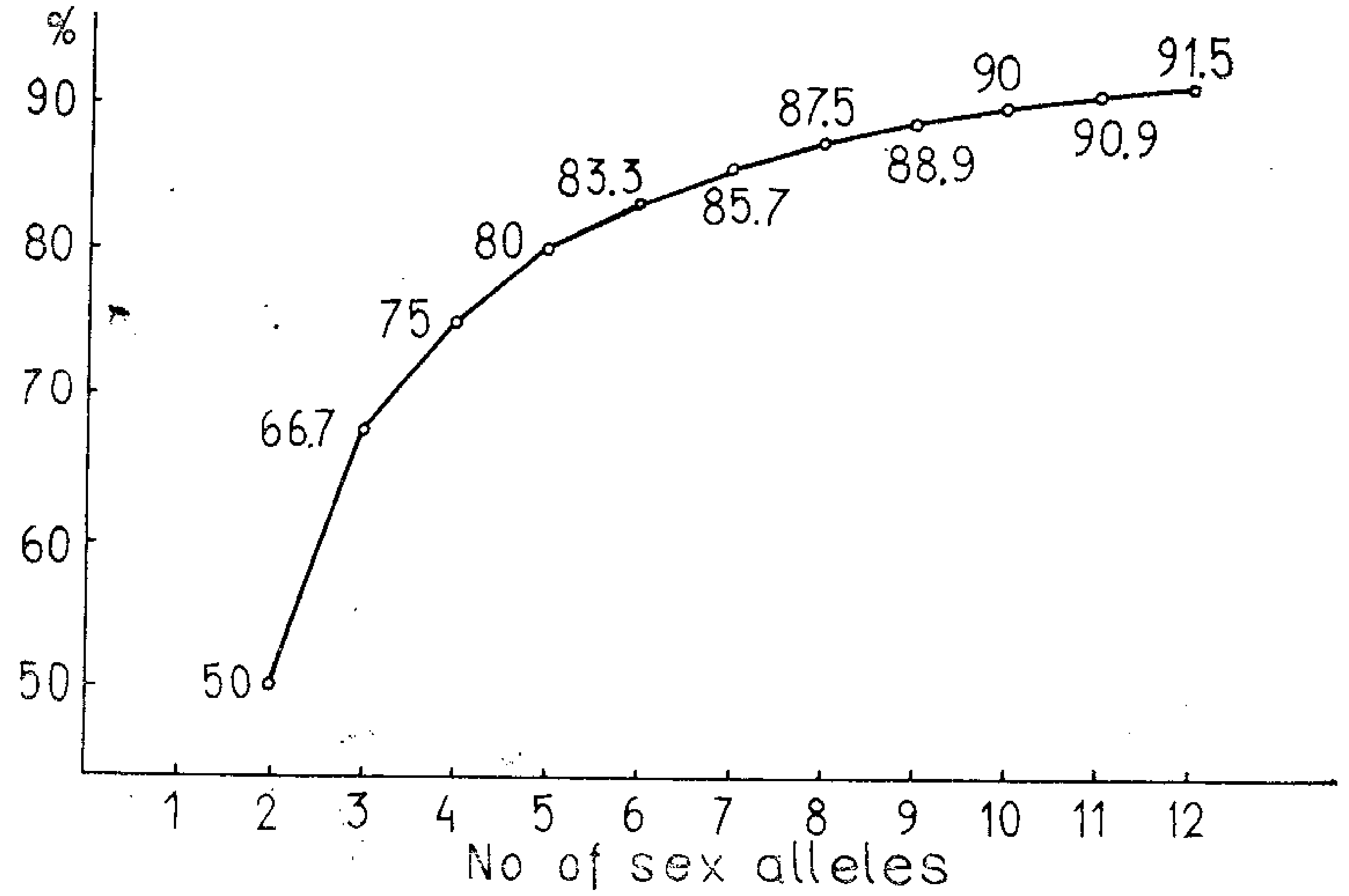

FIG. 4. Relation between the number of sex alleles in a honeybee population and the survival rate of brood.

After transforming equation 4, $N = 1/(1 - S)$ or $N = 100/(100 - S\%)$; the number of sex alleles in a population (N) is the reciprocal of the proportion of homozygous eggs (a) :

$$N = \frac{100}{100 - S\%} = \frac{100}{b} = \frac{1}{a} \tag{5}$$

The corrected rate of survival (82·1%) due to the action of sex alleles on Kangaroo Island indicates that 6 alleles were present in the population, since $N = 100/(100 - 82{\cdot}1) = 5{\cdot}58$.

Frequencies of sex alleles in subsequent generations

When the frequencies of different sex alleles in a population are equal, the frequencies of homozygotes resulting from a random cross are all equal. Each allele is then eliminated from the population in the same proportion, and no change in its frequency occurs. But this is not so when the frequencies of different alleles are different (e.g. p, q, r). After homozygotes are eliminated from the offspring (equation 2), a new pool of individuals (genetypes) $1 - p^2 - q^2 - r^2$ is established. All the survivals are heterozygotes of two different alleles. Therefore, to calculate the new frequency of a given allele in the remaining pool of alleles, the frequency of individuals with that allele must be divided by 2 (because they are heterozygotes) and expressed as a proportion of the remaining individuals. Taking into account equations 2 and 3, the frequency of a given allele in the next surviving generation is :

$$p' = \frac{pq + pr}{1 - p^2 - q^2 - r^2} \quad \text{and} \quad q' = \frac{pq + qr}{1 - p^2 - q^2 - r^2} \tag{6}$$

and similarly for r'.

Results for subsequent generations may be obtained by repeated application of these relations. We have :

$$pq + pr = p(q + r) = p(1 - p) = p - p^2 \quad \text{and} \quad pq + qr = q - q^2.$$

Thus

$$p' = \frac{p - p^2}{1 - p^2 - q^2 - r^2} \quad \text{and} \quad q' = \frac{q - q^2}{1 - p^2 - q^2 - r^2} \tag{7}$$

and similarly for r'.

TABLE 2. Frequencies of sex alleles, and survival of brood, in subsequent generations of a population with three unequally distributed alleles.

Generation	*Frequencies of alleles* X_1	X_2	X_3	*% surviving brood* ($S\%$)
	p	q	r	
P	·1500	·3500	·5000	60·50
F_1	·2107	·3761	·4132	64·34
F_2	·2585	·3646	·3769	65·82
F_3	·2912	·3520	·3568	66·40
F_6	·3274	·3361	·3365	66·66
F_{10}	·3330	·3335	·3335	66·67
Fn	·3333	·3333	·3333	66·67

Equation 6 was used to calculate the frequencies of 3 sex alleles in subsequent generations, presented in Table 2. The frequency of any one allele cannot exceed 0·5 (the sum of all other alleles in the parental generation); this could happen only if homozygotes exist, and these are eliminated from the offspring generation in the proportion of the square of the frequency of the allele in the parental generation. An allele of low frequency (0·15) is thus eliminated in still lower proportion (0·0225), and that of high frequency (0·5) in a proportionately much higher proportion (0·25). Therefore (Table 2) the allele X_1 of low frequency increases its frequency in the subsequent generations, and X_3 of high frequency decreases its frequency. Finally the alleles reach an equilibrium state, in which the frequency of each allele is $1/N$, i.e. 0·3333.

The survival rate of brood in a population with unequal frequencies of alleles is always lower than in a population with equally distributed alleles (Table 2): in P, $S\% = 60{\cdot}5\%$; in F_n, $S\% = 66{\cdot}7\%$.

It must be assumed that, after many generations of mating at random, the frequencies of the different sex alleles in the honeybee population on Kangaroo Island are equal.

Introduction of new sex allele into a population

When there are few sex alleles in a population, and the survival rate of brood is therefore low, the introduction of new alleles would be desirable. But introduction of many new colonies could well change the character of the isolated population, which is not desirable. Can the situation be improved by introducing new alleles in low proportions ?

Let us assume a population with alleles X_1, X_2 X_3, . . . X_n, to which a new X_{n+1} is introduced. The frequencies of the alleles in the whole population are designated $p_1 + p_2 + p_3 \ldots p_n + q = 1$. If the alleles of the initial population with N alleles were in equilibrium, then $p_1 = p_2 = p_3 \ldots = p_n = p$. Thus equation 6 for the frequency of an allele in the next generation may be simplified :

$$p' = \frac{(N-1)\,p^2 + pq}{1 - Np^2 - q^2} \quad \text{and} \quad q' = \frac{Npq}{1 - Np^2 - q^2} \qquad (8)$$

TABLE 3. Frequencies of sex alleles, and survival of brood, after introducing a new allele, in a proportion of 5%, into a population with few ($n = 3$, on left) or many ($n = 10$, on right) alleles. $X_2 \ldots . Xn$ have the same frequencies as X_1.

Generation	*Frequencies* X_1	X_{n+1}	% *surviving brood* ($S\%$)	*Frequencies* X_1	X_{n+1}	% *surviving brood* ($S\%$)
	p	*q*		*p*	*q*	
Initial	·3333	—	66·67	·1000	—	90·00
P	·3167	·0500	69·66	·0950	·0500	90·73
F_1	·3106	·0682	70·59	·0948	·0524	90·74
F_3	·2952	·1144	72·55	·0943	·0570	90·78
F_5	·2788	·1636	74·00	·0939	·0613	90·81
Fn	·2500	·2500	75·00	·0909	·0909	90·91

But in equations 7 the numerator will not be changed. Frequencies of sex alleles obtained after repeated application of equation 8 are shown on the left of Table 3. One allele, introduced in a low proportion (5%) to a population with 3 alleles, increases its frequency rapidly, and the survival rate of brood improves. After 10 generations the survival of brood is almost that characteristic of four alleles (75·0%). Thus it is worth while to introduce a new allele into populations with few alleles.

Table 3 (right) shows that an allele introduced in the same proportion (5%) into a population with many (10) alleles increases its frequency very slowly, and improvement in the survival of brood is almost undetectable. Therefore it is not worth while to introduce an additional allele into a population with many alleles.

It is possible to introduce to a population with alleles X_1, X_2, X_3, . . .X_n not one, but several new K alleles X_{n+1}, X_{n+2} . . . X_{n+k}. The frequencies in the new combined paternal population are $p_1 + p_2 + p_3 + \dots p_n + q + r + s$, and the total $= 1$. If the frequencies of the alleles in the initial population were in equilibrium ($p_1 = p_2 = \dots p_n = p$) then the frequency of a given allele in the next generation would be :

$$p' = \frac{(N-1)\,p^2 + pq + pr + ps}{1-Np^2 - q^2 - r^2 - s^2} \quad \text{and} \quad q' = \frac{Npq + qr + qs}{1-Np^2 - q^2 - r^2 - s^2}$$

If all the introduced K alleles have an identical frequency, $q = r = s = q$, and $q \neq p$, and the frequency of a given allele in the next generation is given by :

$$p' = \frac{(N-1)\,p^2 + Kpq}{1 - Np^2 - Kq^2} \quad \text{and} \quad q' = \frac{Npq + (K-1)\,q^2}{1 - Np^2 - Kq^2} \qquad (9)$$

These formulae can also be presented in the same form as equation 7.

Different frequencies of alleles in the two sexes

Since the drones develop from unfertilized eggs, a queen produces females with sex alleles and frequencies different from those in the males.

A queen X_1X_2 inseminated by an X_3 drone ($X_1X_2.X_3$) produces 0·5 X_1 and 0·5 X_2 alleles. But the several million spermatozoa produced by the drone are all identical, and therefore the frequency is $1{\cdot}0X_3$. After a cross ($X_1X_2.X_3$) is made, females produced are X_1X_3 and X_2X_3 and males X_1 and X_2. The frequencies of alleles in the female generation are now $0{\cdot}25X_1$, $0{\cdot}25X_2$ and $0{\cdot}50X_3$, and in the males $0{\cdot}5X_1$ and $0{\cdot}5X_2$. The three alleles will appear in the males in the next generation. Generally speaking, if there are X_1, X_2, X_3 alleles in a population, with frequencies $p_f + q_f + r_f$ ($= 1$) in the females and $p_m + q_m + r_m$ ($= 1$) in the males, then after a random cross, genotypes of the following frequencies are produced :

$$\begin{aligned} &p_fp_m + q_fq_m + r_fr_m + p_fq_m + p_mq_f + p_fr_m + p_mr_f + q_fr_m + q_mr_f = \\ &p_fp_m + q_fq_m + r_fr_m + p_f(q_m + r_m) + q_f(p_m + r_m) + r_f(p_m + q_f) = 1 \end{aligned}$$

The survival rate of heterozygotes $S = 1 - p_mp_f - q_mq_f - r_mr_f$. The frequency of a given allele in the next generation after eliminating the heterozygotes is given by :

$$p_f' = \tfrac{1}{2}\,\frac{p_fq_m + p_mq_f + p_fr_m + p_mr_f}{1 - p_fp_m - q_fq_m - r_fr_m}$$

or, after transforming,

$$p_f' = \tfrac{1}{2}\,\frac{p_f(q_m + r_m) + p_m(q_f + r_f)}{1 - p_fp_m - q_fq_m - r_fr_m} \qquad (10)$$

Similar expressions give q_f' and r_f'. This is true for the diploid (female) offspring. Since drones develop from unfertilized eggs, the frequency of alleles for haploid male offspring is identical to that of females in the previous generation.

Generalizing, if the frequencies of different alleles are designated p_1, p_2, p_3 and so on, then the frequency of an allele in the next generation :

$$p_{1f}' = \frac{1}{2}\;\frac{p_{1f}(p_{2m} + p_{3m} + p_{3m} + \ldots) + p_{1m}(p_{2f} + p_{3f} + p_{4f} + \ldots)}{1 - p_{1f}p_{1m} - p_{2f}p_{2m} - p_{3f}p_{3m} \ldots} \tag{11}$$

Very often the frequencies of several alleles can be collected into two groups : for example when, to one population, a new one is introduced.

Consider a population with N alleles X_1, $X_2 \ldots X_n$, to which K alleles $X_n + {}_1$, $X_n + {}_2$, $\ldots X_k$ were introduced, so that the frequencies in females are :

$$p_{1f} + p_{2f} + \ldots p_{nf} + q_{1f} + q_{2f} + \ldots q_k = 1,$$

and in males $\quad p_{1m} + p_{2m} + \ldots p_{nm} + q_{1m} + q_{2m} + \ldots q_{nm} = 1.$

If the frequencies were equal within each of the two populations but not between them, then $p_1 = p_2 = \ldots p_n = p$ and $q_1 = q_2 = \ldots q_k = q$. Equation 11 may then be simplified :

$$p_f' = \frac{1}{2}\;\frac{p_f\,[(N-1)\,p_m + Kq_m] + p_m\,[(N-1)p_f + Kq_f]}{1 - Np_fp_m - Kq_fq_m}$$

and, after transformation,

$$p_f = \frac{(N-1)\,p_fp_m + \tfrac{1}{2}K(p_fq_m + p_mq_f)}{1 - Np_fp_m - Kq_fq_m}$$

and, by analogy,

$$q_f = \frac{\tfrac{1}{2}N(p_fq_m + p_mq_f) + (K-1)\,q_fq_m}{1 - Np_fp_m - Kq_fq_m} \tag{12}$$

The simplest example of the introduction of a new allele into a population with diploid females and haploid males is a population originating from one queen inseminated by one drone $X_1X_2.X_3$. Here $N = 2$ and $K = 1$. The situation may be treated as one population with two alleles, equally distributed within each sex but differently between them (p_f, p_m), to which a population is added with 1 allele differently distributed between the two sexes (q_f, q_m). Equation 12 would be reduced to :

$$p_f' = \frac{p_fp_m + \tfrac{1}{2}(p_fq_m + p_mq_f)}{1 - 2p_fp_m - q_fq_m}$$

and

$$q_f' = \frac{p_fq_m + p_mq_f}{1 - 2p_fp_m - q_fq_m} \tag{12a}$$

Alleles X_1 and X_2 are absent in the parental generation in the male, and allele X_3 in the female. Therefore $p_m = 0$ and $q_f = 0$. No homozygotes are produced in the F_1 offspring (survival rate of brood is 1·00), and therefore the above formulae for F_1 are reduced to $p'_f = p_fq_m/2$ and $q'_f = p_fq_m$.

In F_1, $q_m = 0$, but starting with F_2 all alleles are present in both sexes, and therefore the above equations are repeatedly applied (IBRA Table 5). This is a typical shift from a three-allele cross (P) to a population with 3 alleles unequally distributed. The survival rate of brood drops from 100% to 66·7%, characteristic for 3 alleles. Equations 7, 10, 11 and 12 may be transformed into :

$$p_f' = \frac{1}{2}\,\frac{p_f(1-p_m)+p_m(1-p_f)}{1-p_fp_m-q_fq_m-r_fr_m} = \frac{1}{2}\,\frac{p_f-p_fp_m+p_m-p_fp_m}{1-p_fp_m-q_fq_m-r_fr_m}$$

Thus

$$p_f' = \frac{\dfrac{p_f+p_m}{2}-p_fp_m}{1-p_fp_m-q_fq_m-r_fr_m}$$

or

$$p_f' = \frac{\dfrac{p_f+p_m}{2}-p_fp_m}{1-Np_fp_m-Kq_fq_m} \tag{13}$$

and similarly for q_f' and r_f', and so on. Equation 13 is quite different from the usual one for populations with heterogametic sex, where no selection occurs: $p_f' = (p_f+p_m)/2$. In the last case the gene frequency in the female offspring is the mean of the gene frequencies of their male and female parents. But this is not so with the sex alleles in the honey-bee.

Now consider the difference ($\triangle$) between the frequencies of a given sex allele in the two sexes :

$$\triangle p = p_f - p_m \qquad \text{and} \qquad \triangle p' = p_f' - p_m'$$

Since the frequency of an allele in drones is equal to that in females of the previous generation,

$$p_m' = p_f \qquad \text{and so} \qquad \triangle p' = p_f' - p_f$$

Hence, subtracting p_f from (13),

$$\triangle p = \frac{\frac{1}{2}(p_f-p_m)-p_fp_m}{1-Np_fp_m-Kq_fq_m} - p_f$$

$$= \frac{\frac{1}{2}p_f+\frac{1}{2}p_m-p_fp_m-p_f+Np_f^2p_m+Kp_fq_fq_m}{1-Np_fp_m-Kq_fq_m}$$

$$= \frac{\frac{1}{2}p_m-\frac{1}{2}p_f+Kp_fq_fq_m-p_fp_m(1-Np_f)}{1-Np_fp_m-Kq_fq_m}$$

Since $Np_f+Kq_f=1$, $1-Np_f=Kq_f$, and so

$$\triangle p' = \frac{Kp_fq_f(q_m-p_m)-\frac{1}{2}(p_f-p_m)}{1-Np_fp_m-Kq_fq_m} \tag{14}$$

and by analogy

$$\triangle q' = \frac{Np_fq_f(p_m-q_m)-\frac{1}{2}(q_f-q_m)}{1-Np_fp_m-Kq_fq_m}$$

This equation is also very different from that for populations with heterogametic sexes where no selection occurs : $\triangle q' = -(q_f - q_m)/2$. In the latter case the difference in gene frequency between two sexes is halved in each generation, and the sign is changed; the change in gene frequency is oscillatory. Equation 14 shows that this may or may not occur with the sex alleles in the honeybee. The sign and value of the difference between sexes in a population depends upon constant N and K values and changeable p and q values.

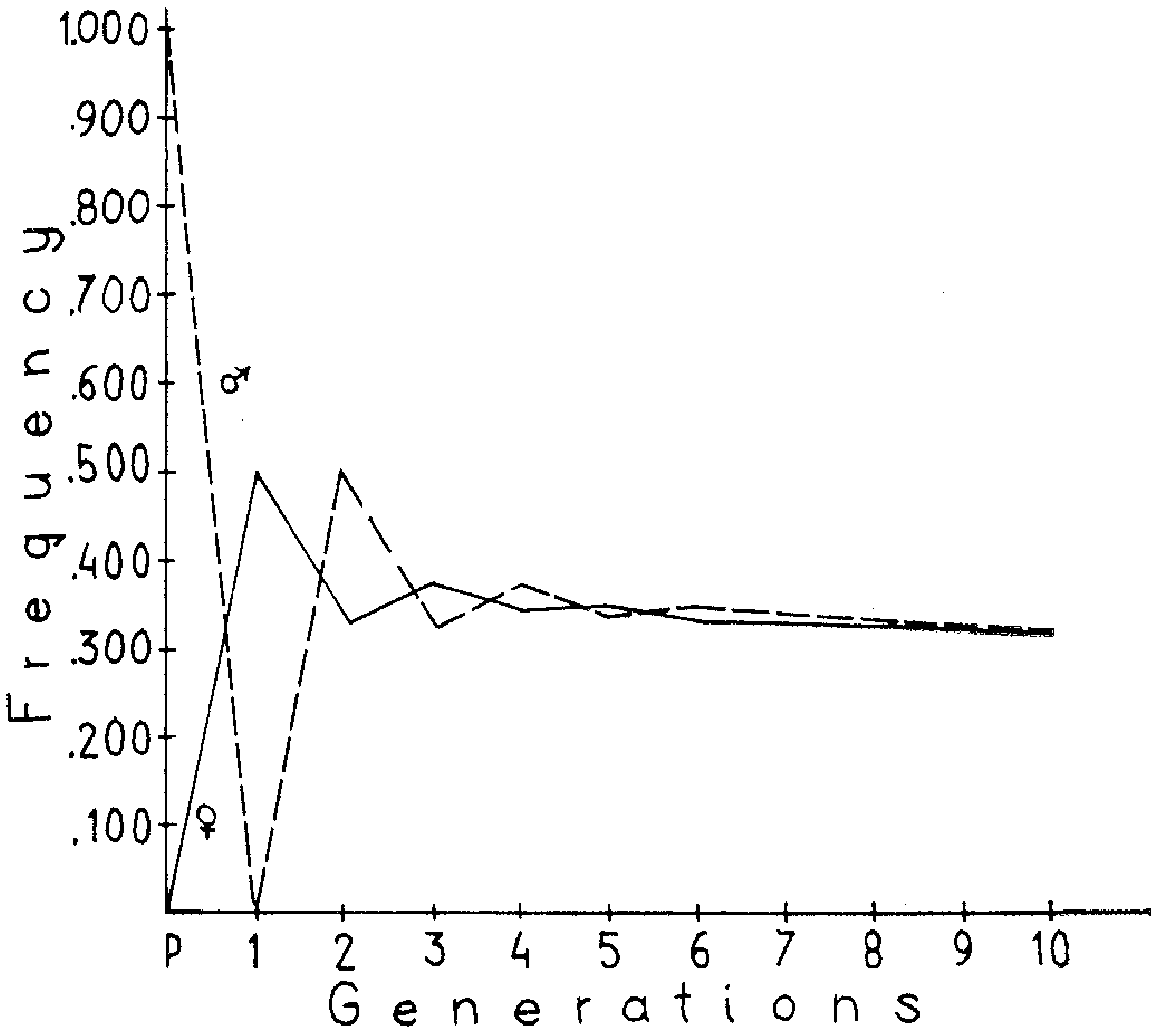

FIG. 5. Frequency of X_3 allele in subsequent generations of males (- - - -) and females (———) originating from an $X_1X_2.X_3$ cross.

Fig. 5 shows that, with three alleles, there is a diminishing oscillation of frequencies of the same allele between the two sexes until the sixth generation. The frequencies in both sexes next diminish (IBRA Table 5) to the level at which $1/N = 0 \cdot 3333$. Until then, however, the frequency of the same allele is always higher in one sex than in the other.

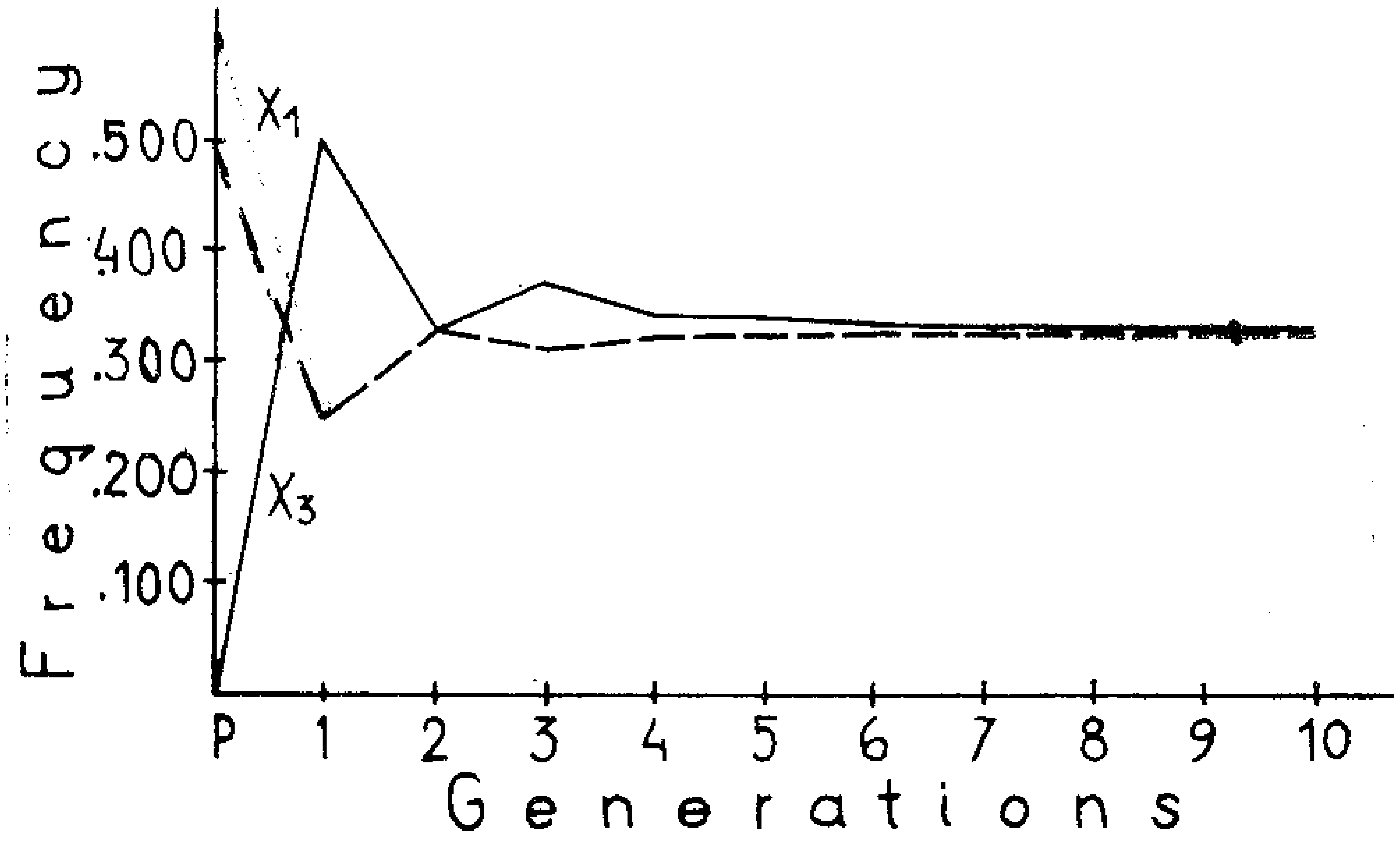

FIG. 6. Frequency of X_1 (- - - -) and X_3 (———) alleles in subsequent generations of females originating from an $X_1X_2.X_3$ cross.

Fig. 6 shows that there is also oscillation between different alleles (X_1 and X_3) in the same sex. But, after few generations, both the alleles approach the $1/N$ level without oscillation. During this process the frequency in the female of the X_1 allele is lower than in the male, whereas that of the X_3 allele is higher (IBRA Table 5).

In nature a queen mates not with 1 drone but with 8-10. If a population is created by a queen X_1X_2 mated to 8 drones X_3, X_4, . . .X_{10}, then the situation is like a population with $N = 2$ alleles to which 8 new K alleles were introduced (equation 12). In the parental generation $p_m=0$ and $p_f=0{\cdot}500$; $q_m=0{\cdot}125$ and $q_f=0$. In F_1, $p_m=0{\cdot}500$ and $p_f = 0{\cdot}250$; $q_m = 0$, and $q_f = 0{\cdot}0625$.

With many alleles, oscillation between the sexes is lower (IBRA Table 6) than in a population with three alleles. After the oscillation ceases (i.e. after F_4), the frequencies are lower in females than in males in the high-frequency group (X_1, X_2), but higher in the low-frequency group ($X_3 \ldots X_{10}$). Thus the female alleles are the leading ones in approaching the $1/N$ level frequency. Equalization of the frequency of different alleles occurs slowly. The survival rate of brood produced by the parents is 100% (three-allele crosses only). It drops rapidly to 75% in the F_1 generation and then increases slowly towards the level where $S\% = 100(N - 1)/N = 90\%$. Thus it reaches eventually a higher survival rate than in a population originating from a queen mated to one drone. The character of the changes of the survival rate of brood (IBRA Table 6) is similar to that in a population created by a queen inseminated by one drone (IBRA Table 5).

Introduction of new queen into a population

In any attempt to increase the survival rate of brood in a population with few alleles, more than one allele will generally be introduced. The new alleles may be introduced in one sex only, as when one or more colonies are introduced and their drones are allowed to mate with the queens of the initial population, but no new virgin queens are allowed to be reared in the introduced colonies. The formula for a queen inseminated with several drones may be applied. If the distribution of sex alleles is equal within the initial population, and within the introduced drones, but different between the two populations, a typical situation for two groups of alleles occurs, characteristic of equation 12.

Usually one or more queens will be introduced, and male as well as female offspring will be reared freely. Drones mated to the queens will introduce alleles additional to those of the queens. Thus three groups of alleles will be present in the new combined populations : p_1X_1, p_2X_2, . . . p_nX_n of the initial population, $q_1X_{n+1} \ldots q_kX_k$ introduced by the queens, and additionally $r_1X_{k+1} \ldots r_uX_u$ introduced by the drones mated to the queens.

Spermatozoa in the spermatheca of introduced queens cannot fertilize other eggs in the initial population, and eggs produced by the introduced queens cannot be fertilized by spermatozoa of drones present in the initial population. Crosses can occur only between progeny of the two populations, and therefore the progeny must be treated as the parental generation (P) of the new population. The survival rate of brood, and frequencies of different alleles in subsequent generations, may be calculated from equations 10, 11 or 12. But when the frequencies of the three groups were equal within the groups but different between them, then :

$$p_1 = p_2 = \ldots p_n = p; \quad q_1 = q_2 = \ldots q_k = q; \quad r_1 = r_2 = \ldots r_u = r.$$

The survival of brood $S = 1 - Np_fp_m - Kq_fq_m - Ur_fr_m$. Simplifying equations 10 and 11, the frequency of a given allele in the next generation is :

$$p' = \tfrac{1}{2}\,\frac{p_f[(N-1)p_m + Kq_m + Ur_m] + p_m[(N-1)p_f + Kq_f + Ur_f]}{1 - Np_fp_m - Kq_fq_m - Ur_fr_m} \qquad (15)$$

and similarly for q' and r'. After transforming equation 15, the frequency of a given allele in the next generation may be presented as follows :

$$p' = \frac{(N-1)\,p_f p_m + \tfrac{1}{2}K(p_f q_m + p_m q_f) + \tfrac{1}{2}\,U(p_f r_m + p_m r_f)}{1 - Np_f p_m - Kq_f q_m - Ur_f r_m}$$

and by analogy

$$q' = \frac{\tfrac{1}{2}\,N(p_f q_m + p_m q_f) + (K-1)\,q_f q_m + \tfrac{1}{2}\,U(q_f r_m + q_m r_f)}{1 - Np_f p_m - Kq_f q_m - Ur_f r_m} \qquad (16)$$

$$r' = \frac{\tfrac{1}{2}\,N(p_f r_m + p_m r_f) + \tfrac{1}{2}\,K\,(q_f r_m + q_m r_f) + (U-1)r_f r_m}{1 - Np_f p_m - Kq_f q_m - Ur_f r_m}$$

To increase the number of alleles, and thus the survival of brood, without changing the character of the initial population, it is desirable to introduce the smallest number of queens with the greatest number of alleles.

The simplest example is the introduction into a population of a single queen, inseminated from a single drone. Consider a population with 3 alleles X_1, X_2, X_3, into which a queen $X_4X_5 \,.\, X_6$ was introduced in a proportion of 1% (IBRA Table 7). Then $N = 3$, $K = 2$ and $U = 1$, and the frequencies of alleles in the combined population are : $p_m = p_f = 0{\cdot}3300$ for each of the 3 alleles of the initial population, $q_m = 0$ and $q_f = 0{\cdot}0050$ for both alleles introduced by the queen, and $r_m = 0{\cdot}0100$ and $r_f = 0$ for allele introduced by the drone mated to the queen. Only offspring of this population will mate freely, and therefore the frequencies of alleles in the P generation are : $p_m = p_f = 0{\cdot}3300$; $q_m = 0{\cdot}0050$; $q_f = 0{\cdot}0025$; $r_m = 0$, $r_f = 0{\cdot}0050$. Introduction of a queen into initial population even in such a low proportion changes the frequency of all alleles in that population (IBRA Table 7). But oscillation is absent between the sexes in the high-frequency group (X_1, X_2, X_3), and it disappears with F_2 even in the low-frequency group (X_4, X_5, X_6). Nevertheless, introduction of new alleles into a population in equilibrium causes non-oscillatory differences in frequencies of alleles between the sexes.

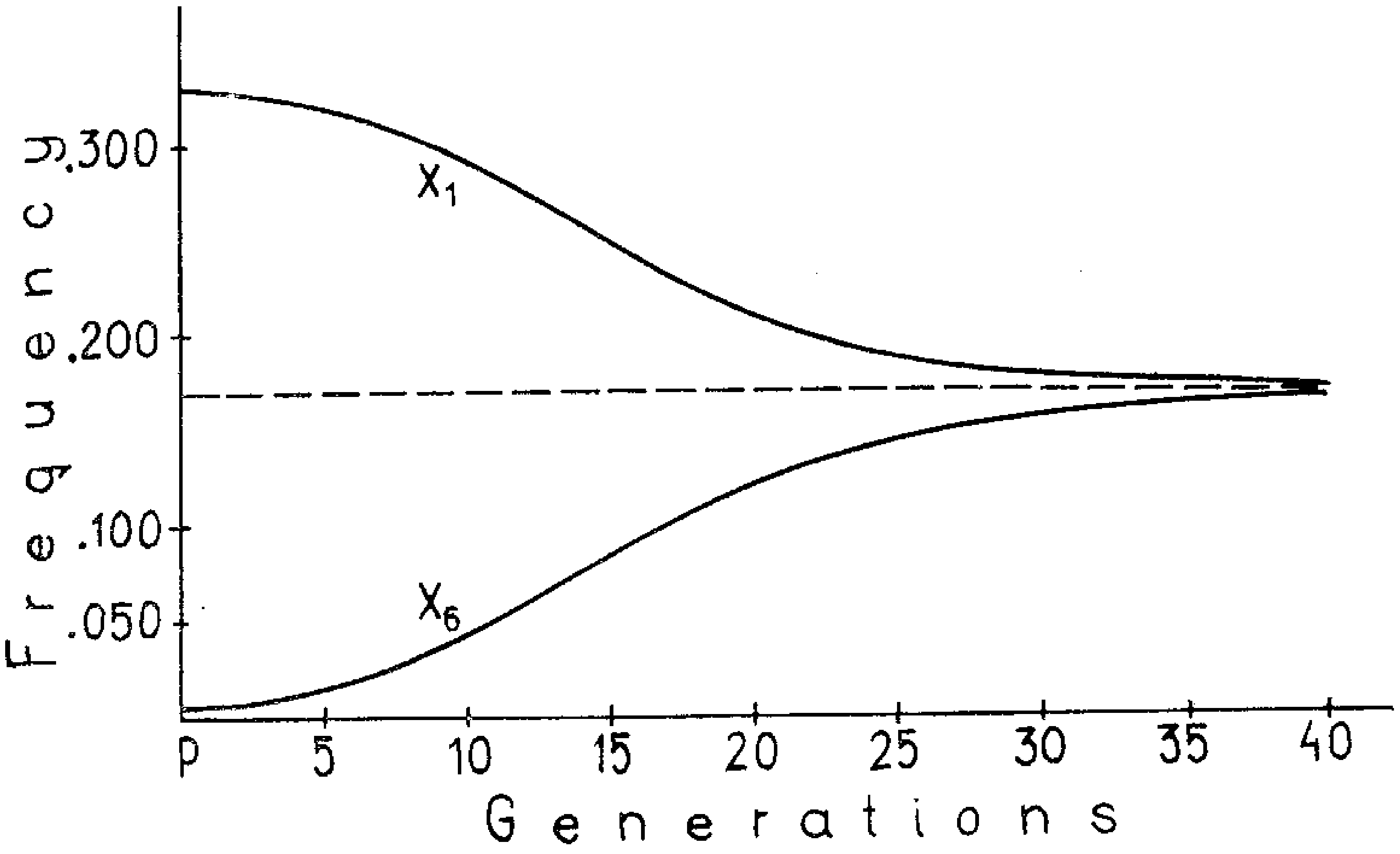

FIG. 7. Frequencies of allele X_1 of the initial population with 3 alleles and of an introduced X_6 allele, after a queen inseminated with one drone ($X_4X_5.X_6$) is introduced into that population in a 1% ratio. The dotted line shows the equilibrium level.

The difference reaches its highest value in the F_{14} generation ($\triangle = 0{\cdot}0090$ to $0{\cdot}0093$) and then decreases gradually until a new equilibrium level is reached, $p = q = r = 0{\cdot}1667$.

Fig. 7 shows that the new equilibrium in frequency of different alleles is not reached linearly. (The frequencies of allele X_4 introduced by the queen, and allele X_6 introduced by the drone, are so similar that they cannot be separated in Fig. 7.) Very slow changes occur with any allele when the frequencies are low. The three introduced alleles reach a total of 10% (0·1003) in the ninth generation. But when frequencies are higher the changes are more rapid, and a further increase of 10% is attained after only 4 generations. This rate of change is maintained until the total reaches a frequency of 40% in F_{22}. But with high frequencies the rate of change slows down greatly again.

The survival rate of brood increases after the introduction of three new alleles from 66·6% in the initial population to 83·3% in F_{38}, characteristic of 6 alleles. Fig. 8 shows that the survival rate of brood in subsequent generations changes in a similar way to the frequencies of alleles. Slow changes occur with the lowest and the highest values of allele frequency, and the most rapid changes with medium frequencies.

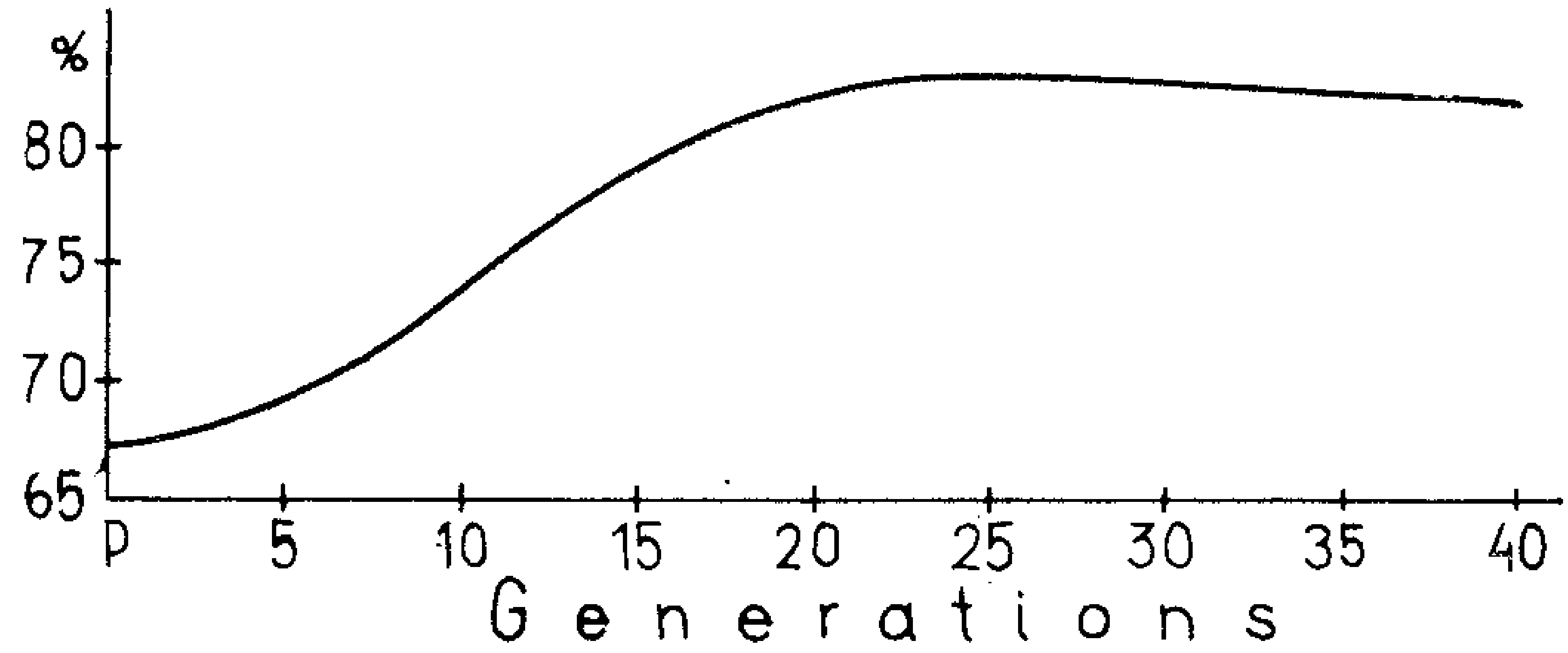

FIG. 8. Percentage survival rate of brood for the conditions represented by Fig. 7.

More alleles are likely to be introduced when the queen introduced in a proportion of 1% is inseminated by several drones, e.g. 5 (IBRA Table 8). Then $N = 3$, $K = 2$, $U = 5$, and the frequencies in the parental generation are $p_m = p_f = 0{\cdot}3300$ for the initial population, $q_m = 0{\cdot}0050$ and $q_f = 0{\cdot}0025$ for alleles introduced by the queen and $r_m = 0$ and $r_f = 0{\cdot}0010$ for alleles introduced by each drone mated to the queen. Repeated application of equation 15 shows that the frequencies of alleles introduced by each drone mated to the queen increase very slowly. But the frequency of alleles introduced by the queen increases much more quickly (Fig. 9). As a result it passes the $1/N = 0{\cdot}1000$ equilibrium level, reaching its highest value (0·1250) in F_{26}. Because of the low frequencies of alleles introduced by drones in the early generations, the whole pool of alleles is more like a population with 5 alleles (3 of the initial population + 2 introduced by the queen), and at equilibrium level $1/N = 0{\cdot}2000$. The frequency of allele X_4 introduced by the queen decreases in further generations to a 0·1000 equilibrium level. The difference between sexes in frequencies of alleles introduced by the queen show interesting changes. The frequency of allele X_4 is at first higher in females; the difference between the sexes is highest in F_{13}, $\triangle = 0{\cdot}09$, then it decreases, until it is almost zero in F_{26} and F_{27}. Afterwards the frequency of allele X_4 is lower in females, with the highest difference between sexes in F_{32} ($\triangle = 0{\cdot}0011$). Finally, the frequencies of alleles introduced by the queen approach the $1/N$ level in both sexes.

Comparing the results of introducing a queen inseminated by one drone (Fig. 7) and by a few drones (Fig. 9), the sum of frequencies of all introduced alleles in F_{10} or even in F_{20} is only a little higher in the population to which the queen inseminated by a few drones was introduced. But, in further generations, alleles introduced by a queen inseminated by a single drone reach in F_N a total frequency of 0·5000, whereas those introduced by a queen inseminated by several drones reach a total frequency of 0·7000. As a result, survival rate of brood in the two populations respectively is 73·3% and 73·5% in F_{10}, 82·0% and 84·6% in F_{20}, and finally 83·3% and 90·0% in F_N.

Thus the number of sex alleles, and consequently the brood survival rate, may be improved by introducing a single queen. This would hardly change the other characteristics of that population, which are not eliminated from it.

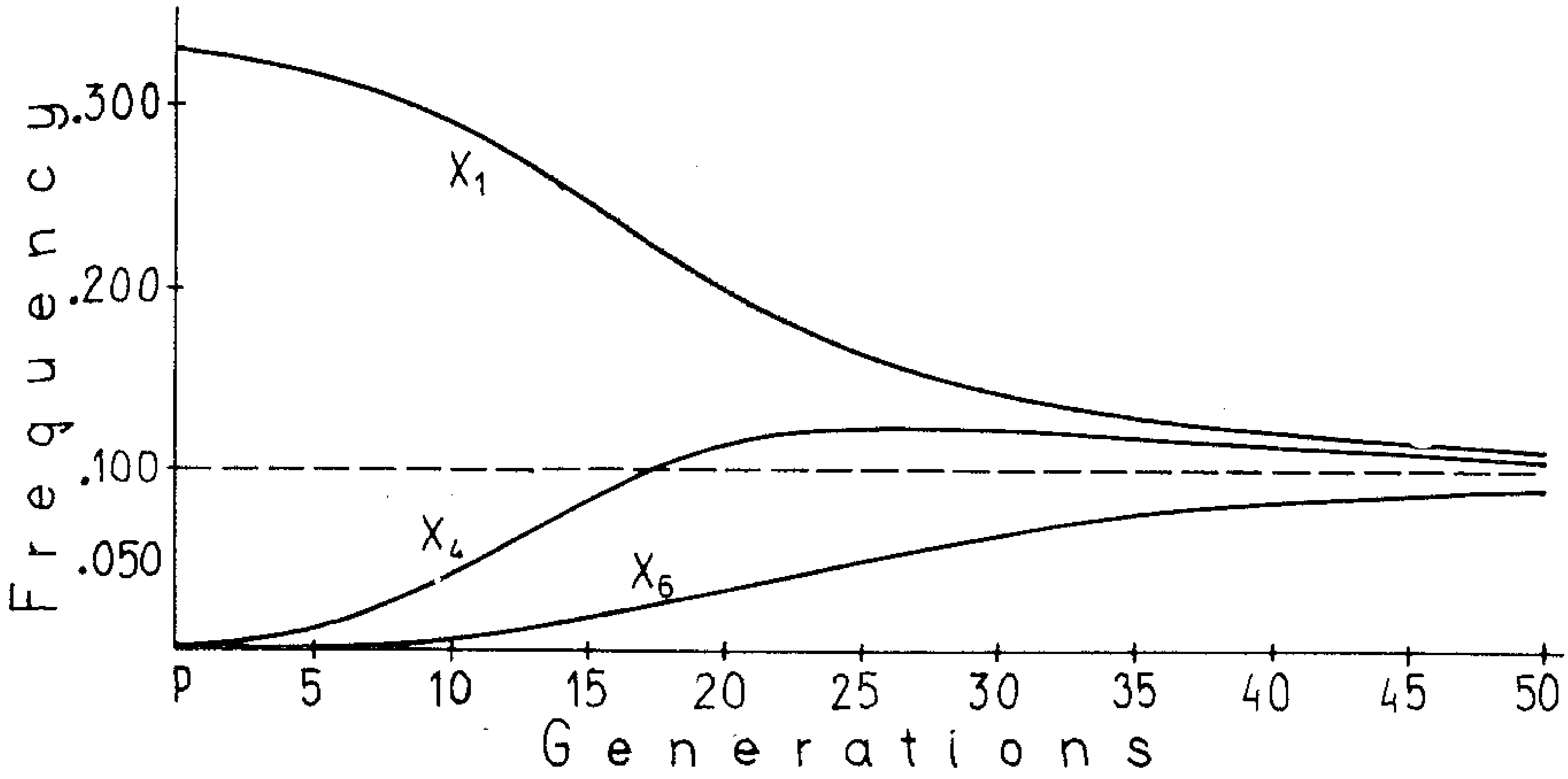

FIG. 9. Frequencies of three alleles in subsequent generations of a population with 3 initial alleles (X_1, X_2, X_3) into which one queen inseminated with 5 drones ($X_4X_5.X_6 \ldots . X_{10}$) is introduced in a proportion of 1%.

X_1 = allele of initial population.
X_4 = allele introduced by the queen.
X_6 = allele introduced by one of the drones mated to the queen.
dotted line = equilibrium level.

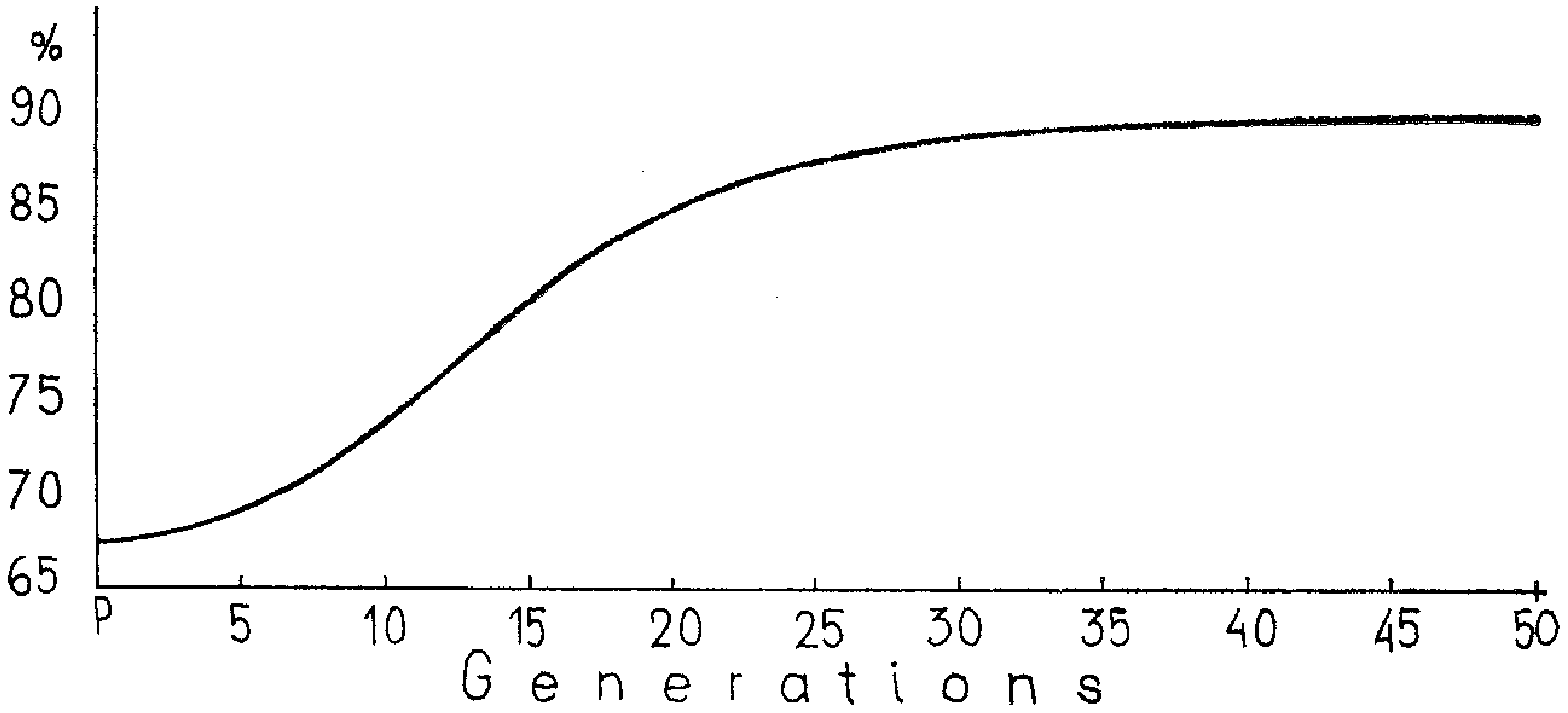

FIG. 10. Percentage survival of brood in subsequent generations of the population presented in Fig. 9.

Comments and Conclusions

After isolation for nearly 100 years, the number of sex alleles in the honeybee population on Kangaroo Island was found to be reduced; brood is scattered, and the colonies do not normally achieve very high populations. Nevertheless, the climate and bee forage on the island are good, and colonies kept commercially are capable of yielding 30-60 kg honey a year.

The low number of alleles may be due to a number of factors. Firstly, the queens initially taken to the Island were apparently few, so they may not have carried all the 12 alleles known to exist in the honeybee. Secondly, commercial queen rearing, and later queen rearing by officers of the S. Australia Department of Agriculture, may have led to some inbreeding and thus to a reduction in the number of alleles. Thirdly, in 1957 and also probably earlier, there were intensive forest fires in the sanctuary; these must have destroyed a high proportion of the wild colonies, with the result that repopulation of the main area of the sanctuary would have been by swarms from the Government apiaries in the Sanctuary. Despite this low number of alleles the bees flourish, conditions on the island being well suited for honeybees.

The results of this study suggest that there may be difficulties in maintaining isolated populations of pure strains of honeybees unless the variability in the sex alleles can be maintained. This problem, which would be accentuated if the climate and bee forage were not optimal, should be considered when new sanctuaries are established for maintaining specific ecotypes of honeybees. It is satisfactory to introduce new alleles, provided their proportions are kept small compared to those of alleles in the existing population; the frequency of the new alleles will increase after several generations of random mating, and heterozygosity in the population may thus be maintained.

Such introductions should not be made on Kangaroo Island. This is a unique sanctuary of a pure strain of *Apis mellifera ligustica*, and the bees are of extraordinary value for scientific research and for practical beekeeping.

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